ITALIAN JOURNAL OF FOOD SCIENCE

Rivista italiana di scienza degli alimenti

IJFS

Volume XXIX
Number 2
2017

CHIRIOTTI EDITORI

ITALIAN JOURNAL OF FOOD SCIENCE

(RIVISTA ITALIANA DI SCIENZA DEGLI ALIMENTI) 2nd series

Founded By Paolo Fantozzi under the aeges of the University of Perugia
Official Journal of the Italian Society of Food Science and Technology
Società Italiana di Scienze e Tecnologie Alimentari (S.I.S.T.Al)
Initially supported in part by the Italian Research Council (CNR) - Rome - Italy
Recognised as a "Journal of High Cultural Level"
by the Ministry of Cultural Heritage - Rome - Italy

Publisher:
Alberto Chiriotti - Chiriotti Editori srl, Viale Rimembranza 60, I-10064 Pinerolo, Italy - Tel. +39 0121 393127 - Fax +39 0121 794480 e-mail: alberto@chiriottieditori.it - URL: www.chiriottieditori.it

Aim:

The Italian Journal of Food Science is an international journal publishing original, basic and applied papers, reviews, short communications, surveys and opinions on food science and technology with specific reference to the Mediterranean Region. Its expanded scope includes food production, food engineering, food management, food quality, shelf-life, consumer acceptance of foodstuffs. Food safety and nutrition, and environmental aspects of food processing.
Reviews and surveys on specific topics relevant to the advance of the Mediterranean food industry are particularly welcome.
Upon request and free of charge, announcements of congresses, presentations of research institutes, books and proceedings may also be published in a special "News" section.

Review Policy:

The Co-Editors with the Editor-in-Chief will select submitted manuscripts in relationship to their innovative and original content. Referees will be selected from the Advisory Board and/or qualified Italian or foreign scientists. Acceptance of a paper rests with the referees.

Frequency:

Quarterly - One volume in four issues. Guide for Authors is published in each number and annual indices are published in number 4 of each volume.

Impact Factor:

Impact Factor: 0.504 published in 2015 Journal of Citation Reports, Scopus CiteScore 2016: 0.84.
IJFS is abstracted/indexed in: Chemical Abstracts Service (USA); Foods Adlibra Publ. (USA); Gialine - Ensia (F); Institut Information Sci. Acad. Sciences (Russia); Institute for Scientific Information; CurrentContents®/AB&ES; SciSearch® (USA-GB); Int. Food Information Service - IFIS (D); Int. Food Information Service - IFIS (UK); EBSCO Publishing; Index Copernicus Journal Master List (PL).

IJFS has a publication charge of € 350.00 each article.
Subscription Rate: IJFS is now an Open Access Journal and can be read and downloaded free of charge at http://www.ijfs.eu
Journal sponsorship is € 1,2010.00

OPINION PAPER

CHARACTERIZATION OF SEMOLINA BIOPOLYMER FILMS ENRICHED WITH ZINC OXIDE NANO RODS

SHIMA JAFARZADEH*[a], FAZILAH ARIFFIN[b], SHAHROM MAHMUD[c], ABD KARIM ALIAS[d], ALI NAJAFI[e] and MEHRAJ AHMAD[f]

[a]PhD candidate, Food Biopolymer Research Group, Food Technology Division, School of Industrial Technology, University Sains Malaysia, 11800 Minden, Penang, Malaysia

[b]Associate Professor, Food Biopolymer Research Group, Food Technology Division, School of Industrial Technology, School of Industrial Technology, University Sains Malaysia, 11800 Minden, Penang, Malaysia

[c]Associate Professor, Nano Optoelectronic Research (NOR) Lab, School of Physics, University Sains Malaysia, 11800 Minden, Penang, Malaysia

[d]Professor, Food Biopolymer Research Group, Food Technology Division, School of Industrial Technology, University Sains Malaysia, 11800 Minden, Penang, Malaysia

[e]PhD candidate, Food Biopolymer Research Group, Food Technology Division, School of Industrial Technology, University of Damghan, Semnan, Iran

[f]Senior lecturer, Institute of Nutrition (INMU), Mahidol University, 999 Phutthamonthon 4 Rd, Salaya, Nakhon Pathom 73170, Thailand

*Corresponding author. shimajafar@yahoo.com

ABSTRACT

This research aimed to develop biopolymer-based antimicrobial films as food packaging that will consequently reduce environmental pollution caused by the accumulation of petroleum origin food packaging. Zinc oxide nanorods (ZnO-nr) were incorporated as the antimicrobial component in nanocomposite films based on semolina, which were prepared by solvent casting. SEM and XRD were used to characterize the resulting films. The mechanical, barrier, optical, physical and antimicrobial properties of the films were also analyzed. The addition of ZnO-nr reduced the solubility, WVP, and elongation at break while increased the tensile strength and modulus of elasticity of the nanocomposite films compared with the control film. The apparent surface color and UV transmittance of the semolina films was greatly influenced by the amount of ZnO-nr. The nanocomposite films exhibited 0% UVA in transmittance the near infrared spectra. Furthermore, the ZnO-nr semolina films exhibited strong antimicrobial activity against *Staphylococcus aureus*.

Keywords: antimicrobial, packaging, zinc oxide nano rod, semolina

1. INTRODUCTION

A large number of biodegradable polymers have been studied to develop edible films and finally lower the amount of waste produced through non-degradable petroleum-based food packaging activities (THARANATHAN, 2003; JAFARZADEH *et al*., 2016). These edible films not only provide physical protection to foods but also prevent the mass transfer of moisture, oxygen, carbon dioxide, lipids, flavors, and aromas into and from food products (MARCUZZO *et al*., 2010). Given their nutritive value and superior properties, protein-based biopolymers have attracted considerable interest for the development of edible films (GENNADIOS and YADA, 2004). These polymers are excellent oxygen barriers and they render certain mechanical properties to the films (SOTHORNVIT *et al*., 2009).

Different types of proteins are used as components of biodegradable packaging (Khwaldia *et al*., 2010). Among these proteins, the protein from wheat is a potential component of packaging materials because of its cost effectiveness, biodegradability, renewability, and favorable film-forming and adhesive/cohesive properties (TÜRE *et al*., 2013). Semolina is a type of wheat whose flour contains high gluten content (QUAGLIA, 1988). The gluten increases the nutrient value of edible films. Semolina grain is extra hard, translucent, light colored, and exhibits antioxidant activities (ONYENEHO and HETTIARACHCHY, 1992; JAFARZADEH *et al*., 2017b). In addition, semolina extracts suppress radical-induced liposome lipid peroxidation and show radical cation-scavenging activity (ONYENEHO and HETTIARACHCHY, 1992).

However, the poor mechanical and water sensitivity of biopolymers limit their application in food packaging. Nanoparticles, which reinforce biopolymers through the formation of nanocomposites, have been recently employed to overcome these limitations (DUFRESNE, 2006). One of the most successful applications of nanotechnology in the field of packaging concerns the development of "nanocomposites"(UNALAN *et al*., 2014). The nanoparticles dispersed in the biopolymer matrix considerably reinforce the mechanical, thermal, optical, and physicochemical properties of nanocomposites compared with pristine biopolymer (PETERSSON and OKSMAN, 2006). Given their large specific surface area and high surface energy, nanofillers exhibit excellent interfacial interactions with polymer branches and consequently enhance polymer properties significantly (KOVACEVIC *et al*., 2008). Zinc oxide (ZnO) has been widely applied as a functional filler in UV absorbers in pharmaceuticals, cosmetics, coating materials, and pigments (KUMAR and SINGH, 2008; Li *et al*., 2009; Yu *et al*., 2004). ZnO nanoparticles can potentially prevent infectious diseases through the antimicrobial effects of ZnO (LI *et al*., 2009; Li *et al*., 2010; RAJENDRA *et al*., 2010; ZHANG *et al*., 2008). The size, morphology, crystallinity, composition, and shape of ZnO nanoparticles affect their intrinsic properties (SHAHROM and ABDULLAH, 2006; JAFARZADEH *et al*., 2017a). LIN *et al*. (2009) reported that ZnO nanorods (ZnO-nr) exhibit optimal UV-absorption activity. Semolina displays excellent properties for edible film production, and ZnO-nr are potent materials in reinforcing the mechanical, physicochemical, and barrier properties of semolina. Despite these advantages, ZnO-nr-reinforced semolina remains poorly understood. Studies published on semolina thus far are limited. To the best of our knowledge, there is no information regarding of the preparation blend films from semolina, Zno-nr as filler and glycerol and sorbitol as plasticizer. In the present study, we hypothesized that low-concentration ZnO-nr addition into semolina films improves the hydrophobicity of the films and that the resulting biopolymeric films exhibit UV-blocking, low WVP and antimicrobial properties. Given their favorable antimicrobial activities, the proposed films can be used as food packaging especially for cheese. In this study was used ZnO-nr as fillers to prepare semolina film

bionanocomposites and characterized the morphology, physicochemical, mechanical, and barrier properties of the prepared films.

2. MATERIALS AND METHODS

2.1. Materials

Semolina flour (14.2% protein, 18.5% gluten) was purchased from the local market in Tehran, Iran and then stored in a dry and cool place until the tests. Food-grade glycerol was obtained from SIM Company Sdn. Bhd. (Penang, Malaysia), whereas food-grade liquid sorbitol was purchased from LiangtracoSdn. Bhd. (Penang, Malaysia). The magnesium nitrate used to control humidity was purchased from Sigma Aldrich (Kuala Lumpur, Malaysia), and ZnO-nr was synthesized through the catalyst-free combust-oxidized mesh process as described by SHAHROM and ABDULLAH (2007).

2.2. Preparation of bionanocomposite films

Semolina flour (4g) was dispersed by magnetic stirring in 80 mL of distilled water (based on water or water/ethanol) at room temperature, and the pH of the dispersion was adjusted to 8 with 1M NaOH. Similarly, various concentrations of ZnO powder (1%, 2%, 3%, 4%, and 5%; w/w of total solid) and 2g of a sorbitol and glycerol (3:1) mixture were dispersed in 20 mL of distilled water for 30min followed by sonication in an ultrasonic bath (Marconi model, Unique USC 45 kHz, Piracicaba, Brazil) (JAFARZADEH *et al*., 2017c). Subsequently, the dispersions of semolina flour and ZnO-nr plasticizer were mixed and stirred for 1 h at 90 °C. For the preparation of nanocomposite films, the homogenous mixtures were poured into plates and the solvents were allowed to evaporate at room temperature for 24h.
The films were dried under controlled conditions in a humidity chamber (25 °C and 58% relative humidity (RH). A control film was prepared in a similar manner except for the addition of nanoparticles. The dried films were peeled and stored at 23 ± 2 °C and 58% RH until use.

2.3. Characterization studies

2.3.1 Determination of film thickness

The films were equilibrated at 25 °C and 58% RH in a humidity chamber for 2 days. The thickness of the nanocomposite films was determined as the mean of measurements made at five random points. Measurements were obtained using a micrometer (Model No. 2046-08; Mitutoyo Tokyo, Japan).

2.3.2 Water solubility

The solubility of the semolina/ZnO-nr films in deionized water was calculated as the ratio of the solubilized material in water to the initial dry weight of the film (RHIM *et al*., 1999). The initial dry weight of films 2.5cm × 2.5cm in dimension was obtained after dehydration for 3 days at 25 °C in a desiccator with phosphorus pentoxide (0% RH). The samples were placed in a beaker with 80 mL of deionized water (18 MX) and then gently shaken at 40 rpm for 1 h at room temperature. The remaining pieces of the films were separated using a filter paper (Whatman No. 1) and then dried to a constant weight in an oven at 60 °C for

24h. Finally, the weight of the dried insoluble material was determined. The weight of the water-soluble material was calculated by subtracting the weight of the insoluble dry matter from that of the initial dry matter. The film samples were weighed to the nearest 0.0001g before and after drying. The solubility of the films was determined in triplicate.

2.3.3 Moisture content

To measure the moisture content of the bionanocomposite films, approximately 50mg of the films were conditioned at 58% RH and 25 ℃ for 2 days. Subsequently, the films were dried at 105 °C for 1 day (until equilibrium weight was attained). The moisture content was obtained using the following equation:

$$Moisture\ content = \frac{Mi - Mf}{Mt} \times 100$$

Where *Mi* and *Mf* are the initial and final weights (mg) of the dried samples, respectively. The weight of each sample was measured three times.

2.3.4 Water vapor permeability (WVP)

WVP tests for the semolina films were performed gravimetrically following the ASTM Standard Method E96-05 (ASTM, 2005).

2.3.5 Mechanical properties

A minimum of seven 100mm × 25mm films were conditioned at 25Ċ and 58% RH for at least 48 h in an environmental test chamber (Sang Woo Co., Korea). A texture analyzer (TA-XT2, Stable Micro Systems, Surrey, UK) was used to measure the tensile strength [TS (MPa)], Young's modulus [YM (MPa)], and elongation at break [EB (%)] of the films in accordance with the ASTM Standard Method D882-10 (ASTM, 2010). The initial grip separation was set at 50mm, and the crosshead speed was set at 0.5 mm/s.

2.3.6 Optical properties (color and Light transmission)

We studied the transmittance of the films (in triplicate) at 200 and 800 nm, by using the UV–vis spectrophotometer model UV-1650PC (Shimadzu, Tokyo, Japan). Biofilms were sectioned (60mm × 4mm) and directly placed in a spectrophotometer test cell. An empty glass plate served the reference.

The color of the biofilms was determined by using a colorimeter (Hunter Lab system, model Miniscan XE, USA). The CIELab scale was applied to measure the following parameters: L* (luminosity), a* (red to green), b*(yellow to blue), Chroma (C*), and hue (h*). Measurements were obtained in five different points in each nanocomposite film (RHIM *et al*., 1999).

2.3.7 Film morphology

The conditioned bionanocomposite samples were vacuum coated with gold for field-emission scanning electron microscopy. The surface microstructure of the nanocomposite films was visualized using a Leo Supra 50 VP field-emission scanning electron microscope (Carl-Ziess. SMT, Oberkochen, Germany) equipped with an Oxford INCA 400 energy dispersive.

We used a Phillips CM12 transmission electron microscope and Siemens D5000 X-ray diffractometer to investigate the crystallinity of the semolina nanocomposite films. In addition, energy-dispersive X-ray spectroscopy (EDX) was conducted under 15 kV incident electron energy.

2.3.8 Antimicrobial assay

The antimicrobial activity of the ZnO-nr-reinforced film was evaluated using the agar diffusion method as described by MAIZURA *et al*. (2007). The test for zone of inhibition on solid media was applied to determine the antimicrobial effects of the films against common foodborne pathogens and spoilage bacteria, such as the Gram-positive *Staphylococcusaureus*. Circular samples (5mm) were sterilized under UV radiation for 2h to eliminate surface contamination and were subsequently placed on nutrient agar plates that had been previously smeared with 100 μL of inoculum containing approximately 10^6–10^7 CFU/mL *S. aureus*. The plates containing the films were stored at 37°C for 24h. Thereafter, we measured the zone of inhibition produced with the nanocomposite films.

2.3.9 Statistical analysis

ANOVA and Tukey's post-hoc tests were used to evaluate the mean values of the physical, optical, mechanical, barrier, and antimicrobial properties of the prepared semolina films at the 5% significance level. Statistical analysis was conducted using SPSS version 22.0.

3. RESULTS AND DISCUSSION

3.1. Thickness

Table 1 shows the thickness of the control semolina film and those reinforced with various concentrations of ZnO-nr. The thickness of the films significantly increased with increasing ZnO-nr concentration ($p < 0.05$). This result can be attributed to the increased solid content of the films (AHMAD *et al*., 2012).

Table 1. Mechanical, water vapor permeability, thickness of semolina nanocomposite films.

ZnO-nr (%w/w)	TS (MPa)	EB (%)	YM (MPa)	Thickness(mm)	WVP × 10^{-7} [g m^{-1} h^{-1} Pa^{-1}]
Control	3.40± 0.105^{e}	59.43±1.79^{a}	63.12±2.27^{f}	0.143±0.005^{e}	8.61±0.304^{a}
1%	3.53±0.098^{e}	52.09±2.79^{b}	73.49±3.30^{e}	0.149±0.001^{cd}	6.71±0.26^{b}
2%	3.85±0.113^{d}	46.87±2.96^{c}	85.64±3.13^{d}	0.152±0.005^{cd}	5.84±0.24^{c}
3%	4.21±0.211^{c}	40.90±3.12^{d}	100.03±4.99^{c}	0.158±0.005^{bc}	5.03±0.23^{d}
4%	4.64±.0262^{b}	33.53±2.52^{e}	117.40±3.07^{b}	0.163±0.001^{ab}	5.03±0.23^{d}
5%	5.13±0.151^{a}	27.61±2.10^{f}	143.51±4.37^{a}	0.166±0.005^{a}	4.40±0.19^{e}

Different letters in each column represent significant difference among semolina films at the 5% level of probability.

3.2. Moisture content and water solubility

Most biopolymers are sensitive to water. However, incorporating lipids and nanoparticles, as well as the enhanced crosslinking in the biofilm, may reduce the sensitivity of the biopolymers to water (PAVLATH and ORTS, 2009).
Figure 1 illustrates the solubility and the moisture content of the control and nanocomposite films. Compared with the control, the nanocomposite films showed lower moisture content. In addition, the solubility of the films decreased with increasing ZnO-nr content. This finding may be attributed to the interaction among the plasticizer, biopolymer matrix, and ZnO-nr, which consequently reduced the amount of hydroxyl groups that react with water, thereby creating a less hygroscopic matrix. Our results were consistent with those of TUNC and DUMAN (2010) and MÜLLER *et al*. (2011).

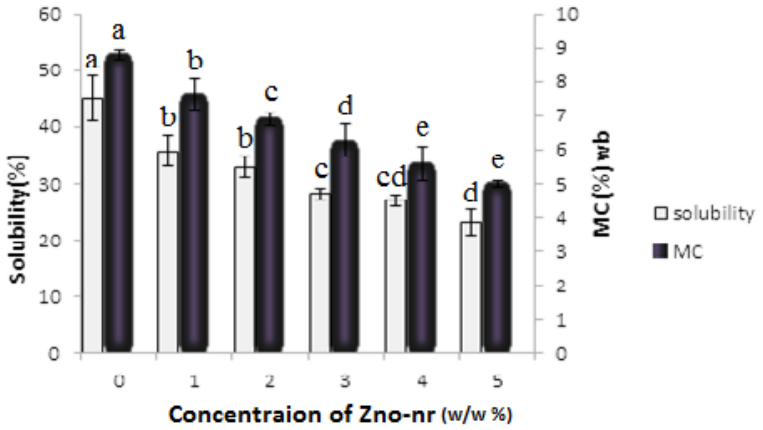


Figure 1. Effects of ZnO-nr on water solubility (empty bars) and moisture content (filled bars) of the semolina films. The bars represent mean (n = 5) ± SD. Different letters on the bars represent significant difference at the 5% level of probability.

3.3. WVP

WVP is a serious problem in the food industry; food packaging must prevent a contact between the food and the environment, and protect food products from any harmful agents. The problem with composite films in the food industry is the relatively high WVP of edible films. Film permeability is controlled by the diffusivity and solubility of water within the film matrix. Thus, nanoscience can be used to develop a material that prevents the migration of water in food products. Table 1 shows the WVP of semolina films as a function of ZnO-nr content.
The WVP of the control semolina film was 8.61×10^{-7}, which was significantly higher than that of the nanocomposite films. The lowest WVP was found in the semolina film incorporated with 5% of Zn0-nr, which is significant when compared to the control or other percentage.
The enhanced water vapor barrier property of the nanocomposite films can be attributed to the impermeability of the ZnO-nr in the polymer matrix to water vapor and the formation of a tortuous pathway for the diffusing water molecules (YU *et al*., 2009). The WVP of the films reinforced with 5% ZnO-nr was significantly reduced by 4.40×10^{-7} compared with that of the control film ($p < 0.05$). These results showed that the water

vapor barrier property of the bionanocomposite films was stronger than that of the biopolymer films. Similar results have been reported for nanocomposite protein films (JAFARZADEH *et al*., 2015).

3.4. Mechanical properties

ZnO-nr significantly affected the mechanical properties of the bionanocomposite films. Table 1 shows the TS, EB, and YM of the bionanocomposite films. The maximum stress that the film can withstand while being stretched or pulled before failing or breaking is known as TS. EB and YM indicate the flexibility and intrinsic stiffness of the films, respectively. Compared with those of the control films, the TS (5.13 MPa) and YM (143.51 MPa) of the bionanocomposite films significantly increased as the amount of ZnO-nr was increased from 1% to 5%. This result indicates that the bionanocomposite films had greater rigidity than the control film.
This result is due to the increased surface interaction between the protein matrix and Zno-nr with a high surface area, as well as the hydrogen bond formation between them (RHIM, 2011).
EAB has a reverse relation to tensile strength in most cases, and YM is directly related to tensile strength. As shown in Table 1, the EB decreased with increasing TS and maximum YM when the 5% ZnO-nr was added. The mechanical properties of the films are closely related to the distribution and density of the intra and intermolecular interactions between the polymer chains in the film matrix. Moreover, the degree of chain elongation and the nature of amino acid sequence might affect the mechanical strength of the protein-based films (SHELLHAMMER and KROCHTA *et al*., 1977).This finding is similar to those of SOTHORNVIT *et al* (2009).

3.5. Color

The surface color of the bionanocomposite is a critical parameter because it affects the general appearance and appeal of the food packaging to consumers (BOURTOOM and CHINNAN, 2008).
Table 2 presents the color properties of the semolina films and their nanocomposite counterparts.

Table 2. Colorimetric parameters for the transparency of semolina films.

ZnO-nr (%w/w)	L^*	a^*	b^*	C^*
Control	94.84±0.016^{a}	–0.794±0.017^{b}	4.06±0.12^{e}	4.11±0.12^{e}
1%	84.06±0.116^{b}	–0.74±0.090^{ba}	12.86±0.10^{d}	12.86±0.10^{d}
2%	82.20±0.009^{c}	–0.684±0.033^{ba}	13.34±0.012^{c}	13.35±0.012^{c}
3%	78.11±0.08^{d}	–0.674±0.024^{ba}	13.345±0.20^{c}	13.36±0.20^{c}
4%	74.86±0.70^{e}	–0.44±0.064^{ba}	15.24±0.004^{b}	15.26±0.004^{b}
5%	71.68±0.014^{f}	–0.35±0.061^{a}	15.82±0.020^{a}	15.84±0.019^{a}

Values represent mean (n = 5) ± SD. Different letters in each column represent significant difference among semolina films at the 5% level of probability.

Incorporation of Zno-nr had a significant effect on the L*-value, b*-value, and C*-value of the resulting film ($p<0.05$). Evidently, the control film was colorless and transparent,

whereas the semolina/ZnO-nr composite films became less transparent with increasing ZnO-nr content from 1% to 5%. This finding indicates that ZnO-nr addition influenced the coloring properties of the biopolymer films. As the content of ZnO-nr was increased, the b* (indicating blueness/yellowness) and C* values of the composite films significantly increased, whereas a* values (indicating greenness/redness) of the bionanocomposite films only slightly increased. By contrast, the L* values (indicating lightness) significantly decreased from 94.84 to 71.68 upon the addition of ZnO-nr into the semolina films.
These results are consistent with those of NAFCHI *et al*. (2013), who found that adding ZnO-nr in sago starch significantly reduces the L* value and increases a* and b* values compared with the control.

3.6. Light transmission

The optical properties of biopolymer films are highly important in food packaging because protection against light is a basic requirement to preserve the food quality.
Fig. 2 shows the UV transmission in the control and nanocomposite films. The control films exhibited a relatively high transmittance within the UV range of 290-400 nm. The addition of ZnO-nr completely prevented UV transmission. NAFCHI *et al*. (2013) reported that adding 5% ZnO-nr into starch film reduces UV transmission to almost 0%. We obtained a similar result but with 3% instead of 5% ZnO-nr. By contrast, YU *et al*. (2009) have recently reported that adding 4% ZnO-nr into starch film allows 3.4% UV light transmission. Moreover, the transmission of visible to IR (> 400 nm) spectra decreased by > 50% after adding ZnO-nr. The different behavior of ZnO in the present study can be attributed to the nanorod morphology of the particles (LIN *et al*., 2009). These findings suggest the applicability of ZnO-nr-reinforced biopolymer films as UV-blocking films in the packaging industry.

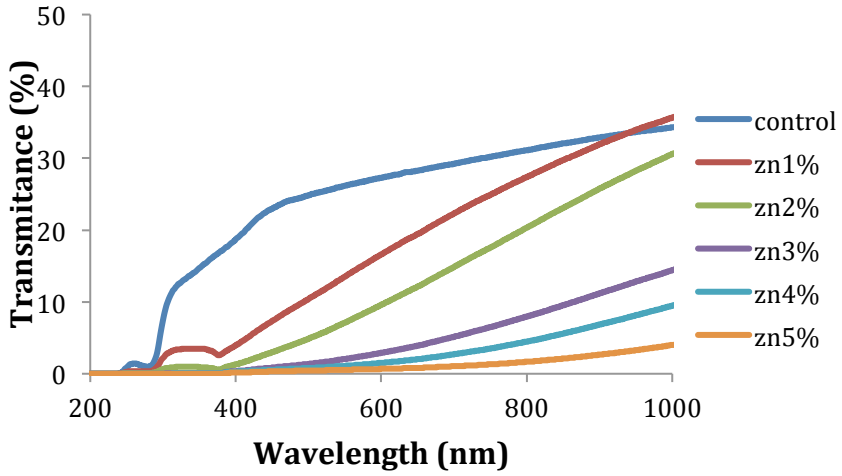


Figure 2. UV-vis transmittance of semolina nanocomposite films at 25 °C.

3.7. XRD analysis

XRD analyzes the scattered intensity of an X-ray beam on a material to reveal its crystallographic structure, chemical composition, and physical properties. This technique is widely used to characterize various materials because it is nondestructive and does not require elaborate sample preparation (ESPITIA *et al*., 2013). Thus, we analyzed the control

film and their nanocomposite counterparts through XRD [Figs. 3(a) and 3(b)]. This work presents the results obtained at the maximum amounts of ZnO nanoparticles, where the main characteristic peaks of the ZnO nanoparticles with hexagonal cross section were observed at $2\theta = 31.64°$, 34.32°, 36.14°, 47.44°, 56.53°, 62.82°, *67.92°*. Moreover, the intensity of the major peaks of ZnO-nr increased as the ZnO-nr concentration in the matrix was increased.
Furthermore, the XRD patterns of the nanocomposite films revealed that ZnO-nr affected the crystallinity of the matrix. The addition of ZnO nanoparticles in the matrix produced sharp and strong peaks, indicating greater crystallinity of the nanocomposite films than the control film.

3.8. Transmission electron microscopy (TEM)

Fig. 3(c) shows the TEM images of ZnO-nr. The ZnO-nr crystallites exhibit a rather hexagonal morphology with a diameter of 40-100 nm and a length of 200nm.

3.9. Scanning electron microscopy (SEM) and EDX

SEM is the most widely applied technique to determine the shape, size, morphology, and porosity of matrices. Fig. 3 shows the SEM images of the semolina films and ZnO-nr-reinforced films; the prepared ZnO-nr are evidently a nanostructure. TEM also revealed that the nanorods are cylindrical with hexagonal cross section. The control film exhibited a smooth and compact surface morphology, whereas the nanocomposite films showed a slightly rough surface. SEM images revealed that ZnO-nr particles were homogenously distributed throughout the film surface, which possibly rendered the surface of the nanocomposite films rough. This finding is possibly associated with the protruded film structures resulting from the increased thickness of the films (Table 1). ZnO-nr particles were uniformly dispersed in the nanocomposite films, which triggered an effective force transfer from the protein matrix to the ZnO-nr reinforcing phase. This finding may be attributed to the higher TS of the semolina nanocomposite films with 5% ZnO-nr compared with the other films (Table 1). Fig 3(d) illustrates the EDX spectrum of semolina/ ZnO-nr blend films. If Zno-nr content was increased, their signals could be detected. As shown in Fig. 3 (d), that C, Zn, O and Na elements were identified. This result agreed well with XRD analysis.

3.10. Antimicrobial assay

Fig. 4 shows the antibacterial activity toward the Gram-positive food pathogen *S. aureus* of the semolina films and the nanocomposite films containing various contents of ZnO-nr. The inhibition zone of the control and nanocomposite films significantly increased with increasing ZnO-nr content. The excellent antimicrobial activity of ZnO nanoparticles and the mechanism of action against microorganisms have already been demonstrated by other researchers (LI *et al*., 2009; YU *et al*., 2004). ZHANG *et al*. (ZHANG *et al*., 2010) elucidated the mechanisms underlying the antibacterial activity of ZnO. In specific, ZnO penetrates through the cell wall of the microorganism, reacts with internal components of the cell, and finally reduces the viability of the organism. Moreover, Zn ions may bind to proteins and deactivate them, may interact with the microbial membrane to cause changes in the structure and permeability, and may interact with the microbial nucleic acids to prevent replication. Furthermore, accumulation of ZnO nanoparticles in the microbial membrane causes membrane disintegration and cellular internalization (BRAYNER *et al*., 2006).

ZHANG *et al*. (34) also reported that nano-sized ZnO is more effective than micro-sized ones because the former easily penetrates through the cell wall of microorganisms. Nano rods can act as needles for easy penetration through the cell wall (NAFCHI, 2013).

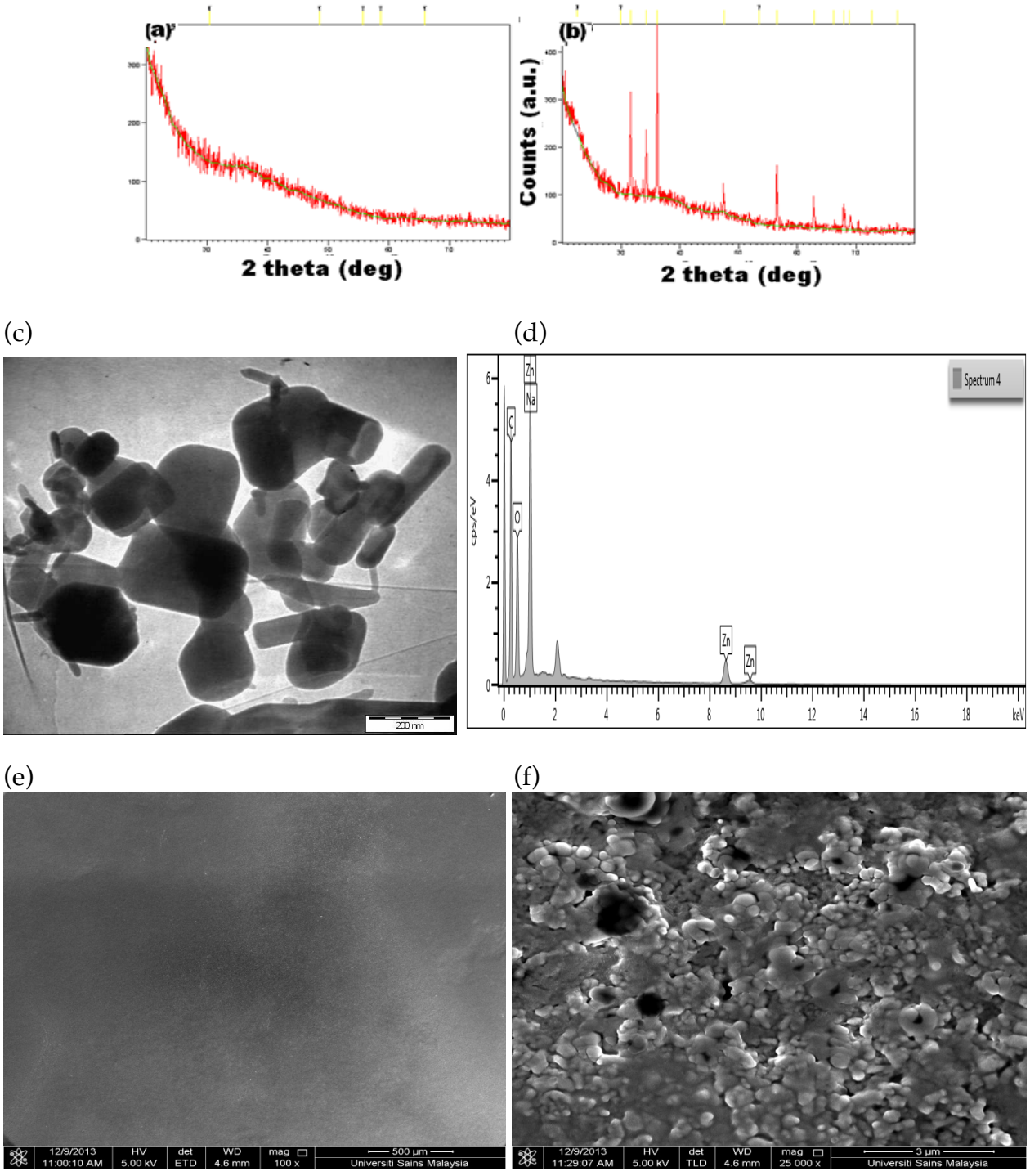


Figure 3. (a) XRD pattern of pure film, (b) 5% ZnO-nr-reinforced semolina, (c) TEM micrograph of ZnO-nr, (d) EDX spectrum (e) FESEM micrograph of pure semolina film surface, and (f) ZnO-nr-reinforced semolina film surface.

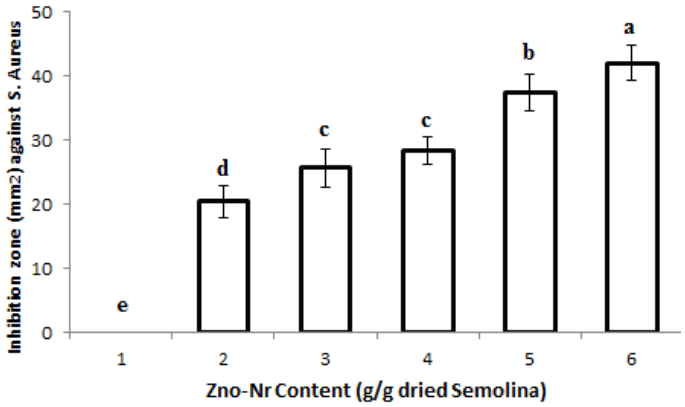


Figure 4. (a) Effects of ZnO-nr contents on the antimicrobial activity of semolina nanocomposite films against *S. aureus*. Inhibition zone = total inhibition area − total film area. The bars represent mean (n = 5) ± SD. Different letters on the bars represent significant difference at the 5% level of probability.

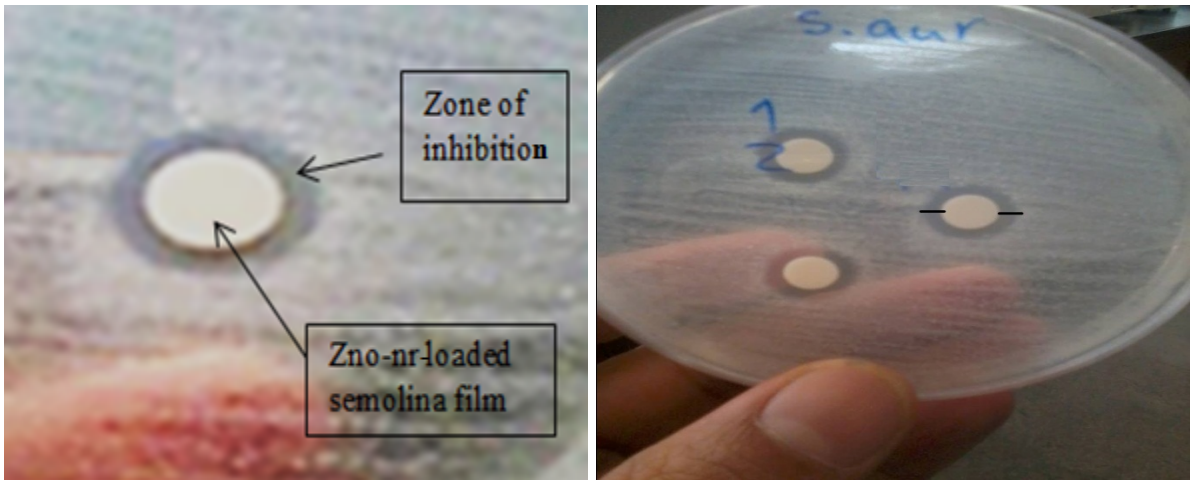


Figure 4. (b) Antimicrobial assay of ZnO-nr supported semolina films.

4. CONCLUSIONS

The present research characterized and created semolina-based nanobiocomposites of ZnO-nr for food packaging purposes. There are some reasons why semolina was employed as a polymeric matrix including its great accessibility in nature, biodegradability, low expenditure, and great gluten content, which enhance the edible films'nutritional properties.

ZnO-nr played an important role in enhancing the physical properties of semolina-based biocomposites. After the incorporation of low levels of ZnO-nr fillers, significant differences were observed in the film properties, particularly in mechanical, barrier, microbial and UV protection activities. The optical properties of bionanocomposites indicated that the UV transmission becomes almost zero with the addition of small

amounts of ZnO-nr to the biopolymer matrix. XRD diffraction shows that the intensity of the crystal facets of (100), (101) and (002) increased with increasing ZnO-nr concentrations in the biocomposite matrix. Moreover, the semolina-based nanocomposite films inhibited the growth of the Gram-positive food pathogen *S. aureus*. The present findings stressed that the biopolymer-based nanocomposite films are environment friendly in films antimicrobial packaging to make an improvement in the shelf life of food as well as viable replacement to petroleum-based or synthetic packaging films. Overall, this study suggests that semolina films incorporated with Zno-nr show a strong potential to be used as active films.

REFERENCES

Ahmad M., Benjakul S., Prodpran T. and Agustini T.W. 2012. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. Food Hydrocolloid. 28:189-199.

ASTM. 2005. Annual Book of ASTM Standards. ASTM, Philadelphia, PA.

ASTM. 2010. Standard test method for tensile properties of thin plastic sheeting D882-10. In: Annual book of ASTM standards. American Society for Testing and Materials, PA, Philadelphia.

Bourtoom T. and Chinnan M.S. 2008. Preparation and properties of rice starch–chitosan blend biodegradable film. Lwt-Food Sci. Technol. 41:1633-1641.

Brayner R., Ferrari-Iliou R., Brivois N., Djediat S., Benedetti M.F. and Fiévet F. 2006. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett. 6:866-870.

Dufresne A. 2006. Comparing the mechanical properties of high performances polymer nanocomposites from biological sources. J. Nanosci. Nanotechno. 6:322-330.

Espitia P.J.P, Soares N.D.F.F, Teófilo R.F., dos Reis Coimbra J.S., Vitor D.M, Batista R.A. and Medeiros E.A.A. 2013. Physical-mechanical and antimicrobial properties of nanocomposite films with pediocin and ZnO nanoparticles. Carbohyd Polym. 94:199-208.

Gennadios A. and Yada R.Y. 2004. Edible films and coatings from proteins. Proteins in food processing. Woodhead Publishing, Cambridge. pp. 442-467.

Gontard N., Guilbert S. and CUQ, J.L. 1992. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. Journal of Food Science 57(1):190-195.

Jafarzadeh S., Alias A.K., Ariffin F., Mahmud S. and Najafi A. 2015. Preparation and characterization of bionanocomposite films reinforced with nano kaolin. Journal of Food Science and Technology pp.1-9.

Jafarzadeh S., Alias A.K., Ariffin F., Mahmud S., Najafi A. and Ahmad M. 2016. Fabrication and characterization of novel semolina-based antimicrobial films derived from the combination of ZnO nanorods and nanokaolin. Journal of Food Science and Technology, 1-9.

Jafarzadeh S., Ariffin F., Mahmud S., Alias A.K., Hosseini S.F and Ahmad M. 2017a. Improved the physical and protective functions of semolina film by embedding blend nanocomposite (ZnO-nr and nano-kaolin). Food Packaging and Shelf life.

Jafarzadeh S., Alias A.K., Ariffin F., Mahmud S., Najafi A. and Sheibani S. 2017b. Characterisation of new biodegradable edible film based on semolina loaded with nano-kaolin.

Jafarzadeh, S., Alias A., Ariffin F. and Mahmud S. 2017c. Characterization of Semolina Protein Film with Incorporated Zinc Oxide Nano Rod Intended for Food Packaging. Polish Journal of Food and Nutrition Sciences.

Khwaldia K., Arab-Tehrany E. and Desobry S. 2010. Biopolymer coatings on paper packaging materials. Compr Rev Food Sci. F. 9:82-91.

Kovacevic E., Stefanovic I., Berndt J., Godde C., Winter J. and Boufendi L. 2008. The nanoparticle formation in hydrocarbon plasmas. Publ. Astron. Obs Belgrade. 84:151-152.

Kumar A.P. and Singh R.P. 2008. Biocomposites of cellulose reinforced starch: improvement of properties by photo-induced crosslinking. Bioresource Technol. 99:8803-8809.

Li J.H, Hong R.Y, Li M.Y, Li H.Z, Zheng Y. and Ding J. 2009. Effects of ZnO nanoparticles on the mechanical and antibacterial properties of polyurethane coatings. Prog. Org. Coat. 64:504-509.

Li X.H, Xing Y.G, Li W.L, Jiang Y.H. and Ding Y.L. 2010. Antibacterial and physical properties of poly(vinyl chloride)-based film coated with ZnO nanoparticles. Food Sci. Technol Int. 16:225-232.

Lin O.H, Akil H.M. and Mahmud S. 2009. Effect of particle morphology on the properties of nanoZnO/polypropylene composites. Adv Compos Lett. 8:77-83.

Maizura M., Fazilah A., Norziah M.H. and Karim A.A. 2007. Antibacterial activity and mechanical properties of partially hydrolyzed sago starchealginate edible film containing lemongrass oil. J. Food Sci. 72:C324-C330.

Marcuzzo E., Sensidoni A., Debeaufort F. and Voilley A. 2010. Encapsulation of aroma compounds in biopolymeric emulsion based edible films to control flavour release. Carbohyd. Polym. 80:984-988.

Muller C.M.O, Laurindo J.B. and Yamashita F. 2011. Effect of nanoclay incorporation method on mechanical and water vapor barrier properties of starch-based films. Ind. Crop. Prod. 33:605-610.

Nafchi A.M, Nassiri R, Sheibani S, Ariffin F. and Karim A.A. 2013. Preparation and characterization of bionanocomposite films filled with nanorod-rich zinc oxide. Carbohyd Polym. 96:233-239.

Onyeneho S.N. and Hettiarachch N.S. 1992. Antioxidant activity of durum wheat bran. J Agr Food Chem. 40:1496-1500.

Pavlath A.E. and Orts W. 2009. Edible films and coatings: why, what and how? pp. 1-23. In: Edible films and coatings for food applications. Embuscado ME, Huber KC. (Eds.), Springer, New York.

Petersson L. and Oksman K. 2006. Biopolymer based nanocomposites: comparing layered silicates and microcrystalline cellulose as nanoreinforcement. Compos. Sci. Technol. 66:2187-2196.

Quaglia G.B. 1988. Other durum wheat products. In: Durum chemistry and technology, AACC International, St. Paul, MN, pp. 263-282.

Rajendra R, Balakumar C, Ahammed H, Jayakumar S, Vaideki K. and Rajesh E. 2010. Use of zinc oxide nano particles for production of antimicrobial textiles. Int. J. Eng. Sci. Tech. 2: 202-208.

Rhim J.W, Wu Y, Weller C.L. and Schnepf M. 1999. Physical characteristics of a composite film of soy protein isolate and propyleneglycol alginate. J. Food Sci. 64: 149-152.

Shahrom M. and Abdullah M.J. 2006. Nanotripods of Zinc Oxide pp. 442-446. In: IEEE Conference on Emerging Technologies - Nanoelectronics, 10-13 January, Singapore.

Shahrom M. and Abdullah M.J. 2007. Tapered head of ZnOnanorods. J. Solid State Sci. Tech. 15:108-115.

Shellhammer T.H. and Krochta J.M. 1997. Whey protein emulsion film performance as affected by lipid type and amount. Journal of Food Science, 62(2), pp.390-394.

Sothornvit R., Rhim J.W. and Hong S.I. 2009. Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films. J. Food Eng. 91:468-473.

Tharanathan R.N. 2003 Biodegradable films and composite coatings: past, present and future. Trends Food Sci Tech. 14:71-78.

Tunc S. and Duman O. 2010. Preparation and characterization of biodegradable methyl cellulose/montmorillonitenanocomposite films. Appl. Clay. Sci. 48:414-424.

Türe H., Gällstedt M., Johansson E. and Hedenqvist M.S. 2013. Wheat gluten/montmorillonite clay multilayer-coated paperboards with high barrier properties. Ind. Crop Prod. 51:1-6.

Yu D., Cai R. and Liu Z. 2004. Studies on the photodegradation of rhodamine dyes on nanometer-sized zinc oxide. Spectrochim Acta A. 60:1617-1624.

Yu J., Yang J., Liu B. and Ma X. 2009. Preparation and characterization of glycerol plasticized-pea starch/ZnO-carboxymethylcellulose sodium nanocomposites. Bioresource Technol. 100:2832-2841.

Zhang L., Ding Y., Povey M. and York D. 2008. ZnOnanofluids – a potential antibacterial agent. Prog. Nat. Sci. 18:939-944.

Zhang L., Jiang Y., Ding Y., Daskalakis N., Jeuken L., Povey M., O'Neill A. and York D. 2010. Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against *E. coli*. J. Nanopart. Res. 12:1625-1636.

Unalan I.U, Cerri G., Marcuzzo E., Cozzolino C.A. and Farris S. 2014. Nanocomposite films and coatings using inorganic nanobuilding blocks (NBB): current applications and future opportunities in the food packaging sector. RSC advances 4(56):29393-29428.

Paper Received May 23, 2016 Accepted October 20, 2016

PAPER

THE LIKELIHOOD OF SHEEP MEAT CONSUMPTION IN TURKEY

A. UZMAY*[a] and G. ÇINAR [b]

[a]Department of Agricultural Economics, Faculty of Agriculture, Ege University, Bornova Izmir 35100, Turkey

[b]Department of Agricultural Economics, Faculty of Agriculture, Adnan Menderes University, Aydin 09100, Turkey

*Corresponding author. Tel.: +90 2323111437; fax: +90 2323881862

E-mail address: ayse.uzmay@ege.edu.tr

ABSTRACT

The aims of this study are threefold: first, to determine the factors affecting the likelihood of sheep meat consumption; second, to determine the reasons for consumers' preferences; and third, to determine consumers' willingness to pay for quality-based labelling. The study conducted interviews with 300 households in Izmir province. According to the results of logistic regression analysis, gender, level of income, number of people in the household, beef consumption, and whether the interviewed individual has the highest income in his or her household affect the probability of sheep meat consumption. While the personal preferences of regular sheep meat consumers vary according to their red meat consumption, willingness to pay based on labelling is $1.62/kg.

Keywords: consumer preferences, logistic regression, sheep meat consumption, willingness to pay

1. INTRODUCTION

According to OECD reports, worldwide consumption of sheep meat is increasing. In the year 2023, average world consumption of sheep meat per capita is expected to reach 1.91 kg, an increase of 12.3% compared to 2014 (OECD, 2015). Recently conducted studies also indicate that consumer demands for different types of meat are changing (BERNUÉS *et al*., 2012, MONTOSSI *et al*., 2013). In particular, factors such as the relationship between red meat and cancer (NORAT *et al*., 2002; CHAO *et al*., 2005), an increase in other health-related concerns, changes in demographic structure, economic growth, and changes in meat's, price, quality and image have been effective in driving this change (BERNABÉU and TENDERO, 2005; GRUNERT, 2006). The negative effect of red meat consumption on cardiovascular health and the recommended diet to maintain a healthy lifestyle are among the important factors affecting the preferences of consumers, especially in developed countries (LICHTENSTEIN *et al*., 2006, DANIEL *et al*., 2010).

There have been several studies of the factors that affect consumer preferences and willingness to pay for sheep meat (GRACIA *et al*. 2011). While some studies examine factors such as price, origin, certification, meat type and size, and feeding method (grain-fed, grass-fed and grain + grass fed) (SAÑUDO *et al*., 2007; FONT I FURNOLS *et al*., 2011; JOY *et al*., 2012; BERNUÉS *et al*., 2012), others primarily focus on the effects of factors such as the environment, animal welfare and food safety (DICKINSON *et al*., 2003; NAPOLITANO *et al*., 2007; NAPOLITANO, 2009; SEPÚLVEDA *et al*., 2011). The results of these studies indicate that origin is a significant factor in meat consumption preferences; in particular, local meat is preferred (KAUR, 2010; FOINT I FURNOLS *et al*., 2011; MEAS, 2014). In addition, studies concerning sheep meat consumption generally observe that origin is an essential factor in preferences and that consumers' willingness to pay is highly affected by the meat's origin (IMAMI *et al*., 2011; GRACIA, *et al*., 2011; HERSLETH *et al*., 2012; MONTOSSI *et al*., 2013). However, BERNEUES *et al*. (2003) determined that, with regard to lamb meat preferences in France and Spain, the attention paid to animal feeding systems may be a stronger factor than the origin of the meat. On the other hand, PRESCOTT *et al*. (2004) report that the peculiar fat and smell of sheep meat are important reasons why it is rarely or never preferred in some countries or regions. It should be further noted that the culture, habits and beliefs of some countries may also affect sheep meat consumption (BONNE and VERBEKE, 2006; FONT I FURNOLS *et al*., 2006; NAKYINSIGE 2012; MONTOSSI *et al*., 2013). Because studies of consumer behaviours related to sheep meat – and the factors that affect these behaviours – are closely related to several disciplines, including psychology, sociology, agronomics, food science and medicine, several relevant studies can be found in the literature. Nevertheless, in comparison to the number of studies conducted on other types of meat, the number of studies focused on lamb/mutton is still relatively low.

Although studies concerning meat consumption in Turkey are available, a robust database containing the production and consumption figures of animal products at the national level is missing (MFAL, 2015). Nevertheless, it was announced by the Meat and Milk Foundation (UKON, 2013) that meat consumption per capita in 2013 was 32.5 kg, with 60% (19.4 kg) of that being poultry meat consumption, 35% (11.4 kg) being bovine consumption and 5% (1.7 kg) being ovine consumption. In Turkey, anaemia is observed in 29% of women and 30% of children younger than 5 years. Annual per capita consumption of red meat in Turkey (29 g) is below the world average (31 g) (FAO, 2014).

There have been several studies of red meat consumption in Turkey and the factors that affect it (ATAY *et al*., 2004; KARAKUŞ *et al*., 2008; KAYA *et al*., 2011; TÜZEMEN, 2012; ERDOĞAN, 2013). However, no study could be found that specifically examines the factors directly affecting sheep meat consumption and consumer preferences. As

mentioned earlier, off-the-book ovine breeding in Turkey and the fact that agricultural policy implementers are discussing whether the consumption gap in red meat can be closed with ovine meat not only require studies of production potential but also require that meat consumption amounts and consumer preferences be researched at both the regional and provincial levels. In this context, the main objectives of this study fall into two categories: first, to determine meat consumption patterns in the province of Izmir and to identify the socio-economic factors that affect the possibility of households' sheep meat consumption, and second, to identify personal reasons for preferring sheep meat among those who consume lamb regularly and to determine their willingness-to-pay based on quality-related labelling.

2. MATERIALS AND METHODS

The metropolis of Izmir is Turkey's third largest province, with a population of 4,061,000. The average household has three members (TSI, 2014). In this context, the population of the study was set to 1,353,667 households. The number of households to be included within the scope of the study was calculated as 296 according to the proportional sampling method (NEWBOLD, 1995, equation 1). Considering proportional losses based on the population's distribution among districts, interviews with a total of 300 respondents were completed.

$$n = \frac{Np(1-p)}{(N-1)\sigma_P^2 + p(1-p)} \quad (1)$$

where n is the sample size, N is the population size (1,353,667), and p is the prediction rate (0.5 for the maximum sample size) and the probability level confidence interval (99% confidence interval, σ_p: 0.02960 for 0.075 margin of error from the equation of $2.58\sigma_p$: 0.075). A total of four districts in Izmir with high population densities were included within the scope. Accordingly, the sample size, determined as 300 people, was distributed per the respective populations of the districts. Thus, 151 people from the Balcova district, 76 people from the Karsiyaka district, 46 people from the Konak district and 27 people from the Menemen district were included in the sample. Because meat variety in hypermarkets is high, the survey was conducted among consumers who shop at hypermarkets in the research area. Moreover, the consumers participating in the survey were always responsible for shopping for their households. The questionnaire used for data collection included four main sections: (a) socio-economical characteristics of respondents (b) meat consumption of the households c) the reasons for consumers' preferences (the reasons why consumers prefer sheep meat / the reasons for do not regularly consume sheep meat) d) willingness to pay for a labelled product. In sections a and b, respondents were asked their age as well as open-ended questions regarding their education level, household, district, occupation, marital status, and amount of meat consumed in their household. However, income groups were divided into three categories to encourage the respondents to answer without hesitation. The scale was determined by the Turkish Statistical Institute's classifications. Therefore, the lowest income category included families of four with incomes at or below the poverty line ($2270). Queries regarding consumer preferences consisted of multiple choice questions. Respondents were asked to select the most appropriate option. Finally, because there are no labelling or certification systems used for

sheep meat in Turkey, we attempted to determine whether consumers would be willing to pay a higher price for labelled (thus quality-assured) sheep meat. In this respect, sheep meat consumers were informed of the importance of labelling, and their willingness to pay for labelled meat was assessed. In this context, consumers in İzmir were told the average current price of sheep meat ($15.71) and were asked whether they would pay extra for labelling. If the answer was positive, then they were asked to indicate the final price they were willing to pay.

In the present study, the factors that affect sheep meat consumption were determined by means of logistic regression analysis. As the dependent variable, while the respondents regularly consuming sheep meat were set as (1), those who do not regularly consume sheep meat were set as (0). In the logistic regression model, where the dependent variable has two categories, independent variables can be discrete, continuous and qualitative. The logistic regression model employed in this study is presented in equation 2.

$$\pi(x) = P(Y = 1 / X = x) = \frac{e^{\beta_0+\beta_1X_1+\beta_2X_2+\ldots+\beta_pX_p}}{1+e^{\beta_0+\beta_1X_1+\beta_2X_2+\ldots+\beta_pX_p}} = \frac{1}{1+e^{-(\beta_0+\beta_1X_1+\beta_2X_2+\ldots+\beta_pX_p)}} \quad (2)$$

where X is the data matrix with regard to the independent variable, and when X = x (when the value X is known), the probability of the occurrence is (Y=1) p. β is the constant, β_i is the parameter to be predicted for each explanatory (independent) variable, and X_i indicates the i^{th} independent variable.

The nonlinear logistic regression function given in the equation was subjected to logit conversion and linearized. Designing the model as per the data of the study produced the following equation,

$$g(x) = In\left[\frac{\pi(x)}{(1-\pi(x))}\right] = Ine^{\beta_0+\beta_1X} = \beta_0 + \beta_1X\ldots \quad (3)$$

where b_1 indicates the variation in the dependent variable caused by 1 unit of change in the independent variable x, demonstrating how much change is caused by 1 unit of change in the x in the logistic model (ALDRICH and NELSON, 1984).

Within the scope of the study, the respondents' age, education, gender, marital status, employment status, household income, number of people in the household, whether the respondent contributes the highest income to the household, beef consumption of the household, chicken consumption of the household and whether there are any cardiac patients (family members taking medication) in the household were accepted as the independent variables (Table 1). The -2LLR value obtained for the model was 256.057, significant at a 5% margin of error (Table 2). With this value, the significance of the coefficients pertaining to the variable levels was tested.

In this study, the conditional valuation method is used. To apply this method, an imaginary market is created for a good or a service and people are asked how much they would pay in return for that good or service (CARSON, 2000). The price that consumers were willing to pay for labelled lamb/mutton products was determined by means of the lower bound mean method initially implemented by BLAINE *et al*. (2003).

$$\text{LBM} = \prod_0(P_0) + \sum_{i=1}^{k}\prod_i(P_i - P_{i-1}) \quad (4)$$

where Π_0 is the cumulative percentage of willingness to pay, P_0 is the lowest payment boundary and K is the number of boundaries.

Table 1. Variables used in logit model and descriptive statistics of the variables.

Dependent variable (Y)	Type of Variable	Description	Frequency	Percent(%)
	Dichotomus	0 1	221 79	73.67 26.33
Independent variables (X)		Mean	Standard d.	
The respondents' age (AGE)		41.39	11.2490	-
Number of people in the household (HS)		3.11	1.193	-
Beef consumption of the household kg (Monthly) (BC)		4.22	1.010	-
Chicken consumption of the household kg (Monthly) (CC)		6.20	1.10	-
Gender of the respondent (GEN)	Dichotomus	0: Male 1 :Female	142 158	47.3 52.7
Education of the respondent (EDU)	Ordinal Categorical	0: Otherwise 1: University	161 139	53.7 46.3
Income level of the household (INCM)	Ordinal Categorical	1: x≤ $2270 2: $2271– $3974 3: x ≥$3975	83 175 42	27.7 58.3 14.0
Employment status of the respondent (ES)	Dichotomus	0: No 1: Yes	94 206	31.3 68.7
Marital status of the respondent (MS)	Dichotomus	0: Other 1: Yes	64 236	21.3 78.7
Whether the respondent brings the highest income to the household (HINCM)	Dichotomus	0: No 1: Yes	138 162	46.0 54.0
Whether there are any cardiac patients (family members taking medication) in the household (PIH)	Dichotomus	0: No 1: Yes	239 61	79.2 20.3

3. RESULTS AND DISCUSSIONS

3.1. Factors affecting the likelihood of sheep meat (lamb/mutton) consumption

Among all households included in the study, 26.3% consume sheep meat (lamb/mutton). The average age of the respondents is 41, 78.7% are married, and the total income of 58.3% of the households is between $2,271 and $3,974 per month. A total of 46% of the participating consumers have bachelor's degrees, approximately 53% are women, 54% contribute the highest income to their households, and 69% are employed.
According to the results of the study, among the meat preferences of the households in Izmir, poultry, beef and sheep meat have shares of 55% (6.22 kg/month), 38% (4.22

kg/month) and 7% (0.78 kg/month), respectively. None of the families consumed goat meat. On the basis of the types of meat in question, the consumption average of the households is 43.21 kg/year, or 3.60 kg/month. The annual average consumption of red meat, on the other hand, is 27 kg. While beef constitutes 89% of red meat consumption, sheep meat constitutes 11%. The average consumption of the 79 households that regularly consume sheep meat is 1.48 kg/month (while the same average among all households included in the sample is 0.78 kg/month). While 36.7% of the families that regularly consume sheep meat prefer sheep meat to beef, 20.3% of them prefer to consume solely sheep meat. On the other hand, among the whole population included in the study, 5.3% prefer to consume only sheep meat.

Examining studies conducted in other provinces of Turkey demonstrates that consumption patterns in Aydin province, which is also within the Aegean Region (ATAY *et al*., 2004), and Erzurum province, located in the Eastern Anatolia Region (KAYA *et al*., 2011), are similar to the pattern in Izmir province. However, it is also noteworthy that sheep meat consumption increases toward the regions of Central Anatolia and Southeastern Anatolia (KARAKUS *et al*., 2008; TUZEMEN, 2012). According to the findings of the present study conducted in the Izmir region, average annual sheep meat consumption per capita is approximately 3 kg in this province. Although above the world average according to OECD data, this level of consumption is still below the average for Turkey. Furthermore, while consumers in Izmir mostly prefer spring lamb meat, towards Southeastern Anatolia, mutton is preferred (ÖNENÇ and ÖZŞENOĞULLARI, 2009). Indeed, in the present study, 58% of sheep meat consumers in Izmir reported that they prefer lamb.

Comparing – in terms of income groups – the rate of households that regularly consume sheep meat to those that do not demonstrates that while the rate is 10.84% in the lowest income group ($x \leq$ \$2,270), in the middle-income group (\$2,271-\$3,974) and the highest income group ($\$3,975 \leq x$) the rates are 33.14% and 28.4%, respectively. Quantitatively examining the relationship between income groups and consumption amounts demonstrates that 100% of the consumption in the lowest income group is 2 kg or lower. The rates of the households consuming 4 kg and less in the middle and the highest income groups, on the other hand, are 88% and 75%, respectively. The households that consume more than 4 kg have shares of 12% in the medium income group and 25% in the high income group. According to the results of Pearson's chi-squared test (37.329), the income groups and consumption amounts of the households were significant at the level of 0.01. In a study conducted by PEARCE (2013), the findings of previous studies concerning Australia, the USA, EU and UK were evaluated, and it was suggested that the number of lamb meat consumers is higher in the medium income group. The highest rate of regular sheep meat consumers, compared to those who do not consume sheep meat in Turkey (33.14%), is also found in the medium income group.

The rate of households consuming sheep meat in the districts of Bornova, Karsiyaka, Konak and Menemen, compared to the total number of households, varies between 25% and 28%. The results of Pearson's chi-squared test (0.126) indicate that there is no significant difference between the districts of İzmir and sheep meat consumption at the level of 0.05.

According to the results of the logistic regression model (Table 2), the respondent's gender, whether he or she contributes the highest income to the household, total income of the household, amount of beef consumption, and number of people in the household were determined to affect the likelihood of sheep meat consumption.

Table 2. Statistical results of logit model.

	B	S.E.	Wald	df	Sig.	Exp(B)
AGE	0.004	0.020	0.034	1	0.854	1.004
GEN	-1.648	0.619	7.088	1	0.008*	0.192
EDU	-0.153	0.402	0.144	1	0.704	0.859
MS	0.086	0.522	0.027	1	0.870	1.090
ES	0.287	0.391	0.537	1	0.464	1.332
HINCM	-2.005	0.646	9.624	1	0.002*	0.135
PIH	-0.471	0.459	1.053	1	0.305	0.625
INCM	1.505	0.365	16.969	1	0.000*	4.503
CC	-0.116	0.162	0.514	1	0.474	0.890
BC	-1.316	0.233	31.770	1	0.000*	0.268
HS	0.701	0.178	15.571	1	0.000*	2.016
Constant	1.063	1.616	0.433	1	.511	2.895
	B	S.E.	Wald	df	Sig.	Exp(B)
Variables in the Equation Model Summary	-1.001	0.132	57.910	1	0.000*	0.367
Model Summary	-2 Log likelihood		Cox & Snell R Square		Nagelkerke R Square	
	256.057(a)		0.254		0.369	

Hosmer and Lemeshow Test: Chi-square 13,801, df 8, p(0.087>0.05).

Women's probability of consuming sheep meat is 80.8% (0.192-1) less than that of men. As a matter of fact, according to the conclusions of the study conducted by PRÄTTÄLÄ *et al*. (2006) in countries with different cultures and economies (Finland and Baltic countries), it was reported that, in general, women consume less meat than men, and they usually prefer to consume vegetables and fruits. In addition, other studies (KUBBERAD *et al*., 2002; SANTOS and BOOTH, 1996; HUGHES, 1995) report that, in particular, younger women tend to consume mostly white meat. UREÑA *et al*. (2008) reported that women and men display different attitudes and behaviours in buying food; in terms of lifestyle, women have a more positive attitude toward buying organic food and men tend to pay higher prices for organic food.

Households with total incomes above $3,975 are 350% (1- 4.503) more likely to consume sheep meat than households with total incomes of less than $2,271. The likelihood of consuming sheep meat increases in line with the increasing income of the household. However, it is noteworthy that the likelihood decreases by 86.5% (0.135-1) when considering sheep meat consumption in terms of the person who contributes the highest income to the household (Table 2). Furthermore, SHIFLETT *et al*. (2007) reported that in the USA, income has a positive effect on lamb meat consumption per capita.

In May 2015, beef carcass and lamb carcass prices in Izmir were $8.40/kg and $8.22/kg, respectively (UKON, 2015). The average prices in hypermarkets, on the other hand, are $15.35/kg for beef meat and $15.72/kg for lamb meat, indicating that because the recent prices of beef and lamb/mutton are quite close to each other, factors other than price affect

consumers' preferences for red meat. A one-kilo decrease in beef consumption in a household in Izmir increases the likelihood of sheep meat consumption by 73% (0.268-1). In a study by BYRNE *et al*. (1993), pork prices were found to affect demand for lamb meat, while the prices of chicken and beef were determined to have no effect on sheep meat demand. Pork is not consumed in Turkey because of religious beliefs. Because there is no habit of consuming goat meat in Izmir, this increases the chance of beef and lamb/mutton being consumed as each other's substitutes.
The presence of one additional person in a household increases the likelihood of consuming sheep meat by 101.6% (1-2.016).
Educational status, age, whether there are any cardiac patients in the household, employment status and poultry consumption of the household do not affect sheep meat consumption in a statistically significant way. On the other hand, while chicken consumption, the existence of a cardiac patient in the household and having a bachelor's degree are negatively correlated with sheep meat consumption, the age of the consumer and his or her marital status are positively correlated with sheep meat consumption (Table 2). According to the findings of a study conducted by RUSSEL and COX (2004), the meat preferences of middle-aged and older individuals differ; in comparison to younger individuals, elders' perceptions of processed food, roasted chicken and lamb and pork chops are more positive. In addition, PEARCE (2013) examined the results of studies conducted in Australia and the USA, EU and UK and reached the generalization that consumers older than 35 consume more lamb meat. In the present study, grouping the respondents into two groups (those that are younger and older than 35) produced no statistically significant difference in terms of lamb/mutton consumption. The rates of sheep meat consumption in groups of people both younger than and older than 35 are fairly similar and are between 26% and 27%. According to the results of Pearson's chi-squared test (0.034), there is no significant difference between age and sheep meat consumption at 0.05. On the other hand, the total rate of sheep meat consumption among families that have members 25 years of age or younger is 64%; lamb/mutton is consumed in 30.7% of these households. In families in which all members are older than 25, the rate of sheep meat consumption is 18.5%. Thus, the results of Pearson's chi-squared test (5.313) were significant at 0.05. While the rate of families with members 55 years of age and older is 18% among all families, 26% of them regularly consume sheep meat. The results of Pearson's chi-squared test (0.006) were not significant at 0.05. Accordingly, it was determined that in Izmir, the rate of families that have young members and regularly consume sheep meat is 12.2% higher than families that also regularly consume sheep meat but do not have young members.
The marital status of consumers in Izmir was determined to have no statistically significant effect on sheep meat consumption (Table 2). While PRÄTTÄLÄ *et al*. (2006), similarly, could find no effect of marital status in Finland and Latvia on meat consumption, the authors determined that married people in Estonia and Lithuania consume meat more frequently.

3.2. Individual Reasons for sheep meat consumption

As for the reasons why consumers prefer sheep meat, they were under the impression that lamb/mutton is healthier (29.1%) because sheep in Turkey are mostly fed in pastures, and it is believed that sheep breeding requires less medicine than bovine breeding. They also noted that sheep meat is considered tastier (41.8%) and is preferred for reasons of habit (29.1%) However, when grouping sheep meat consumers on the basis of their red meat consumption (as beef-heavy red meat consumption, lamb/mutton-heavy red meat consumption and exclusively lamb/mutton consumption) significant differences among

the groups were determined. Thus, the results of Pearson's chi-squared test (20.453) were significant at 0.01. While the group that exclusively consumes sheep meat as its red meat prefers sheep meat because of the belief that it is healthier (62.5%), in the beef-heavy consumption group, the preference for consuming sheep meat is less related to the perception of it being healthier (14%). In a study conducted by FONT I FURNOLS *et al*. (2011), it was reported that the belief that lamb/mutton is healthier is based on the fact that feeding with fresh grass is healthier, more natural and more environmentally friendly than intensive pellet feeding. In addition, consumers' perception that sheep meat is healthier is based on the fact that sheep are fed in pastures. It is also reported that consumers prefer animals bred in highland pastures to those bred in lowland pastures (IMAMI *et al*., 2011; HERSLETH *et al*., 2012; MONTOSSI *et al*., 2013).
Approximately 77% of the participating sheep meat consumers stated that choosing meat of domestic origin is important to them. In some studies, consumers that considered meat origin to be important were older; in addition, gender has a distinctive effect on purchasing decisions (VERBEKE *et al*., 2000; FONT I FURNOLS *et al*. 2011). However, no significant difference among consumers' age group (young, middle-aged or older groups) in Izmir was found in terms of regular consumers' preferences for meat origin. The results of Pearson's chi-squared test (2.170) were significant at 0.05.

3.3. The respondents who do not regularly consume sheep meat

Consumers' reasons for not consuming sheep meat include the lack of appeal of the specific smell and taste of sheep meat (53%), the consideration that it is fattier than beef and therefore unhealthy (35%), and not having the habit of consuming it because their families did not consume sheep meat (12%). In addition, according to the findings of several studies (PRESCOTT *et al*, 2001, PRESCOTT *et al*., 2004), the lack of the habit of consuming sheep meat and mutton and the factor of taste are important in terms of consumer preferences; factors such as how the animals are fed and deodorization by using spices are also worth considering.

3.4. Willingness to pay for a labelled product

When the consumers who prefer sheep meat were asked how much their willingness to pay would increase if the lamb/mutton were sold with labels indicating its quality, some of the consumers (13 respondents, 16.45%) stated that the price of sheep meat was already too high and that they could not pay more. As for the extra amount the willing respondents would pay for labelled products, this was calculated as $1.62/kg by means of Blaine's method, as presented in Equation 3 (Table 3). DICKINSON *et al*. (2003) determined that 35% of consumers in the USA and 37% of consumers in Canada would be willing to pay $1.35 and $1.85, respectively, for certification of meat traceability, animal welfare and advanced food safety and that the demographic characteristics of the consumers affect this willingness. LYFORD *et al*. (2010), on the other hand, reported that among consumers from Australia, Japan, the USA and Ireland, the willingness to pay for quality is highest in Japan; the willingness to pay is higher among consumers aged 25 to 35 in all four countries; and the effects of the other demographic variables are relatively lower. In a study conducted by SÁNCHEZ *et al*. (2001) in Spain, it was determined that while willingness to pay more for lamb is based on meat origin, for beef it depends on food quality.

Table 3. Willingness to pay and the lower bound mean (LBM)*

Consumers (number)	Payment willingness TL/ kg	%	Cumulative percentage %
1	8	1.52	1.52
2	7	3.03	4.55
6	6	9.09	13.64
9	5	13.64	27.28
13	4	19.70	46.98
15	3	22.73	69.71
15	2	22.73	92.42
5	1	7.58	100.00
66		100.00	LBM= TL3.56/kg ($1.62/kg)

*1 US$ = 2.192 TL.

4. CONCLUSIONS

The present study addressed preferences and willingness-to-pay among sheep meat consumers. According to the findings of the study, the likelihood of consuming sheep meat in the Izmir province is affected by gender, level of income, number of people in the household, beef consumption and whether the respondent is the member who contributes the highest income to the household. According to the model results, household income positively affects the possibility of sheep meat consumption. On the other hand, when the three income groups were compared, it was determined that the highest sheep meat consumption ratio (33%) was in the middle-income group ($2271– $3974). In this context, we can recognize the importance of developing marketing strategies aimed specifically at high-income groups. As goat meat and pork are not consumed in Izmir, and the prices of sheep meat and beef have recently become very similar, it was determined that factors other than price affect consumer preferences related to red meat. However, if, from the perspective of consumers, parity between cattle meat and sheep meat develops on behalf of sheep meat, the demand for sheep meat among low-income groups will also increase. In this context, analyses of the effects of supply-increasing policies in sheep breeding can be tested by new studies.

One of the important findings of this study is that women negatively affect the possibility of sheep meat consumption. If women are to prefer sheep meat, it is important to disseminate information about the change in fat ratio in the meat according to different carcass sizes and different rations, and about the frequencies with which sheep are allowed to forage.

One of the most prevalent reasons for not consuming sheep meat regularly is dislike of its smell and taste. Companies should test different meat types in the market according to consumer preferences (spiced and sauced meat, different methods of feeding, etc.)

The results of this study showed that the majority of consumers are willing to pay more for meat that uses quality-based labelling. In this context, if companies develop standards according to quality and especially if this becomes a legal obligation consumption of sheep meat will increase.

In conclusion, the possibility of sheep meat consumption may increase in Izmir, which represents the Aegean region. However, it is also understood from these results that the

increase in the possibility of consumption depends on a series of precautions that must be taken during the production stage.

ACKNOWLEDGEMENTS

This study (Project No 2012-ZRF-005) has been supported by the Ege University Scientific Research Commission.

REFERENCES

Aldrich J.H. and Nelson F.D. 1984. Linear Probability, Logit, and Probit Models. Thousand Oaks, CA: SAGE Publications, 95 p.

Atay O., Gökdal Ö., Aygün T. and Ülker H. 2004. Aydın ili Çine ilçesinde kırmızı et tüketim alışkanlıkları, 4. Zootekni Bilim Kongresi, İsparta 1: 348-354.

Bernabéu R. and Tendero A. 2005. Preference structure for lamb meat consumers. A Spanish case study. Meat Science. 71(3): 464-470.

Bernués A., Olaizola A. and Corcoran K. 2003. Labelling information demanded by European consumers and relationships with purchasing motives, quality and safety of meat. Meat Science 65(3):1095-1106.

Bernués A., Ripoll G. And Panea B. 2012. Consumer segmentation based on convenience orientation and attitudes towards quality attributes of lamb meat, Food Quality and Preference 26(2):211-220.

Blaine W.T., Lichtkoppler F.R. and Standbro R. 2003. An assessment of residents' willingness to pay for green space and farmland preservation conservation easements using the contingent valuation method (CVM), Journal of Extension 41(4).

Bonne K. and Verbeke W. 2006. Muslim consumer's motivations towards meat consumption in Belgium: qualitative exploratory insights from means-end chain analysis. Anthropology of Food 5:2-24.

Byrne P.J., Capps O.C. and Williams G.W. 1993. U.S. Demand for lamb: the other red meat. Journal of Food Distribution Research 24(1):158-166.

Carson R.T. 2000. Contingent valuation: A user's guide. Environmental Science and Technology 34(8):1413-1418.

Chao A., Thun M.J., Connell, C.J., McCullough M.L., Jacobs E.J., Flanders W.D., Rodriguez C., Sinha R., and Calle E.E. 2005. Meat Consumption and Risk of Colorectal Cancer. JAMA. 293(2): 172-182.

Daniel C.R., Cross A.J. Koebnick C. and Sinha R. 2010. Trends in meat consumption in the USA, Public Health Nutrition 14(4):575-583.

Dickinson D.L., Hobbs J.E. and Bailey A.V. 2003. Comparison of US and Canadian consumers' willingness to pay for red-meat traceability, Paper prepared for presentation at the American Agricultural Economics Association Annual Meetings, Montreal, Canada.

Erdoğan N. 2013. Hayvansal Gıdaların Tüketim düzeyi ve tüketici tercihlerinin belirlenmesi üzerine bir araştırma, Afyon Kocatepe Üniversitesi personeli örneği, Hayvan Sağlığı Ekonomisi ve İşletmeciliği Anabilim Dalı, YL Tez No.2013-013, p. 82.

Food and Agriculture Organization of the United Nations (FAO). 2014. Food and Nutrition in Numbers, http://www.fao.org/publications/card/en/c/ 9f31999d-be2d-4f20-a645-a849dd84a03e/. Accessed May 2016.

Font i Furnols M., San Julián R., Guerrero L., Sañudo C., Campo M.M., Olleta J.L., Oliver M.A., Cañeque V., Álvarez I., Díaz M.T., Branscheid W., Wicke M., Nute, G.R. and Montossi F. 2006. Acceptability of lamb meat from different producing systems and ageing time to German, Spanish and British consumers'. Meat Science 72 (3):545-554.

Font i Furnols M., Realini C., Montossi F., Sañudo C., Campo M.M., Oliver M.A., Nute G.R. and Guerrero L. 2011. Consumer's purchasing intention for lamb meat affected by country of origin, feeding system and meat price: A conjoint study in Spain, France and United Kingdom. Food Quality and Preference 22:443-451.

Gracia A., de Magistris T. and Nayga R.M. Jr. 2011. Willingness to pay for a local food label for lamb meat in Spain. 2011 International Congress, August 30-September 2, Zurich, Switzerland 114607, European Association of Agricultural Economists.

Grunert K.G. 2006. Future trends and consumer lifestyles with regard to meat consumption. Meat Science 74 (1):149-160.

Hersleth M., Naes T., Rodbotten M., Lind V. and Monteleone E. 2012. Lamb meat importance of origin and grazing system for Italian and Norwegian consumers. Meat Science 90:899-907.

Hughes, D. (1995). Animal welfare, British Food Journal. 97(10):3-7 Available from: http://dx.doi.org/10.1108/00070709510104529. Accessed May 2016.

Imami D., Chan-Halbrendt C., Zhang Q., and Zhllima E. 2011. Conjoint analysis of consumer preferences for lamb meat in central and southwest urban Albania. International Food and Agribusiness Management Review 14(3):111-126.

Joy M., Sanz A., Ripoll, G., Panea B., Ripoll-Bosch R., Blasco J. and Alvarez-Rodriguez J. 2012. Does forage (grazing vs. hay) fed to ewes before and after lambing affect suckling lambs performance, meat quality and consumer purchase intention?. Small Ruminant Research 104:1-9.

Karakuş K., Aygün T. and Alarslan E. 2008. Gaziantep ili merkez ilçede kırmızı et tüketim alışkanlıkları. Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi. J. Agric. Sci. 18(2):113-120.

Kaur B. 2010. Consumer preference for goat meat in Malaysia: Market opportunities and potential. Journal of Agribusiness Marketing 3:40-55.

Kaya E.T., Külekçi M., Sezgin A. and Kumbasaroğlu H. 2011. Erzurum ilinde (Aziziye, Palandöken ve Yakutiye ilçeleri) Kırmızı et tüketim alışkanlığı ve buna etki eden faktörlerin belirlenmesi, Şanlıurfa Tarım Ekonomisi Kongresi, Available from: http://www.tarekoder.org/wp-content/ uploads/2011/12/ cilt_I.pdf

Kubberad E., Ueland O., Rodbotten M., Wested F. and Risvik E. 2002. Gender specific preferences and attitudes towards meat. Food Quality and Preference, 13(5):285-294.

Lichtenstein A.H., Appel L.J., Brands M. *et al*. 2006. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation 114, 82-96.

Lyford C., Thompson J., Polkinghorne R., Miller M. Nishimura T., Neath K., Allen P. and Belascoa E. 2010. Is willingness to pay (WTP) for beef quality grades affected by consumer demographics and meat consumption preferences?. Australasian Agribusiness Review 18:1-17.

Meas T. 2014. The Effects of Country of Origin Image and Patriotism on Consumer Preference for Domestic Versus Imported Beef. Theses and Dissertations, Agricultural Economics. Paper 27. P. 81. Available from: http://uknowledge.uky.edu/agecon_etds/27. Accessed May 2016.

Montossi F., Font-i Furnols M., Del Campo M., Julian R. S., Brito G. And Sañudo C. 2013. Sustainable sheep production and consumer preference trends: Compatibilities, contradictions, and unresolved dilemmas. Meat Science 95(4):772-789.

Nakyinsige K., Man Y.B.C. and Sazili A.Q. 2012. Halal authenticity issues in meat and meat products. Meat Science 91:207-214.

Napolitano F., Braghieri A., Caroprese M., Marino R., Girolami A. and Sevi A. 2007. Effect of information about animal welfare, expressed in terms of rearing conditions, on lamb acceptability. Meat Science 77:431-436.

Napolitano F. 2009. Meat liking, animal welfare and consumer willingness to pay. Ital. J. Anim. Sci. 8 (2):469-476.

Newbold P. 1995. Statistics for business and economics. Prentice-Hall International, 867p, New Jersey.

Norat T., Lukanova A., Ferrari P. and Riboli E. 2002. Meat consumption and colorectal cancer risk: Dose-response meta-analysis of epidemiological studies. International Journal of Cancer 98(2):241-256.

OECD 2015. Meat consumption (indicator). Available from: https://data.oecd.org/agroutput/meat-consumption.htm. Accessed May 2016.

Önenç A. and Özşenoğulları, H. 2009. Türkiye'de koyun etinin tüketim olanakları, Türkiye Koyunculuk Kongresi, Ege Üniversitesi Zootekni Bölümü, İzmir, s.132-137.

Pearce K.M. 2013. Maintaining our share of the lamb meat pie. Nuffield Scholar, Nuffield Australia, Project No: 1210, p. 72.

Prättälä R., Paalanen L., Grinberga D., Helasoja V., Kasmel A. and Petkeviciene J. 2006. Gender differences in the consumption of meat, fruit and vegetables are similar in Finland and the Baltic countries. European Journal of Public Health 17(5):520-525.

Prescott J., Young O. and O'Neill l. 2001. The impact of variations in flavour compounds on meat acceptability: a comparison of Japanese and New Zealand consumers, Food Quality and Preference 12 (4):257-264.

Prescott J., Young, O., Zhang S. and Cummings T. 2004. Effects of added "flavour principles" on liking and familiarity of a sheep meat product: a comparison of Singaporean and New Zealand consumers. Food Quality and Preference 15:187-194.

Republic of Turkey Ministry of Food, Agriculture and Livestock (MFAL). 2015. Livestock strategy, General Directorate of Animal Husbandry, Available from: http://www.tarim.gov.tr/HAYGEM/Belgeler/Stratejisi.pdf Accessed Juni 2016.

Russell C.G. and Cox D.N. 2004. Understanding middle-aged consumers' perceptions of meat using repertory grid methodology. Food Quality and Preference 15(4):317-329.

Santos M.L.S. and Booth D.A. 1996, Influences on meat avoidance among British students. Appetite 27:197-205.

Sánchez M., Sanjuán A.I. and Akl G. 2001. The influence of personal attitudes and experience in consumption on the preferences for lamb and veal, Presentado en el 71th European A. of Agricultural Economics Seminar, Zaragoza (España).

Sañudo C., Alfonso M., Julián R.S., Thorkelsson G., Valdimarsdottir T., Zygoyiannins D., Stamataris C., Piasentier E., Mills C., Berge P., Dransfield E., Nute G.R., Enser M. and Fisher A.V. (2007). Regional variation in the hedonic evaluation of lamb meat from diverse production systems by consumers in six European countries. Meat Science. 75: 610-621.

Sepúlveda W.S., Maza M.T. and Pardos L. 2011. Aspects of quality related to the consumption and production of lamb meat. Consumers versus producers. Meat Science 87(4):366-372.

Shiflett J.S., Purcell W., Marsh D. and Rodgers P. 2007. Analysis of Lamb Demand in the United States, Amerikan Lamb Board, p.89. Available from: http://www.lambcheckoff.com/wp-content/uploads/2012/04/juniper-2007.pdf.

Turkish Statistical Institute (TSI) (2014). Seçilmiş Göstergelerle İzmir, p. 112. Available from: http://www.tuik.gov.tr/ilGostergeleri/iller/IZMIR.pdf

Tüzemen E. (2012). Konya İli Selçuklu İlçesinde Kırmızı Et Tüketim Alışkanlığı ve Buna Etki Eden Faktörlerin Belirlenmesi, Selçuk Üniversitesi Fen Bilimleri Enstitüsü YL Tezi Tarım Ekonomisi Anabilim Dalı, s. 61.

UKON. 2013. Sektör raporu, Available from: http://www.ukon.org.tr/ raporlar/pdf/ etvesutkurumu 2013yilisektoreldegerlendirmeraporu.pdf. Accesed April 2016.

UKON. 2015. Et ve süt kurumu piyasa bülteni, Available from: Available from: http://www.ukon.org.tr/raporlar/pdf/ etvesutkurumuhaftalikpiyasabulteni2015.04.pdf. Accessed May 2016.

Ureña F., Bernabéu R. and Olmeda M. 2008. Women, men and organic food: differences in their attitudes, and willingness to pay. A Spanish case study International Journal of Consumer Studies 32:18-26.

Verbeke W., Ward R. W. And Viaene J. 2000. Probit analysis of fresh meat consumption in Belgium: exploring BSE and television communication impact. Agribusiness 16(2):215-234.

Paper Received August 23, 2016 Accepted November 24, 2016

PAPER

A STUDY ON THE CONTENT OF TERPENIC COMPOUNDS IN THE CULTIVAR 'MORAVIAN MUSCAT' (*VITIS VINIFERA* L.)

M. BARON, J. SOCHOR, B. PRUSOVA*, L. TOMASKOVA and M. KUMSTA
Department of Viticulture and Enology, Faculty of Horticulture, Mendel University in Brno, Valtická 337, CZ-691 44 Lednice, Czech Republic
*Corresponding author. Tel.: +420 519367259; fax: +420 519367222
E-mail address: prusova.bozena@email.cz

ABSTRACT

Terpenic represent a very interesting group of aromatic substances. They occur in aromatic grapevine cultivars and create muscat and flower aromas. In 2013, their contents as well as contents of other aromatic substances were determined in juices made of the Moravian Muscat cultivar after 5, 12 and 24 of maceration. Pedigree: Muscat Ottonel x Prachtraube. As Control, fresh juice analysed immediately after the pressing was used. The aim of this experiment was to find out which terpenic compounds are present in grapes of this cultivar and which length of maceration would be the most suitable for making wine. Attention was paid also to levels of monoterpens, phenols, ethyls, alcohols and acids as well as to basic analytical parameters. Results of a chemical analysis indicated that an optimum is the maceration of grapes for 24 hours because after this time interval the highest amount of terpenic substances was released.

Keywords: terpenes, maceration, antioxidant activity, volatile compounds, analytical parameters

1. INTRODUCTION

Terpenic compounds are members of an important group of aromatic compounds and are characterized by floral, muscatel or fruity aromas that are synthesized in berries and stored in their skin. This aroma of a wine depends on its content of volatile compounds, over 680 of which have been identified in wines from some white grape varieties (PEINADO *et al*., 2004). Although terpenes mainly give off a pleasant aroma some of them may show a negative effect on quality of wine. So, for example, yeast of the genus Streptomyces may synthetize sesquiterpenes either on cork or in the barrel. Their presence may jeopardize the sensory quality of wine (JACKSON, 2008). The aroma of young wines is the product of a biochemical and technological sequence. Its formation derives from the grapes and juice production (grape de-stemming, crushing, and pressing technology), and is decisively influenced by the fermentation procedure. All of these parameters will determine the complexity of the wine aroma. (TAO *et al*., 2008) Its quality and quantity influenced by the cultivar, soil, climate and viticultural practices. (RIBÉREAU-GAYON *et al*., 2006, SANTIAGO *et al*., 2011) The experiment monitored the occurrence of terpenic compounds in juice samples after different periods of maceration. Terpenes contribute to some white wines aroma, especially these produced from Muscat grapes and others aromatic ones of high terpene contents (Gewürtztraminer, Traminer, Huxel, Sylvaner). (DZIADAS and JELEŃ, 2010) The variety which was chosen is 'Muscat Moravsky'. Pedigree: Muscat Ottonel x Prachtraube. It was chosen for its good reputation in the South Moravia (Czech Republic). There are many preconcentration techniques to obtain volatile compounds, such a terpenes, subsequently analyzed by gas chromatography. The most widely used are Headspace sorptive extraction techniques (HSSE), such a SPE or SPME, purge and trap technique – sample is stripped by inert gas, which is concentrated in sorption column, steam distillation, continuous distillation and extraction. All this preconcentration techniques needs expensive equipment, or their performance needs high temperature, which can lead to terpene cyclization. Technique liquid-liquid extraction (LLE) used in this experiment is easy to perform, even it use high temperature. For purpose of this experiment, where we compare the length of maceration of similar samples is this sample preparation sufficient. The product of terpene cyclization is α-terpinol, so in each sample we can compare its increasing. The aim of this experiment was to found out if (and to which extent) the length of maceration shows an effect on the increase in the content of terpenic and other compounds in produced wine. Terpenes, because of their high concentrations and low aroma thresholds, are the principal components responsible for the characteristic aroma of a wine (CARBALLEIRA LOIS *et al*., 2001).

2. MATERIALS AND METHODS

2.1. Sampling site and procedure

Grapes used in the experimental part of this study originated from vineyards situated in the locality Velké Bílovice (wine-growing subregion of Velké Pavlovice). In this region, the average annual precipitations and temperature are 550 mm and 9.5 °C, respectively. Phenological data of the grapes and vine in year 2013:
Shooting: (BBCH05) – 25.4.2013,
Full antesis: (BBCH 65) – 12.6.2013,
Verasion: (BBCH 81) – 9.8.2013,
Ripening: (BBCH 88) – 11.9.2013.

Rootstock CR2 typical for Czech Republic was used. The Czech grape training is modificated German training specially Rhone_Hessen, nevertheless fruitfull wood is horizontal tying. Clasps planting: 1x1.2 mB. High of trunk is 0.75 m. Berries was collected random from the top, middle, and bottom of selected clusters. In order to obtain representative sample, colored berries was not favoured over greens. Berries were stored in a sealed plastic bags in the refrigerator until processing.

2.2. Experimental variants of maceration and processing of samples

In this experiment, grapes of the variety Moravian Muscat' were used. These grapes were harvested on the 11 September 2013. Their sugar content was 19 °NM (i.e. 19 kg of natural sugars in 100 liters of juice). Harvested grapes were crushed and destalked in a stainless destalking-crushing machine, macerated for 0 (Variant 1); 5 (Variant 2); 12 (Variant 3) and 24 hours (Variant 4) at the temperature of 14 °C, and finally pressed.
Wine was made from each juice sample and used for the estimation of the following parameters: pH and contents of alcohol, titratable acidity and sugars, respectively. In individual wine samples, the content of aromatic compounds was estimated as well.

2.3. Estimation of total titratable acidity (EEC No 2676/90)

The content of total titratable acidity was estimated by titration in an automatic titrator TITROLINE EASY (manufacturer SI Analytics GmbH, Germany). Titrations were performed with NaOH (0.1mol.L^{-1}) as the titration reagent. . For analyses, a 10 ml sample was used; this sample was diluted with 10 ml of distilled water. Because of a subsequent formol titration, the sample was not titrated up to the usual pH value of 7.0 but up to the value of 8.1 (the resulting deviation was thereafter considered to be a systematic error). The detection of pH was assured by means of a pH-electrode SenTix 21. After the end of titration, the consumption of NaOH solution in milliliters was read, with the accuracy of two decimal positions, on the titrator's display. The content of total titratable acidity (in g.L^{-1}) was calculated as follows: the amount of consumed NaOH solution was multiplied by the factor of the NaOH solution used for the titration; the product of this multiplication was thereafter multiplied by and by the coefficient 0.75.

2.4. Estimation of pH

The pH value was estimated in an undiluted sample using a pH-meter WTW pH 526 and a pH electrode SenTix 21 (both manufactured by the company WTW, Germany).
Estimation of residual sugar and alcohol
Contents of residual sugar and alcohol were estimated in the apparatus ALPHA. The ALPHA apparatus is a compact FTIR analyzer that uses the ATR sampling technique. This technique helped to process samples before the analysis. Before the first measurement, the spectrometer was thoroughly rinsed with deionized water and the background was determined using a blank sample (i.e. of deionized water). For analyses, 1 ml samples were taken with a syringe; of this sample, 0.5 ml was used for rinsing of the system while the remaining volume of 0.5 ml was analyzed three times. Depending on the calibration used, the measured values were evaluated automatically using a special software.

2.5. Estimation of contents of aromatic compounds in berries by means of gas chromatography

2.5.1 Preparation of samples

Contents of aromatic compounds present in berries were estimated after their extraction by an organic resolvent. In each sample, altogether 100 g of berries were mixed with 100 μL of 1M $K_2S_2O_5$ solution (to prevent oxidation) and 10 μL of the GC internal standard. Thereafter, the mixture was homogenised in a manual mixer and the juice was separated from the mush using a filter paper. The pH value of the juice was adjusted to 3.0 with 10 M H_3PO_4. The adjusted juice was thereafter poured into a volumetric flask of the volume of 25 ml. The extraction was performed after the sample incubation in the boiling water bath for 1 hour. It is necessary to mention in this context that each heating results in a disintegration of glycosides and a release of aromatic compounds. The sample heating promote also the terpenic cyclization, but this preparation of sample is easy to perform and for purpose of this experiment is convenient. The product of terpene cyclization is *α*-terpinol, so in each sample we can compare its increasing. The extraction was performed using 1 ml of methyl terc-butyl ether that contained 1 % of cyclohexane. After the separation, the phase of organic matter was dried up with anhydrous magnesium sulphate and used for the GC-MS analysis.

2.5.2 Analysis of aromatic compounds by means of gas chromatography

Concentrations of individual volatile compounds in wine were determined according to until now unpublished method of extraction with methyl-t-butylether. Into a 25-ml volumetric flask, 20 ml of wine was pipetted together with 50 μl of 2-nonanol solution in ethanol; this compound was used as an internal standard (in concentration of 400 $mg \cdot L^{-1}$) and 5 ml of a saturated $(NH_4)_2SO_4$ solution. The flask content was thoroughly stirred and thereafter 0.75 ml of the extraction solvent (MTBE with an addition of 1% cyclohexane) was added. After another thorough stirring and separation of individual phases, the upper organic layer was placed into a micro test tube together with the produced emulsion centrifuged and the clear organic phase was dried up with anhydrous magnesium sulphate. Extract samples, adjusted in this way, were thereafter used for the GC-MS analysis.
Instruments: Shimadzu GC-17A, Autosampler: AOC – 5000, Detector: QP-5050A, Software: GCsolution. Program: LabSolutions, GC MS solution.Version 1.20, Conditions of separation: column: DB-WAX 30m x 0.25mm; 0.25μm stationary phase (polyethylene glycol). Voltage of the detector 1.5 kV. Individual compounds were identified on the base of MS spectrum and retention time using NIST 107 library, which contains 107,886 spectra.

2.6. Estimation of antioxidant activity by the DPPH method

150 μL volume of the reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl - DPPH·) was incubated with 15 μL of wine sample. The absorbance was measured at 505 nm for 10 minutes and the output ratio was calculated as a difference between absorbance values measured at the 10^{th} minute and the 2^{nd} minute of the assay procedure.

3. RESULTS AND DISCUSSIONS

For experiments, aromatic cultivar Moravian Muscat was used. The weight of 50 berries was 67.8 g at harvest time. The juice was quartered, each of these four parts was macerated

for a different time interval and thereafter used for wine making. The following qualitative parameters were estimated in each part of experimental juice: weight of berries, sugar content, content of titratable acids, pH, content of yeast assimilable nitrogen and aromatic compounds. Contents of aromatic compounds were estimated also in wine made from individual parts of berries. The aim of this experiment was to find out if the length of the maceration period influenced the content of terpenic substances in final wine product.

3.1. Estimation of basic analytical parameters

Values of basic analytical parameters are presented in Fig. 1.
The lowest content of alcohol was determined in Variant 1 (9.79 $g.L^{-1}$); this value was also correlated with the highest level of residual sugars (8.55 $g.L^{-1}$). The lowest content of residual sugars and the highest content of alcohol was found out in Variant 4 (4.68 and 12.47 $g.L^{-1}$, respectively).
The highest and the lowest contents of total titratable acids (i.e. 12.47 $g.L^{-1}$and 5.79 $g.L^{-1}$) were was found out in Variants 1 and 4, respectively. It this context it can be concluded that the longer the period of maceration, the lower the amount of total titratable acids in produced wine. This means that their contents decreased with the period of maceration. On the other hand, however, the pH value increased with the period of maceration. The highest pH (3.36) was found out in Variant 4.

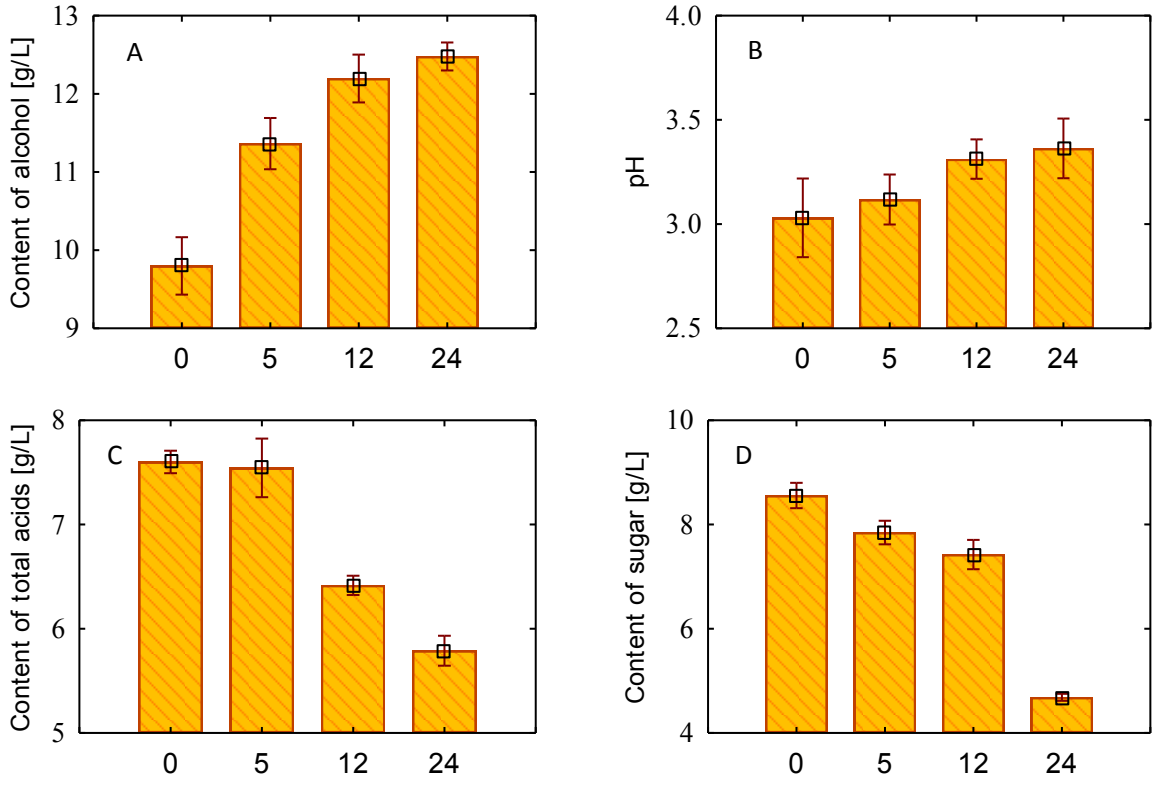


Figure 1. A - Content of alcohol, B - pH, C - content of total acids, D - content of sugar; in period of maceration: 0, 5, 12 and 24 hours.

3.2. Monoterpenes

Linalool is a naturally occurring terpene alcohol that has a characteristic floral scent with spicy and lemon tones. It can be found in the pulp of berries of muscat cultivars. Its content does not change too much during the alcoholic fermentation. Linalool is changed because its oxidation to α-terpineol that occurs in grapes only in smaller amounts. It can be identified only with difficulties and it does not influence the aroma of wine. The odour of nerol resembles roses and thyme but it is considered to be a little fresher. In the course of fermentation, the content of this monoterpene decreases and it is gradually changed to α-terpineol. It has a scent of roses and citrus fruit. A rose-like scent of this compound participates significantly in the aroma of muscat wines; however, it was not identified in other varieties. During the alcoholic fermentation, its content is slowly decreasing. In the course of wine ageing, geraniol is transformed to α-terpineol (JACKSON, 2008).
In general, the content of monoterpenes increased in dependence on the duration of macerations. The content of linalool doubled within 24 hours of maceration. The content of ho-trienol increased from 78.6 to 155.5 $\mu g.L^{-1}$ while that of α-terpineol rose only from 81.7 to 142.4 $\mu g.L^{-1}$ and those of nerol and geraniol rose from 5 to 17.1 $\mu g.L^{-1}$ and from 20 to 49.8 $\mu g.L^{-1}$, respectively.
Results are presented in Table 1.

Table 1. Content of monoterpens.

Substance		Time of maceration							
		0 hours		5 hours		12 hours		24 hours	
		Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
Linalool	[$\mu g.L^{-1}$]	362.40	7.49	381.25	5.73	491.63	7.48	724.21	8.48
Ho-trienol	[$\mu g.L^{-1}$]	78.67	1.67	92.52	1.39	117.61	1.80	155.55	3.18
α-terpineol	[$\mu g.L^{-1}$]	81.73	0.47	89.17	2.03	85.00	2.55	142.41	3.73
ß-citronellol	[$\mu g.L^{-1}$]	2.96	0.07	4.98	0.03	5.02	0.03	8.05	0.09
Nerol	[$\mu g.L^{-1}$]	5.08	0.10	5.92	0.07	8.03	0.12	17.17	0.29
Geraniol	[$\mu g.L^{-1}$]	20.07	0.70	26.35	0.60	29.60	0.69	49.81	1.06
Epoxylinalol 1	[$\mu g.L^{-1}$]	129.25	3.03	144.96	4.40	187.47	4.27	346.00	3.46
Epoxylinalol 2	[$\mu g.L^{-1}$]	78.67	1.67	106.08	2.08	131.77	2.05	206.61	2.40
2,6-dimetyl-3,7-Octadiene-2,6-diol	[$\mu g.L^{-1}$]	607.01	6.01	701.95	6.95	954.45	9.45	1181.55	17.81

3.3. Phenols

Volatile phenolic substance are produced step by step in the course of wine making by means of enzymatic decomposition of phenolic acids present in berries. The principal compounds are the following: 4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol and 4-ethylguaiacol. In white wines, vinylphenols (4-vinylphenol and 4-vinylguajacol in concentrations ranging from 10 to 490 and from 70 to 1,150 $\mu g.L^{-1}$, respectively) are predominating while in reds the principal compounds are ethylphenols (4-ethylphenol up to 6 000 $\mu g.L^{-1}$). In case that the content of ethylphenol is higher than 400 $\mu g.L^{-1}$ (sigma 4-ethylphenol + 4-ethylguaiacol), the quality of wine may be negatively influenced; this is usually manifested as an unpleasant wine aftertaste resembling horse sweat (RAPP and VERSINI, 1996).
The content of phenols is presented in Table 2.

The content of 4-vinylguaiacol increased in the course of the whole maceration period. After 24 hours, the initial value of 118.5 μg.L^{-1} increased to 24 as much as 292.9 μg.L^{-1}. The content of 4-vinylphenol was fluctuating; however, the difference between the initial and final values, i.e. at the beginning and to the end of maceration (0 h and 24 h) was 992 μg.L^{-1}.

Table 2. Content of phenols.

Substance		Time of maceration							
		0 hours		5 hours		12 hours		24 hours	
		Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
Methionol	[μg.L^{-1}]	0.39	0.01	2.58	0.03	1.48	0.03	0.51	0.01
2-Methyltetrathio-phen-3-on	[μg.L^{-1}]	0.68	0.02	0.90	0.04	0.59	0.01	0.51	0.01
4-Vinylguajacol	[μg.L^{-1}]	118.58	2.42	208.03	4.43	267.96	6.03	292.85	4.41
4-Vinylfenol	[μg.L^{-1}]	155.43	4.15	1029.88	15.47	653.60	14.90	1147.81	28.79
4-Ethylguajacol	[μg.L^{-1}]	10.93	0.23	10.07	0.21	9.87	0.15	8.11	0.18
4-Ethylfenol	[μg.L^{-1}]	16.94	0.26	16.11	0.49	13.13	0.13	12.16	0.18
1,1-Diethoxyetan	[μg.L^{-1}]	565.00	22.60	211.15	4.94	630.00	12.60	677.49	7.77

3.4. Alcohol and ethyls

According to Dennis (DENNIS *et al*., 2012) the content of precursors of acetate esters is dependent on their concentration in the juice and on the technology of processing of grapes. These precursors involve above all alcohols. Their concentration increases with the duration of per-fermentation maceration. These precursors are thereafter transformed to aforementioned ethyls and these influence the aromatic profile of wine.
The content of ethylacetate increased within the whole period of maceration, from the initial value of 32.6 mg.L^{-1} to that of 59.7 mg.L^{-1}. Contents of ethyl butyrate and ethyl octanoate increased by 150 and 120 μg.L^{-1}, respectively. The content of isoamyl acetate increased by 92 % to the value of 7.6 μg.L^{-1}. As far as 2-phenylethyl acetate was concerned, its content increased 6 times within the first 5 hours and thereafter decreased to the level that corresponded with 2.5 multiple of its initial concentration. The initial content of 1-hexyl acetate was 261.7 μg.L^{-1}and decreased to 43.7 μg.L^{-1} within the first 5 hours; thereafter, its level increased to final 199 μg.L^{-1} at the end of the 24 hour period of maceration. The content of alcohols is presented in Table 3.
As far as individual alcohols are concerned, the content of methanol is also important. Its level increased within the whole period of maceration. Within 24 hours, this value increased from 12 to 42 mg.L^{-1}. Within the first 5 hours, the content of (E)-3-hexen-1-ol increased. However, after 12 hours it began to decrease again and reached approximately the initial level. The content of (Z)-3-hexen-1-ol increased within the whole 24-hour period of maceration: the initial value of 71 μg.L^{-1} doubled and was as much as 146 μg.L^{-1}.
The content of alcohols is presented in Table 4.

Table 3. Content of ethyls.

Substance		Time of maceration							
		0 hours		5 hours		12 hours		24 hours	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ethyl acetate	[mg.L^{-1}]	32.63	0.74	36.59	0.87	47.32	0.54	59.70	1.20
Ethyl propionate	[µg.L^{-1}]	109.27	1.27	109.08	1.87	107.71	1.24	85.55	1.81
Ethyl isobutyrate	[µg.L^{-1}]	28.90	0.17	42.14	1.06	46.92	0.92	46.69	0.54
Ethyl butyrate	[µg.L^{-1}]	402.18	8.51	470.00	18.80	505.00	8.66	550.80	9.35
Ethyl hexanoate	[µg.L^{-1}]	518.22	8.05	228.47	4.79	536.90	8.34	573.68	9.84
Ethyl octanoate	[µg.L^{-1}]	489.36	2.83	512.00	10.24	624.24	12.24	608.91	12.76
Ethyl decanoate	[µg.L^{-1}]	54.45	0.95	62.16	0.96	1.00	0.02	125.87	2.56
Ethyl lactate	[mg.L^{-1}]	5.56	0.12	2.71	0.01	2.72	0.03	2.61	0.01
Diethyl uccinate	[mg.L^{-1}]	0.60	0.00	1.01	0.02	1.71	0.01	1.99	0.06
Diethylmalate	[mg.L^{-1}]	3.14	0.07	3.50	0.04	3.09	0.08	2.47	0.05
Monoethyl succinate	[mg.L^{-1}]	2.62	0.04	5.41	0.19	4.63	0.05	3.32	0.10
Gama-butyrolactone	[mg.L^{-1}]	7.70	0.09	21.36	0.25	20.35	0.43	19.70	0.53
Isoamyl acetate	[mg.L^{-1}]	3.94	0.10	5.49	0.10	5.82	0.07	7.60	0.08
2-Phenylethyl acetate	[µg.L^{-1}]	151.50	2.31	932.45	22.12	552.35	12.42	384.28	13.45
1-Propyl acetate	[µg.L^{-1}]	66.33	0.67	39.78	0.78	43.14	1.51	90.70	3.20
Isobutyl acetate	[µg.L^{-1}]	116.61	2.94	198.53	4.04	249.00	9.96	283.05	4.34
1-Hexyl acetate	[µg.L^{-1}]	261.73	7.94	43.71	0.25	93.69	2.37	199.00	0.00
(Z)- 3-Hexen-1]-yl acetate	[µg.L^{-1}]	6.04	0.12	14.19	0.21	14.28	0.24	14.19	0.16

Table 4. Content of alcohol.

Substance		Time of maceration							
		0 hours		5 hours		12 hours		24 hours	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Methanol	[mg.L^{-1}]	12.34	0.31	13.91	0.29	32.57	0.57	42.11	1.29
Isoamylalcohol	[mg.L^{-1}]	56.19	0.32	136.65	4.78	134.64	4.71	109.92	3.33
Isobutylalcohol	[mg.L^{-1}	6.33	0.07	28.08	0.48	25.00	0.25	22.17	0.78
2-Phenylethanol	[mg.L^{-1}]	9.73	0.20	68.21	1.21	52.29	0.81	19.80	0.00
1-Propanol	[mg.L^{-1}]	19.69	0.41	9.96	0.24	12.63	0.15	35.14	0.36
1-Butanol	[µg.L^{-1}]	1240.67	14.42	1247.15	29.19	1967.58	38.58	7130.90	146.01
1-Hexanol	[µg.L^{-1}]	1508.00	45.24	1292.80	12.80	915.85	19.19	888.00	20.78
(E)-3-Hexen-1-ol	[µg.L^{-1}]	84.15	1.47	114.00	3.42	101.00	1.00	88.16	2.01
(Z)-3-Hexen-1-ol	[µg.L^{-1}]	71.76	1.10	126.00	5.04	147.46	2.53	146.03	1.71
3-Methyl-1]-pentanol	[µg.L^{-1}]	6.02	0.03	18.68	0.40	13.95	0.49	11.11	0.19
Benzylalkohol	[µg.L^{-1}]	111.72	2.28	123.22	3.23	305.00	0.00	410.63	2.38
2,3-Butandiol	[mg.L^{-1}]	346.61	5.37	552.57	6.29	1309.57	26.81	1497.02	14.82
Propandiol	[mg.L^{-1}]	6.60	0.07	8.94	0.19	23.64	0.50	19.05	0.29

3.5. Antioxidation activity

As compared with red wines, a lower antioxidant capacity of white ones is caused by a lower content of phenolic compounds (VINSON and HONTZ, 1995). A higher content of phenolic in red wine results is caused by the period of maceration during which phenolic compounds are released from skins, seeds, stalks and pulp of berries (FUHRMAN *et al.*, 2001). Because in white wines the maceration usually does not take place, their content of phenolic substances is limited and also their antioxidant activity is reduced (LAMUELA-RAVENTOS and DE LA TORRE-BORONAT). For that reason the maceration represents an interesting (and natural) step when producing white wines because it enables extraction of phenolic compounds and, thus, production of wine with strong antioxidant properties.
In the course of the first 12 hours of maceration, the antioxidant activity (as measured by the DPPH assay, increased and thereafter remained without changes (i.e. constant) during 24 hours of measuring. After 12 hours, it was even more than three times higher than at the beginning. Dependence of antioxidant activity on length of maceration is depicted in Fig. 2.

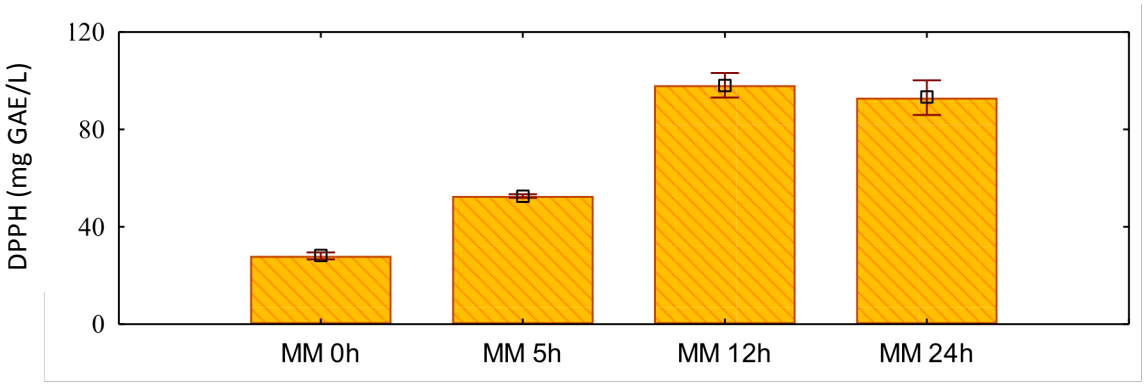


Figure 2. Antioxidation activity.

3.6. Time of maceration

Correlations existing between contents of individual aromatic substances and the time of maceration are presented in Table 5. They are expressed as the Pearson's correlation coefficient and characterize the tightness of individual relationships. Values between 0.1 and 0.3 indicate a weak correlation while those between 0.4 and 0.6 and between 0.7 – 0.8 indicate medium and strong correlations, respectively. Values above 0.9 mean that the correlation is very strong.

Table 5. Correlations existing between contents of individual substances and the period of maceration. Significant correlations ($p < .05000$) are in red.

Linalool	0.982	Ethyl butyrate	0.939	1-Propanol	0.712
Ho-trienol	0.998	Ethyl hexanoate	0.467	1-Butanol	0.926
α-terpineol	0.891	Ethyl octanoate	0.823	1-Hexanol	-0.894
ß-citronellol	0.961	Ethyl decanoate	0.531	(E)-3-Hexen-1-ol	-0.169
Nerol	0.963	Ethyl lactate	-0.679	(Z)-3-Hexen-1-ol	0.784
Geraniol	0.977	Diethyl succinate	0.951	3-Methyl-1-pentanol	0.105
Epoxylinalol 1	0.969	Diethylmalate	-0.834	Benzylalcohol	0.967
Epoxylinalol 2	0.994	Monoethyl succinate	-0.039	2,3-Butandiol	0.935
2,6-dimetyl-3,7-octadiene-2,6-diol	0.990	Gama-butyrolactone	0.575	Propandiol	0.745
Methionol	-0.250	Isoamyl acetate	0.969	Acetic acid	0.884
2-Methyltetrathiophen-3-on	-0.698	2-Phenylethyl acetate	-0.019	Propionic acid	0.569
4-Vinylguaiacol	0.903	1-Propyl acetate	0.587	Butyric acid	0.953
4-Vinylfenol	0.711	Isobutyl acetate	0.923	Isobutyrřic acid	0.967
4-Ethylguaiacol	-0.966	1-Hexyl acetate	-0.004	Isovaleric acid	-0.315
4-Ethylphenol	-0.941	(Z)- 3-Hexen-1-yl acetate	0.655	2-methylbutanoic acid	-0.003
1,1-Diethoxyetane	0.532	Methanol	0.962	Hexanic acid	0.347
Ethyl acetate	0.994	Isoamylalcohol	0.386	Octanoic acid	0.144
Ethyl propionate	-0.899	Isobutylalcohol	0.451	Decanoic acid	-0.111
Ethyl isobutyrate	0.779	2-Phenyletcanol	-0.110	DPPH GA	0.880

4. CONCLUSIONS

In these experiments, interesting volatile compounds characterizing the cultivar Moravian Muscat. Wine production from aromatic cultivars such a Moravian Muscat is a complex process depended on the health condition of grapes, temperature and length of the maceration period. Results of this study indicate that the content of individual compounds is changing in the course of maceration. For that reason it is important to pay attention to its length and create favourable conditions enabling the development of wine character. The content of terpenic compounds (especially of monoterpenes) is increasing above all with the increasing time of maceration. This increases not only the antioxidant activity of wine but also the content of ethyls that can show a negative effect on the aromatic profile of produced wine. However, the content of total acids decreases. The only exception represents the content of acetic acid that markedly increases with the length of maceration so that the quality of produced wine is deteriorated.

REFERENCES

Carballeira Lois L., Cortés Diéguez S., Gil de la Peña M.L. and Fernández Gómez E. 2001. SPE-GC determination of aromatic compounds in two varieties of white grape during ripening. Chromatographia 53(1):S350-S355.

Dennis E.G., Keyzers R.A., Kalua C.M., Maffei S.M., Nicholson E.L. and Boss P.K. 2012. Grape Contribution to Wine Aroma: Production of Hexyl Acetate, Octyl Acetate, and Benzyl Acetate during Yeast Fermentation Is Dependent upon Precursors in the Must. J Agric Food Chem. 60(10):2638-2646.

Dziadas M. and Jeleń H.H. 2010. Analysis of terpenes in white wines using SPE–SPME–GC/MS approach. Analytica Chimica Acta 677(1):43-49.

Fuhrman B., Volkova N., Suraski A. and Aviram M. 2001. White Wine with Red Wine-like Properties: Increased Extraction of Grape Skin Polyphenols Improves the Antioxidant Capacity of the Derived White Wine. J Agric Food Chem. 49(7):3164-3168.

Herjavec S., Tupajic P. and Majdak A. 2001. Influence of malolactic fermentation on the quality of riesling wine. Agriculturae Conspectus Scientificus 66(1):59-64.

Jackson R.S. 2008. Wine Science: Principles and Applications. Elsevier Science.

Lamuela-Raventos R.M. and de la Torre-Boronat M.C. Beneficial effects of white wines. (0378-6501 (Print)).

Peinado R.A., Moreno J., Bueno J.E., Moreno J.A. and Mauricio J.C. 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chemistry 84(4):585-590.

Rapp A. and Versini G. 1996. Volatile phenolic compounds of wine. Dtsch Lebensm-Rundsch 92(2):42-48.

Ribéreau-Gayon P., Dubourdieu D., Don?che B. and Lonvaud A. 2006. Handbook of Enology, The Microbiology of Wine and Vinifications. Wiley.

Santiago A.V.C., Munoz R. and Garcia R.G. 2011. Molecular Wine Microbiology. Elsevier Science.

Tao Y., Li H., Wang H. and Zhang L. 2008. Volatile compounds of young Cabernet Sauvignon red wine from Changli County (China). Journal of Food Composition and Analysis 21(8):689-694.

Vinson J.A. and Hontz B.A. 1995. Phenol Antioxidant Index: Comparative Antioxidant Effectiveness of Red and White Wines. J. Agric. Food. Chem. 43(2):401-403.

Paper Received July 3, 2016 Accepted October 28, 2016

PAPER

TRIGLYCERIDES VARIABILITY IN DONKEY MILK

M. BONONI[1]*, F. TATEO[1] and A. TATEO[2]

[1]Department of Agricultural and Environmental Science, University of Milan, Via Celoria 2, Milan, Italy

[2]Department of Veterinary Medicine, University "Aldo Moro" of Bari, Valenzano, Ba, Italy

*Corresponding author. Tel.: +39 0250316538

E-mail address: monica.bononi@unimi.it

ABSTRACT

The distribution of triacylglycerols (TAGs) in donkey milk and of fatty acids in the glycerol backbone affect assimilation and lipolysis, so the utility of analytical methods to characterise fat fractions is proved. In this study an optimised gas chromatography/on-column injector method was used to study the TAG variability of various milk species. This method is useful for quality control aimed at standardising formulated donkey milk and was used to compare the TAG composition of lyophilised donkey milk distributed on the Italian market. Three TAG groups, based on variability degree, were detected and the most characterising TAG fraction was identified.

Keywords: donkey milk, GC-OCI technique, quality control, triglycerides variability

1. INTRODUCTION

The use of donkey milk for human consumption is justified by various nutritional values and its tolerability (BARŁOWSKA *et al.,* 2011; DO NASCIMENTO RANGEL *et al.,* 2015; MALISSIOVA *et al.*, 2016 POLIDORI and VINCENZETTI, 2013). The presence of bioactive and functional components has been largely reviewed (MARTINI *et al.,* 2014; SALIMEI *et al.,* 2004; SALIMEI, 2011; SALIMEI and FANTUZ, 2012;). Moreover, the consumption of donkey milk is an alternative when there is an intolerance to cow's milk (HØST and HALKEN, 2004; LARA-VILLOSLADA *et al.,* 2005; MONTI *et al.,* 2007; RESTANI *et al.,* 2009). Previous studies (CHIANESE *et al.,* 2010; GUO *et al.,* 2007; MARCONI and PANFILI, 2002; SUMMER *et al.,* 2004) support the optimisation of infant formula milk and the production of nutraceutical compounds in donkey milk for the following reasons: (1) the lipid content in donkey milk is essentially lower than in milk from humans or cows; (2) the carbohydrate content in donkey milk is comparable with human milk but higher than in cow's milk; and (3) the protein content in donkey milk is higher than in human milk but lower than in cow's milk. This research is part of a larger project concerning the industrial formulation of humanised milk and nutraceutical compounds using lyophilised donkey milk. The standardisation of these commercial products is possible only if they are derived from ingredients characterised by standard parameters.

Groups have analysed the triacylglycerol (TAG) composition to identify the fatty acids in the glycerol backbones (GASTALDI *et al.,* 2010), but the analytical methods used for this aim are not suitable for routine industrial quality control. Some authors also reported comparative results for the fatty acid and TAG compositions of various milks species (BLASI *et al.,* 2008; BRECKENRIDGE and KUKSIS, 1967; CHIOFALO *et al.,* 2011; COSSIGNANI *et al.,* 2011; DUGO *et al.,* 2005; GANTNER *et al.,* 2015GASTALDI *et al.,* 2010; JENSEN, 1999; MARTEMUCCI and D'ALESSANDRO, 2012; ZOU *et al.,* 2013). There is often not ideal peak resolution in TAG analysis using high performance liquid chromatography with an evaporative light scattering detector (HPLC/ELSD) (ZOU et al., 2013) and there are difficulties with TAG analysis when using HPLC/atmospheric pressure chemical ionisation (APCI)/mass spectrometry (MS) techniques with correction factors to estimate the proportion of the different TAGs in each type of milk (GASTALDI *et al.,* 2010).

Various authors reported comparative results concerning the fatty acid composition and TAG distribution of the fatty acids in the glycerol backbone of various milk species, but the analytical methods used for TAG analysis are not useful for routine quality control (BLASI *et al.,* 2008; BRECKENRIDGE and KUKSIS, 1967; CHIOFALO *et al.,* 2011; COSSIGNANI *et al.,* 2011; DUGO *et al.,* 2005; GANTNER *et al.,* 2015; GASTALDI *et al.,* 2010; JENSEN, 1999; MARTEMUCCI and D'ALESSANDRO, 2012; ZOU *et al.,* 2013).

This paper discusses the advantages of adopting a rapid gas-chromatography (GC) method based on the identification sequence of peaks, with each one representing the total carbon number (CN) of a single TAG, in order of increasing molecular weight.

The triglyceride composition of lyophilised donkey milk products available on the Italian market was compared and the characterisation of donkey milk by this easy method was useful for the purposes of industrial quality control and to determine the fat variability composition in these lyophilised donkey milk.

2. MATERIALS AND METHODS

2.1. Experimental design

A method that was able to produce a complete pattern of TAGs by direct injection was first reported in the Commission Regulation (EC) No 213/2001 to determine the genuineness of butter. Then, the Authors modified the operative conditions to produce the first repeatability data (BONONI *et al.*, 2001) and adopted the modified method for the identification of synthesised TAGs used as support in butter flavours (TATEO and BONONI, 2003). The same method was also applied for quality control of vegetable and animal fats (TATEO and BONONI, 2002; TATEO and BONONI, 2004).
Some Authors (LOZADA *et al.*, 1995; MOLKENTIN and PRECHT, 1994; MOLKENTIN and PRECHT, 1995) quantitatively determined the TAG composition of milk using capillary columns. In the present paper, we used a Petrocol capillary column with bonded phase, hydrogen as the carrier gas, optimised operative conditions for an on-column injector (OCI), and programmed oven temperatures. The optimisation of the chromatographic conditions was necessary strictly to obtain repeatability data that were useful for various quality control aims.
We used the proposed method to analyse a mass sample of donkey milk from a traditional farming Area of Martina Franca in Apulia (Italy) and the TAG data were compared with milk samples from humans, cows, sheep and goats obtained with the same method.
The lyophilised donkey milk from the farming area of Martina Franca was compared with other lyophilised donkey milk products available from the specialised Italian market.

2.2. Milk samples from different species

First, five samples of milk from different species were compared to highlight the significant differences in the TAG composition of the fat fractions using the proposed method (described below). These samples were:

a) Donkey milk from an autochthonous breed from the Apulia region (Martina Franca), produced by donkeys bred using a semi-extensive method at the farm "Masseria Lamacarvotta" in Laterza (Italy);
b) Cow, sheep, and goat milk selected from Italian farms and collected at the mid lactation stage;
c) Human milk kindly provided by the Neonatology Unit (Milkbank) at the Santa Chiara Hospital in Trento (Italy).

All five samples were stored at 4°C strictly during the time to the laboratory and then were immediately frozen at -20°C before lyophilisation. Subsequently, the samples of milk were lyophilised using a ScanVac CoolSafe 110-4 Pro Freeze Dryer (Labogene) at -105°C.

2.3. Lyophilised, commercially available donkey milk

Four samples of lyophilised donkey milk, commercially available in Italy, were compared with the donkey milk produced from the Martina Franca breed and lyophilised in our pilot equipment, and also with a donkey milk available in Italy that was lyophilised in our laboratory using the same conditions adopted for the liquid samples presented in Table 1. This liquid donkey sample was sterilized by uperisation.

2.4. GC analysis of triglycerides

For the analysis of the TAG profiles in donkey, human, cow, sheep, and goat milk fat, ~5 mL of water was added to ~1-2 g of the lyophilised sample. The sample was vortexed for 1 min, and then sonicated in an ultrasonic bath for 5 min with 2 mL of isooctane (Sigma Aldrich). The mixture was centrifuged at 4000 rpm for 5 min. The isooctane phase (2 mL) was introduced manually in the on-column injector (OCI) at 40°C. The TAG analyses were performed on a HRGC 5160 Mega Series (Carlo Erba Instruments) equipped with a bonded phase poly (dimethyl siloxane) Petrocol EX 2887 capillary column (Supelco) with dimensions of: 5 m x 0.53 mm i.d. and 0.1 μm film thickness. The oven temperature program was 150°C, increased to 200°C at a rate of 10°C min^{-1} and then increased to 340°C at a rate of 5°C min^{-1} (held for 30 min). The detector temperature (FID) was 350°C and the carrier gas was H_2 at 20 kPa pressure. An anhydrous butter fat standard certified by the Community Bureau of Reference (CRM 519) was used for peak identification.

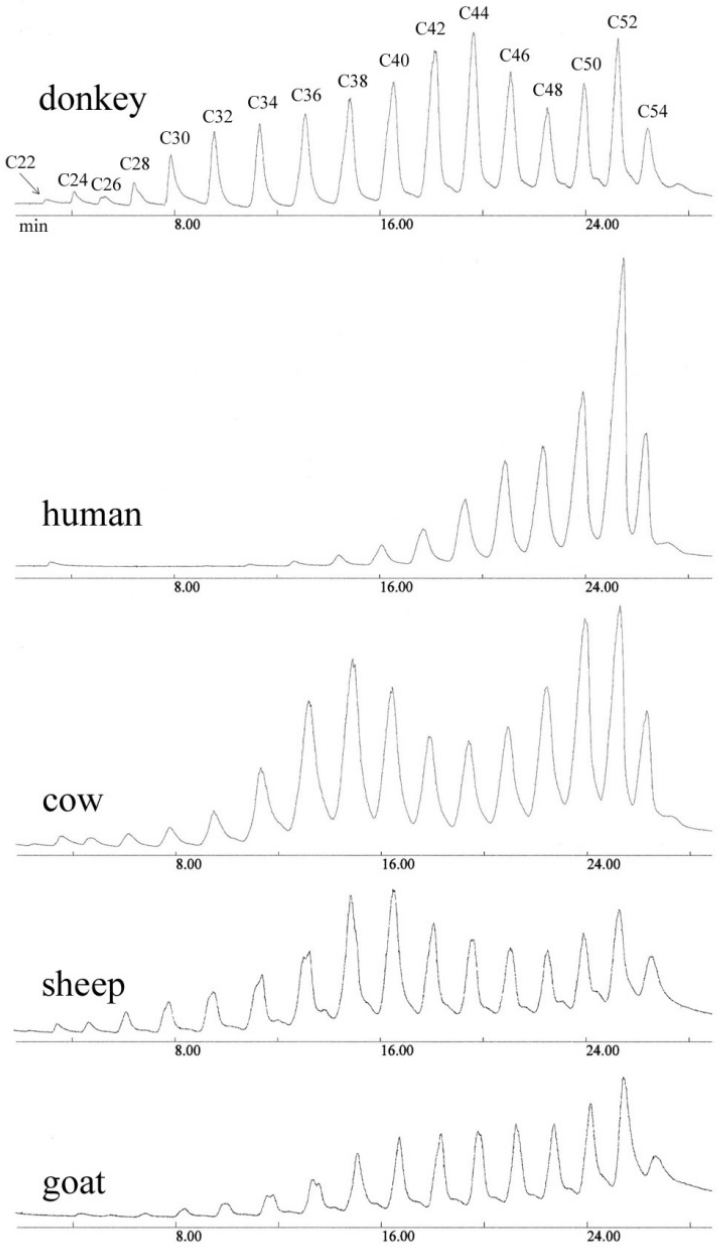
Table 1. Triacylglycerol composition (TAG expressed as % of peaks identified with total carbon number) of donkey milk produced in farm "Masseria Lamacarvotta" (Laterza, Italy) compared with human, cow, sheep and goat milk samples.

TGA	Donkey	Human	Cow	Sheep	Goat
C22	0.01	n.d.	0.07	0.06	n.d.
C24	0.49	0.34	0.56	0.65	0.36
C26	0.21	n.d.	0.46	0.85	n.d.
C28	0.65	n.d.	0.86	1.80	0.38
C30	1.65	n.d.	1.27	2.93	0.89
C32	2.72	n.d.	2.33	4.35	1.72
C34	3.44	0.20	5.30	6.05	3.59
C36	4.57	0.56	10.04	8.69	5.80
C38	6.13	1.11	12.44	13.15	8.21
C40	7.82	2.24	9.67	13.62	9.22
C42	10.15	4.26	6.17	8.77	9.75
C44	12.49	7.39	5.75	7.25	10.16
C46	9.83	11.17	6.55	6.40	10.02
C48	6.90	12.68	8.59	5.57	9.60
C50	8.76	17.79	12.50	6.81	11.75
C52	16.22	32.71	12.08	8.46	15.23
C54	7.59	9.12	5.35	4.60	3.33
C56	0.37	0.42	n.d.	n.d.	n.d.

3. RESULTS

The mean TAG compositions (Table 1) were derived from three replicate GC-OCI analyses of samples of donkey milk from the Martina Franca farm and of samples from other species. Examples of the GC-OCI traces of milk from donkey, human, cow, sheep and goat are shown in Fig. 1.

Figure 1. Examples of GC-OCI traces produced for fat fraction of milk of different species and expressed as total carbon number (CN).



The NMKL Procedure No. 5 (Nordic Committee on Food Analysis, 1997) was used to evaluate the relative Repeatability Standard Deviation (RSDr) using three samples for each milk species, and the following results were obtained: 0.022 (donkey milk), 0.032 (human milk), 0.018 (cow milk), 0.034 (sheep milk), and 0.011 (goat milk).

The TAG composition (Table 2) of four lyophilised samples present on the Italian market were compared with two lyophilised samples produced from the reference liquid donkey milk of Martina Franca and from the liquid donkey milk distributed in Italy. From these data, it is possible to deduce average data and to calculate D_{max} values for each TAG class:

the D_{max} values are simply derived from the minimum and maximum values identified in Table 2. To estimate the variability from the mean values, we defined three ranges (<0.3, 0.3-0.6, and >0.6) deduced from the ratio "D_{max}/mean" that identify TAG classes with low, medium, and high variability.

Table 2. TAG composition of the fat fraction of four lyophilised donkey milk products distributed in Italy (A-D) compared with the Martina Franca donkey milk (E) and a donkey milk distributed in Italy (F) lyophilised in laboratory pilot equipment.

	A	B	C	D	E	F				
TGA							**average**	**D_{max}**	**D_{max}/average**	
C22	0.15	0.03	0.02	0.04	0.01	0.68	0.16	0.67	4.32	***
C24	0.74	0.56	0.70	0.47	0.49	1.19	0.69	0.72	1.04	***
C26	0.13	0.25	0.24	0.32	0.21	0.20	0.23	0.19	0.84	***
C28	0.54	0.86	0.98	0.66	0.65	0.80	0.75	0.44	0.59	**
C30	1.67	2.31	2.54	1.72	1.65	1.87	1.96	0.89	0.45	**
C32	2.80	3.66	4.22	2.82	2.72	4.30	3.42	1.58	0.46	**
C34	3.79	4.72	5.16	3.65	3.44	4.07	4.14	1.72	0.42	**
C36	5.16	6.32	6.58	4.89	4.57	5.19	5.45	2.01	0.37	**
C38	6.75	8.21	8.23	6.40	6.13	6.89	7.10	2.10	0.30	*
C40	8.54	10.54	9.83	8.19	7.82	8.82	8.96	2.72	0.30	*
C42	11.39	13.61	11.69	10.78	10.15	11.55	11.53	3.46	0.30	*
C44	12.61	14.96	12.71	12.41	12.49	12.94	13.02	2.55	0.20	*
C46	9.06	9.52	9.13	9.13	9.83	9.43	9.35	0.77	0.08	*
C48	6.93	7.14	6.10	6.79	6.90	6.75	6.77	1.04	0.15	*
C50	10.41	6.02	7.10	9.80	8.76	8.92	8.50	4.39	0.52	**
C52	14.19	9.80	10.28	15.20	16.22	12.17	12.98	6.42	0.49	**
C54	4.99	1.45	4.36	6.50	7.59	4.23	4.85	6.14	1.27	***
C56	0.15	0.04	0.13	0.23	0.37	n.d.	0.18	0.33	1.79	***

The variability classes for TAG are expressed as the total carbon number (CN). Data for all samples were derived from five replicates.

D_{max}/average

* < 0.3	little variability
** from 0.3 to 0.6	medium variability
*** > 0.6	high variability

The TAGs characterised by the lower variability are included in six TAG classes from C38 to C48. In each donkey milk sample, the classes with lower variability represented more than 50% of the total fat fraction. In particular, for five samples, the TAGs with lower variability represented 53-58% of the total TAG content, and for sample B they represented 64% of the total fat.

The TAGs characterised by the higher variability are included in four TAG classes (C24, C26, C54, and C56) that represented 5-9% of the total fat fraction for five samples and 2% of sample B, while the TAGs with medium variability (C28, C30, C32, C34, C36, C50, and C52) represented 34-39% of the total fat fraction.

Considering the identification of donkey milk fatty acids on glycerol backbone (GASTALDI *et al.,* 2010) and data in Table 2, we deduced Table 3 that shows the TAGs composition of three variability classes. Table 4 reports fatty acid symbols and names used in Table 3.

Table 3. TGA % composition with lower, medium and higher variability classes in six donkey milk samples of Italian market.

Lower variability

TGA	A	B	C	D	E	F	
C38	6.75	8.21	8.23	6.40	6.13	6.89	Bu-P-Ln, Cy-C-Me, C-La-Po, Co-P-P, C-La-P, La-La-M
C40	8.54	10.54	9.83	8.19	7.82	8.82	C-La-Ln, Co-P-Ln, Cy-M-Ln, C-La-O, C-M-P, Cy-P-P
C42	11.39	13.61	11.69	10.78	10.15	11.55	Cy-P-Ln, C-M-Ln, La-Ln-Ln, Cy-P-O, C-Po-P, C-P-P
C44	12.61	14.96	12.71	12.41	12.49	12.94	Cy-Ln-L, Cy-Ln-O, C-P-Ln, Cy-O-O, C-P-O, La-P-P
C46	9.06	9.52	9.13	9.13	9.83	9.43	C-Ln-Ln, C-Ln-L, C-Ln-O, C-L-O, La-P-Ln, C-O-O, La-P-Pl, La-P-O-, M-P-P
C48	6.93	7.14	6.10	6.79	6.90	6.75	La-Ln-O, La-L-O, Mo-P-L, M-P-L, M-Po-O, M-P-O, M-P-S, P-P-P
Σ	55.28	63.98	57.69	53.70	53.32	56.38	

Medium variability

TGA	A	B	C	D	E	F	
C28	0.54	0.86	0.98	0.66	0.65	0.80	Cy-Cy-La
C30	1.67	2.31	2.54	1.72	1.65	1.87	Cy-C-La
C32	2.80	3.66	4.22	2.82	2.72	4.30	C-C-La
C34	3.79	4.72	5.16	3.65	3.44	4.07	Cy-Cy-Ln, C-C-M, Cy-C-P, C-La-La
C36	5.16	6.32	6.58	4.89	4.52	5.19	Cy-Cl-Ln, Cy-C-Ln, Cy-C-O, Cy-La-P
C50	10.41	6.02	7.10	9.80	8.76	8.92	M-Ln-Ln, Po-P-Ln, P-P-Ln, P-P-L, Po-P-O, P-P-O, P-P-S
C52	14.19	9.80	10.28	15.20	16.22	12.17	P-Ln-Ln, P-Ln-L, Po-Ln-O, P-L-L, P-Ln-O, Po-L-O, P-L-O, Po-O-O, P-O-O-, P-O-S
Σ	38.56	33.69	36.86	38.74	38.01	37.32	

Higher variability

TGA	A	B	C	D	E	F	
C22	tr.	tr.	tr.	tr.	tr.	tr.	
C24	0.74	0.56	0.70	0.47	0.49	1.19	Bu-Bu-P
C26	0.13	0.25	0.24	0.32	0.21	0.20	Cy-Cy-C
C54	4.99	1.45	4.36	6.50	7.59	4.23	Ln-Ln-L, Ln-L-L, Ln-Ln-O, Ln-L-O, L-L-O, Ln-O-O, L-O-O, O-O-O, O-O-S, P-O-Ga, O-S-S
C56	0.15	0.04	0.13	0.23	0.37	n.d.	P-O-Er
Σ	6.01	2.30	5.43	7.52	8.66	5.62	

Table 4. Fatty acid symbols used in Table 3.

Symbol	Notation	Fatty acid
Bu	C4:0	Butyric acid
Co	C6:0	Capronic acid
Cy	C8:0	Caprylic acid
Cl	C10:1	Decenoic acid
C	C10:0	Capric acid
La	C12:0	Lauric acid
Mo	C14:1	Myristoleic acid
M	C14:0	Myristic acid
Po	C16:1	Palmitoleic acid
P	C16:0	Palmitic acid
Ln	C18:3	Linolenic acid
L	C18:2	Linolei acid
O	C18:1	Oleic acid
S	C18:0	Stearic acid
Me	C20:3	Eicosatrienoic acid
Ga	C20:1	Eicosaenoic acid
Er	C22:2	Docosaenoic acid

4. CONCLUSIONS

The determination of the amount of TAG, free fatty acids, and phospholipids in milk from various species has been the objective of many studies (CLAEYS *et al.,* 2014; GANTNER *et al.,* 2015; JENSEN *et al.,* 1990) and the distribution of fatty acids in triglycerides and the glycerol backbone affects the assimilation and consequently the biochemical and the nutritional value (EMKEN *et al.,* 2004; FILER *et al.,* 1969; GASTALDI *et al.,* 2010). Therefore, the influence of the TAG composition is confirmed.

From an analytical point of view, a simple method based on the identification of TAG, expressed as CN, is very useful. The GC method described in this paper for the study of TAG content, expressed as CN, is optimised for the quantitative characterisation of TAG profiles and is useful for industrial quality control of donkey milk. Also, the GC-OCI profiles reported in this paper demonstrated there is a clear differentiation between different species of milk and the TAG pattern may be considered a fundamental characteristic of milk from all species. In this paper, the TAG fraction of donkey milk from the Martina Franca breed was compared with human, cow, sheep, and goat milk samples, and the proposed GC-OCI method produced good reproducible results and permitted an easy characterisation and comparison.

Concerning the TAG composition of donkey milk, previous studies used different methods but not always included the TAG composition. Therefore, this paper gives a contribute to define the extremes of natural variability for donkey milk. We determined the variability of TAG composition, expressed as CN, in lyophilised donkey milk using our optimised method and we detected three degrees of variability for the different TAG classes.

ACKNOWLEDGEMENTS

The Authors thanks Eurolactis Group SA for providing the donkey milk of its production.

REFERENCES

Barłowska J., Szwajkowska M., Litwińczuk Z. and Król J. 2011. Nutritional value and technological suitability of milk from various animal species used for dairy production. Compr. Rev. Food Sci. F. 10:291-302.

Blasi F., D. Montesano, De Angelis M., Maurizi A., Ventura F., Cossignani L., Simonetti M.S. and Damiani P. 2008. Results of stereospecific analysis of triacylglycerol fraction from donkey, cow, ewe, goat and buffalo milk. J. Food Compos. Anal. 21:1-7.

Bononi M., Fossati A., Lubian E., Tateo F. and Fasan S. 2001. Caratterizzazione di trigliceridi di sintesi con funzione di solvente/supporto in aroma burro. Riv. Ital. Sostanze G. 78:465-469.

Breckenridge W.C. and Kuksis A. 1967. Molecular weight distributions of milk fat triglycerides from seven species. J. Lipid Res., 8:473- 478.

Chianese L., Calabrese M.G., Ferranti P., Mauriello R., Garro G., De Simone C., Quarto M., Addeo F., Cosenza G. and Ramunno L. 2010. Proteomic characterization of donkey milk "caseome". J. Chromatogr. A 1217:4834-4840.

Chiofalo B., Dugo P., Bonaccorsi I.L. and Mondello L. 2011. Comparison of major lipid components in human and donkey milk: new perspectives for a hypoallergenic diet in humans. Immunopharm. Immunot. 33:633-644.

Claeys W.L., Verraes C., Cardoen S., De Block J., Huyghebaert A., Raes K., Dewettinch K. and Herman L. 2014. Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. Food Control 42:188-201.

Commission Regulation (EC) No 213/2001 of 9 January 2001 laying down detailed rules for the application of Council Regulation (EC) No 1225/1999 as regards methods for the analysis and quality evaluation of milk and milk products and amending Regulation (EC) No 2771/1999 and (EC) No 2799/1999. Official Journal of the European Communities L 37/1-L 37/99.

Cossignani L., Blasi F., Bosi A., D'Arco G., Maurelli S., Simonetti M.S. and Damiani P. 2011. Detection of cow milk in donkey milk by chemometric procedures on triacylglycerol stereospecific analysis results. J. Dairy Sci. 78: 335-342.

do Nascimento Rangel A.H., Bezerra Galvão J.G., Alves Simplício A., Bezerri Freire R.M. and Patto Novaes L. 2015. Aspectos composicionais e nutricionais do leite de jumenta: uma revisao. Rev. Inst. Laticinios Cândido Tostes, Juiz de Fora 70:160-171.

Dugo P., Kumm T., Lo Presti M., Chiofalo B., Salimei E., Fazio A., Cotroneo A. and Mondello L. 2005. Determination of triacylglycerols in donkey milk by using high performance liquid chromatography couplet with atmospheric pressure chemical ionization mass spectrometry. J. Sep. Sci. 28:1023-1030.

Emken E.A., Adlof R.O., Duval S.M., Shane J.M., Walker P.M. and Becker C. 2004. Effect of triacylglycerol structure on absorption and metabolism of isotope-labeled palmitic and linoleic acids by humans. Lipids 39:1-9.

Filer L.J., Mattson F.H. and Fomon S.J. 1969. Triglycerides configuration and fat absorption by the human infants. J. Nutrition 99:293-298.

Gantner V., Mijić P., Baban M., Škrtić Z. and Turalija A. 2015. The overall and fat composition of milk of various species. Mljekarstvo 65:223-231.

Gastaldi D., Bertino E., Monti G., Baro C., Fabris C., Lezo A., Medana C., Baiocchi C., Mussap M., Galvano F. and Conti A. 2010. Donkey's milk detailed lipid composition. Front. Biosci. E2: 537-546.

Guo H.Y., Pang K., Zhang X.Y., Zhao L., Chen S.W., Dong M.L. and Ren F.Z. 2007. Composition, physiochemical properties, nitrogen fraction distribution, and amino acid profile of donkey milk. Int. Dairy J. 90:1635-1643.

Høst A. and Halken S. 2004. Hypoallergenic formulas – when, to, whom and how long: after more than 15 years we know the right indication! Allergy 59:45-52.

Jensen R.G., Ferris A., Lammi-Keefe C. and Henderson R. 1990. Lipids of bovine and human milks: a comparison. J. Dairy Sci. 73:223-240.

Jensen R.G. 1999. Lipids in Human Milk. Lipids, 34:1243-1271.

Lara-Villoslada F., Olivares M. and Xaus J. 2005. The balance between caseins and whey proteins in cow's milk determines its allergenicity. J. Dairy Sci. 88:1654-1660.

Lozada L., De La Fuente M.H., Fontecha J. and Juarez M. 1995. Consideration of the quantitative aspect of the determination of milkfat triglycerides with split PTV and on-column injection. J. High Res. Chromatog. 18:771-775.

Malissiova E., Arsenos G., Papademas P., Fletouris D., Manoursa A., Aspri M., Nikolopoulou A., Giannopoulou A. and Arvanitoyannis I.S. 2016. Assessment of donkey milk chemical, microbiological and sensory attributes in Greece and Cyprus. Int. J. Dairy Technol. 69:143-146.

Marconi E. and Panfili G. 1998. Chemical composition and nutritional properties of commercial products of mare milk powder. J. Food Compos. Anal. 11:178-187.

Martemucci G. and D'Alessandro A.G.. 2012. Fat content, energy value and fatty acid profile of donkey milk during lactation and implications for human nutrition. Lipids Health Dis. 11:113-126.

Martini M., Altomonte I. and Salari F. 2014. Amiata donkeys: fat globule characteristics, milk gross composition and fatty acids. Ital. J. Anim. Sci. 13:123126.

Molketin J. and Precht D. 1994. Comparison of packed and capillary columns for quantitative gas chromatography of triglycerides in milk fat. Chromatographia 39:265-270.

Molketin J. and Precht D. 1995. Development of a precise capillary GC method for rapid triglyceride analysis of milk fats. Fat Sci. Technol. 97:43-49.

Monti G., Bertino E., Muratore M.C., Coscia A., Cresi F., Silvestro L., Fabris C., Fortunato D., Giuffrida M.G. and Conti A. 2007. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: An *in vivo* and *in vitro* study. Pediatr. Allergy Immu. 18:258-264.

Nordic Committee on Food Analysis. 1997. Estimation and expression of measurement uncertainty in chemical analysis. NMKL Procedure No.5.

Polidori P. and Vincenzetti S. 2013. Use of donkey milk in children with cow's milk protein allergy. Foods 2:151-159.

Restani P., Ballabio C., Di Lorenzo C., Tripodi S. and Fiocchi A. 2009. Molecular aspects of milk allergens and their role in clinical events. Anal. Bioanal. Chem. 395:47-56.

Salimei E., Fantuz F., Coppola R., Chiofalo B., Polidori P. and Varisco G. 2004. Composition and Characteristics of ass's milk. Anim. Res. 53:67-78.

Salimei E. 2011. Animals that Produce Dairy Foods - Donkey. Encyclopaedia of Dairy Sciences (Second Edition), 365-373.

Salimei E and Fantuz F. 2012. Equid milk for human consumption. Int. Dairy J. 24:130-142.

Summer A., Sabbioni A., Formaggioni P. and Mariani P. 2004. Trend in ash and mineral element content of milk from Haflinger nursing mares throughout six lactation months. Livest. Prod. Sci. 88: 55-62.

Tateo F. and Bononi M. 2002. Guida all'analisi chimica degli alimenti – Latte e derivati. Ars Edizioni Informatiche.

Tateo F. and Bononi M. 2003. The repeatability test in GC analysis of triglycerides. Ind. Aliment. 42:500-504.

Tateo F. and Bononi M. 2004. Guida all'analisi chimica degli alimenti – Oli e grassi vegetali. Ars Edizioni Informatiche

Zou X., Huang J., Jin Q., Guo Z., Liu Y., Cheong L., Xu X. and Wang X. 2013. Lipid composition analysis of milk fats from different mammalian species: potential for use as human milk fat substitutes. J. Agr. Food Chem. 61:7070-7080.

Paper Received October 15, 2016 Accepted November 29, 2016

PAPER

CHANGES IN TEXTURE ANALYSIS PARAMETERS OF WINE GRAPE BERRIES AT TWO RIPENESS STAGES: A STUDY ON VARIETAL EFFECT

L. BRILLANTE[a,b], F. GAIOTTI[a], L. LOVAT[a], S. GIACOSA[c], S. RÍO SEGADE[c], S. VINCENZI[a], F. TORCHIO[c], L. ROLLE[c]* and D. TOMASI[a]

[a]CREA-VIT Council for Agricultural Research and Economics, Viticulture Research Center, Conegliano, TV, Italy

[b]University of California Davis, Department of Viticulture and Enology, Davis, CA, USA

[c]University of Turin, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Grugliasco, TO, Italy

[d]University of Padova, Centro Interdipartimentale per la Ricerca in Viticoltura ed Enologia, Legnaro, PD, Italy

[e]Università Cattolica del Sacro Cuore, Istituto di Enologia e Ingegneria Agro-Alimentare, Piacenza, Italy

*Corresponding author. Tel.: +39 0116708558; fax: +390116708749

E-mail address: luca.rolle@unito.it

ABSTRACT

Ripening of grapes is associated with great modifications at both the chemical and physical level. The aim of this work was to describe the changes in physical-mechanical parameters associated to ripening of wine grape berries, as evaluated by texture analysis, in order to understand if these modifications are stable across cultivars, or they are cultivar-specific. Berries from 21 different cultivars were sorted by flotation in different saline solutions, separated in two ripening stages differentiated by the amount of sugars (183 and 217 g L^{-1}) and then analysed. Multivariate and univariate variations in texture analysis parameters were found, which were not constant across the studied grapevine varieties. However, a general behaviour was observed for skin weight, which had the largest variation between the two ripening stages. Other parameters showed significant differences between the ripening stages: skin thickness, berry gumminess, chewiness, and springiness, but the variation was not common to all cultivars. The work therefore evidenced the existence of cultivar-specific differences in the behaviour of physical-mechanical parameters between ripening stages.

Keywords: berry ripening, physical-mechanical properties, texture analysis, skin weight, wine grapes, *Vitis vinifera* L.

1. INTRODUCTION

Ripening of grape berries is a complex process, which happens according to a double sigmoid growth curve composed of three different phases (COOMBE, 1992). During the first period of growth, cell number per berry increases because of mitosis divisions, while cell expansion is limited. Generally, at the end of this period the grapevine reaches the phenological stage of bunch closure. This first growth period is followed by a lag phase during which enlargement slows and the seed develops. The phenological stage at the transition between this second and the third final phase is called *véraison*, which corresponds to the onset of ripening, when berries start to soften and change in colours because of anthocyanin synthesis (in red/black grapes). In the third stage, cells enlarge as a result of solutes (principally glucose and fructose) and water accumulation, and berries approximately double in size (CONDE *et al.*, 2007). This last step is crucial because important changes in secondary metabolites occur. These compounds are responsible for flavour, aroma, colour and mouth feel of grapes as well as wines. Modifications of pectins during this stage cause the progressive loss of firmness in ripe berries (NUNAN, 1998; NUNAN *et al.*, 2001). Such modifications are principally due to an increase in the enzymatic activity of pectin methylesterase, α-galactosidase and β-galactosidase, which has been registered after *véraison* (NUNAN *et al.*, 2001; DEYTIEUX-BELLAU *et al.*, 2008; ORTEGA-REGULES *et al.*, 2008). However, only in recent years, scientific studies have begun to instrumentally measure these visual and tactile changes, as summarized by ROLLE *et al.* (2012).

The structure and composition of skin cell walls directly impact textural characteristics and have been linked to phenol extractability (ORTEGA-REGULES *et al.*, 2006; BINDON *et al.*, 2012; HERNÁNDEZ-HIERRO *et al.*, 2014). Several studies have shown that the mechanical properties of whole berry and berry skin are significantly related to anthocyanin and flavanol extractability (ROLLE *et al.*, 2008; RÍO SEGADE *et al.*, 2011a, RÍO SEGADE *et al.*, 2011b). These studies are based on the use of Texture Analysis (TA) test, which is an effective instrumental texture analysis test for a quantitative evaluation of physical-mechanical characteristics of grape berries (LETAIEF *et al.*, 2008). The technique is rapid and cost-effective since it does not require long times for sample preparation and analysis.

However, literature describing the changes in physical-mechanical parameters according to different berry ripening stages is yet scarce and focuses on a very limited number of winegrape varieties (MAURY *et al.*, 2009; ZOUID *et al.*, 2010; RÍO SEGADE *et al.*, 2011c; ROLLE *et al.*, 2011a).

This study evaluates, on a heterogeneous dataset from 21 different grapevine cultivars, which texture analysis (TA) parameters change between two different sugar contents (i.e. stages of ripening). In particular, the aims of the work were *i*) to study if physical-mechanical parameters, as assessed by TA, can significantly vary between the two sampled ripening groups, *ii*) to evaluate if TA measurements allow to discriminate and classify the two ripening classes, therefore assessing the validity of the methods to describe variations in physical-chemical properties with ripening, and *iii*) to evaluate differences in physical-mechanical modifications with ripening across cultivars.

2. MATERIALS AND METHODS

2.1. Plant material and grape sampling

Vitis vinifera L. grapes from 21 red grapevine varieties were sampled in the CREA-VIT experimental collection (1.2 ha) located in Susegana (TV, Veneto, North-East Italy), in

2011. Sampled cultivars were Ancellotta, Barbera, Bonarda, Cabernet-Franc, Cannonau, Corvina, Croatina, Franconia, Gamay, Malbech, Malvasia Nera di Lecce, Marzemino, Merlot, Montepulciano, Negramaro, Pinot Noir, Primitivo, Raboso, Refosco, Schiava Gentile, and Teroldego. Vines were 15 years old, grafted on SO4 rootstock (interspecific cross between *Vitis riparia* Michx. and *Vitis berlandieri* Planch.), and planted at 3.0 m between rows and 1.5 m between vines. They were Sylvoz pruned and trained with a vertical shoot position system. For each cultivar, samples were composed of about 3 kg of grape berries, which were picked up randomly from ten vines.
The berries were sampled at two ripening stages (time lag of two weeks) of difference, and sorted using a densimetric method by berry flotation in different saline solutions (ROLLE *et al.*, 2011a). The two selected groups, called A (early harvest) and B (full ripeness), had respectively 183±8 and 217±8 g L^{-1} of sugars corresponding about to 11.0 ± 0.5% and 13.0 ± 0.5% potential alcohol content by volume, respectively.
The sorted berries were visually inspected before analysis; those with damaged skins were discarded. For each variety studied, a sub-sample of 36 sorted berries (therefore a total of 756 berries for all cultivars together) was randomly selected for the determination of the physical-mechanical properties.

2.2. Physical-mechanical measurements

Grape berries were singularly weighed (g, BW parameter), with an analytical laboratory balance (Radwag AS 220/X, Radwag, Radom, Poland), and a Texture Profile Analysis (TPA) non-destructive mechanical test was then performed for each of them as described by LETAIEF *et al.* (2008). Analyses were made using a Universal Testing Machine (UTM) TAxT2i texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 5 kg load cell and a HDP/90 platform. A SMS P/35 flat cylindrical probe was used, and the test was carried out on each berry in the equatorial position under 25% deformation, with a waiting period of 2 s between the two compressions and a test speed of 1 mm s^{-1}. All force-deformation curves were acquired at 400 Hz and evaluated using the Texture Expert Exceed software package (Stable Micro Systems). TPA parameters calculated were berry hardness (N, as H), cohesiveness (adimensional, as Co), gumminess (N, as G), springiness (mm, as S), chewiness (mJ, as Ch) and resilience (adimensional, as R). After the TPA test, each berry was manually peeled with a razor blade, and skin weight (g, as SW) and skin thickness (μm, as Sp_{sk}) were singularly measured. For the latter test, the same UTM texture analyzer was used, equipped with a SMS P/2 flat cylindrical probe, and setting a compression test at 0.2 mm s^{-1} test speed (BATTISTA *et al.*, 2015).

2.3. Statistical analysis

Analysis of covariance (ANCOVA) was performed to evaluate the univariate differences in each physical-mechanical parameter between the two ripening classes, treating BW as a nuisance factor. The TA data were also normalised by BW as described in SANTINI *et al.* (2011), and the resulting data were analysed by one-way ANOVA. Robust multivariate analysis of variance (robust MANOVA) was used to investigate multivariate differences for physical-mechanical parameters between the ripening groups.
Multiple logistic regression was used to understand if texture analysis (TA, including TPA and Sp_{sk}) measurements can differentiate the two ripening classes, and results were interpreted to assess if relationships between mechanical parameters and ripening classification are stable across cultivars. The choice of the TA parameters in the model (independent variables) was based on an exhaustive search in order to minimise the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC). Finally,

a cross-validation procedure was used to choose between the minimal AIC and BIC proposed models.
The statistical analysis presented in this work was performed in R v.3.2.0 (R CORE TEAM, 2015). Robust multivariate analysis of variance was performed using the rrcov package (TODOROV and FILZMOSER, 2009), and best subset logistic regression with the 'bestglm' package(McLEOD and XU, 2014).

3. RESULTS

3.1. Differences in physical-mechanical parameters between ripeness levels

To observe differences in physical-mechanical parameters with ripening, the first step was to exclude possible differences in BW between classes because this parameter is correlated to other berry physical-mechanical parameters. As an example, lighter berries have lower S and Ch values than heavier ones (r = 0.88 between BW and S, r = 0.44 between BW and Ch), but also lower skin weight (SW) (r = 0.76). It would be logical to assume that the ripe A group (early harvest) could have different BW than the ripe B group (full ripeness), for some reason such as sugar accumulation, water accumulation or loss, etc. This hypothesis is rejected by an ANOVA test, which excludes a significant difference in BW means between the two groups (p-value = 0.19). The same results were obtained when performing the ANOVA test individually by cultivars: the difference in BW was not significant (p-value > 0.05) between ripe A and B groups for each cultivar sample in the experiment. Analysis was performed by taking into account the multiplicity problem by using the Bonferroni correction.
However, in order to increase the robustness of the statistical analysis, BW was treated as a nuisance parameter and then an ANCOVA test was performed to adjust, for any treatment, group differences in BW before assessing the impact of the ripening stage. The inclusion of BW in the model permits to exclude the effect of berry weight on the observed differences in physical-mechanical parameters.
The test was first performed including the interaction between BW and ripening class in order to assess if the assumption of slope equality is respected, which is necessary to the analysis. Because the interaction term was never significant, the assumption was respected and the interaction term excluded. This also means that the relation between BW and all tested mechanical parameters did not depend on the level of ripening.
In all cultivars pooled, the only significant difference between ripe A and B berries for all studied physical-mechanical parameters were found in SW (p-value < 5^{-04}). The difference remained significant even after a Bonferroni correction accounting for the increase in risk because of the multiple comparisons (p-value < 0.01 after correction).
While BW was not different between the two ripening stages considered, a difference in SW exists and berries in the B group had skins 20 mg (approx. 10% over the mean) heavier than those in the A group. The ANCOVA analysis for SW is presented in Fig. 1.
The analysis was also performed by normalising each TA parameter by BW, as already made in SANTINI *et al.* (2011). However, even when normalising data by BW, these results were confirmed. When TA parameters were analysed singularly in a univariate way, differences between the classes were too little to be significantly noticed, with the exception of SW. Nevertheless, considering the variations in all TA parameters as a whole in a multivariate way (robust MANOVA), significant differences were found in the texture parameters between the two ripening groups, even when SW was removed from the dataset (Wilks' λ = 0.82, p-value < 1^{-05}).

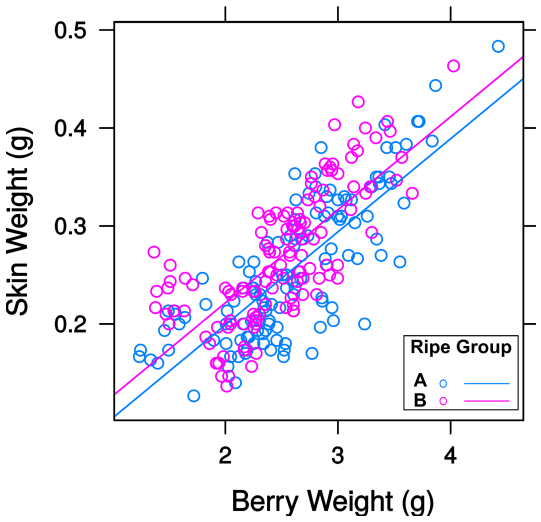


Figure 1. Results of the ANCOVA with equation SW = BW + Mat. The analysis shows differences in SW between the two ripening stage, while controlling for the nuisance effect of BW.

3.2. Classifying berry ripening classes using TA parameters

Because the two ripening classes were significantly different in the berry texture characteristics, the analysis was extended to evaluate if TA parameters were able to discriminate ripe A and B berries. The reason of this analysis was to evaluate the performances of the classification across cultivars, and therefore to know if, at this late stage of ripening, different cultivars show similar changes in TA parameters, or not. If cultivars show similar changes, the performance of the classification should be equal for all cultivars; conversely, if changes in TA parameters are cultivar-specific, the performance should vary across cultivars, with cultivars having greater changes being easier to classify than cultivars with little changes. This was evaluated, in a straightforward and single step approach, using a multiple logistic regression, where the response was the ripening class (which was binary) and the predictors were the physical-mechanical parameters. Because the number of TA parameters was large, feature selection was performed to identify the most informative independent variables. Two models were selected, the first minimised the BIC and just included SW and S as independent variables. Both SW and S parameters were significant in the model (p-value < 0.01). This regression model correctly classified 58% of the observations. The second model minimised the AIC, and contained as variables the SW, G, Ch, and Sp_{sk} (p-value < 0.01 for G, Ch and SW, p-value < 0.1 for Sp_{sk}); the model correctly classified 62% of the observations. It is worth noting that S is correlated to G and Ch (r = 0.61 and 0.38, respectively, p-value < 0.05). This can be a reason why S is excluded in this second model when G and Ch are included. The two models were then compared using cross-validation, which suggested the selection of the second model (AIC minimised). However, the significant effect of S in the model can also be taken into account for a future discussion. The final logistic regression predicting the probability that an observed sample X belongs to the B group has equation 1:

$$\hat{p}(X) = \frac{e^{-2.448-0.008Sp_{sk}+13.205SW-4.243G-1.606Ch}}{1+e^{-2.448-0.008Sp_{sk}+13.205SW-4.243G-1.606Ch}} \quad (1)$$

Where p(X) is the probability of X, *e* is the natural logarithm base, Sp_{sk} is skin thickness, SW is skin weight, G is gumminess, Ch is chewiness.
Table 1 shows coefficient estimate, standard error and *p*-value for the regressions minimising either the AIC or the BIC. Errors do not appear equally spread across all cultivars in the experiment.

Table 1. Coefficients of the logistic regression classifying berries in two ripening classes according to their physical-mechanical characteristics.

	Logistic Regression Minimising AIC (equation 1)		
	Estimate	**Std. Error**	**p-value**
Intercept	-2.448	0.948	9.8e-03
Sp_{sk}	-0.008	0.004	5.5e-02
SW	13.205	3.293	6.1e-05
G	-4.243	1.031	3.9e-05
Ch	-1.606	0.375	1.9e-05
	Logistic Regression Minimising BIC		
Intercept	3.113	1.277	1.5e-02
S	7.820	2.405	1.1e-03
SW	-2.135	0.652	1.1e-03

Sp_{sk} Skin Thickness; SW: Skin Weight; G: Gumminess; Ch: Chewiness; S: Springiness; SW: Skin Weight.

Cultivar-specific errors in the classification are presented in Table 2 (12 samples of three berries for cultivar). In eight of the 21 cultivars (Cannonau-Grenache, Croatina, Franconia, Malbech, Malvasia Nera Di Lecce, Marzemino, Merlot, and Raboso), the classification is not higher than that attended by chance.

Table 2. Percentage of correctly classified samples for the logistic regression in equation 1 across cultivars.

Cultivars	**Correctly classified samples (%)**	**Cultivars**	**Correctly classified samples (%)**
Merlot	50	Cabernet-Franc	67
Raboso	50	Corvina	67
Cannonau	50	Gamay	67
Malvasia Nera di Lecce	50	Montepulciano	67
Croatina	50	Pinot Noir	67
Franconia	50	Ancellotta	75
Marzemino	50	Refosco	75
Malbech	50	Negramaro	75
Bonarda	58	Barbera	83
Schiava Gentile	58	Primitivo	92
Teroldego	58		

Therefore, in these cultivars at this stage of ripening, the changes in physical-mechanical parameters do not consistently vary and do not allow to differentiate between the two classes. In all other cultivars, the results of the classification are sensibly better than chance. Very good results were reached for Primitivo, Barbera, Ancellotta, Negramaro, and Refosco (correct classification equal to 92%, 83%, and 75% for the last three cultivars, respectively). In this last group of cultivars, changes in physical-mechanical properties continue even in a late period of ripening.
Differences in the variation percentage between the two ripening stages are summarised in Fig. 2. The well classified cultivars show higher variation between the two stages than the bad classified cultivars; for bad classified cultivars, variation is close to zero excepting for SW. This suggests that the changes of physical-mechanical properties in the last ripening stages are cultivar dependent.

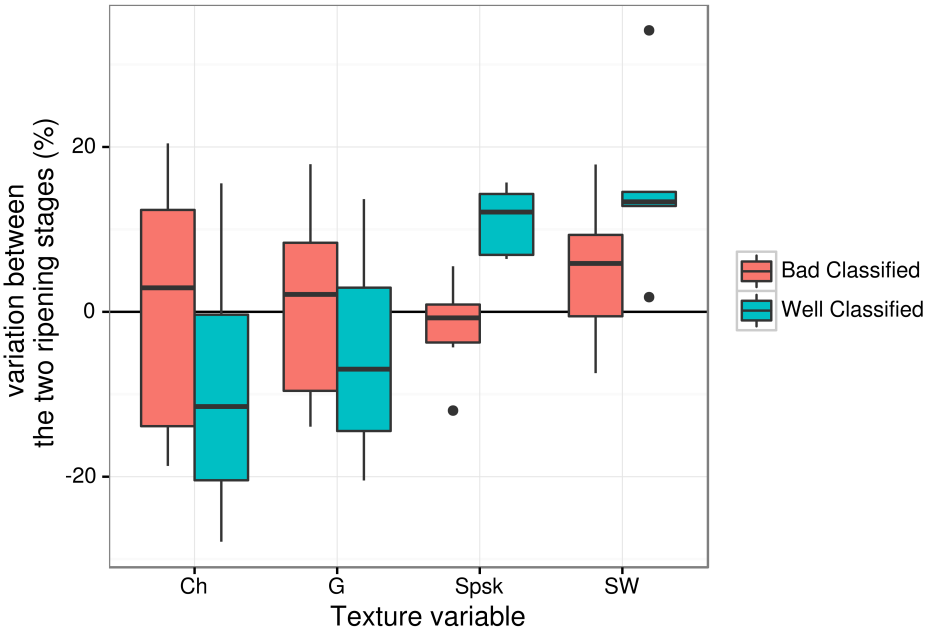


Figure 2. Variation percentage between the two ripening stages for TA parameters and SW.

4. DISCUSSION

The production of high-quality red wines requires the assessment of grape phenolic ripening indexes through the determination of the content of phenolic compounds and of their extractability during winemaking (RÍO SEGADE *et al.*, 2008). Texture analysis has been already used to develop rapid methods for the evaluation of total phenolic content and phenol extractability in grape seeds (ROLLE *et al.*, 2012), and of anthocyanin extractability in grape skins with a good accuracy (ROLLE *et al.*, 2008; RÍO SEGADE *et al.*, 2011a; RÍO SEGADE *et al.*, 2011b). The scientific literature is scarce on the description of changes in physical-mechanical parameters, instrumentally measured by TA, in the late stages of ripening, when variation in sugar content is not huge. In fact, several studies suggested that a steady value is achieved close to ripeness for some mechanical parameters, which could limit their choice as ripeness indicators in grape berries (MAURY *et al.*, 2009). In the present work, this observation is confirmed for a group of the cultivars studied: Cannonau-Grenache, Croatina, Franconia, Malbech, Malvasia Nera Di Lecce,

Marzemino, Merlot, and Raboso. Nevertheless, the observation is not confirmed for other cultivars in this study (Primitivo, Barbera, Ancellotta, Negramaro, and Refosco), which can be easily classified because physical-mechanical properties still change in the late ripening. The reason of this variation can have a genetic origin, and it deserves to be further investigated in future studies. A variety effect was already found in the relationship between flavonoid content and TA parameters (BRILLANTE *et al.*, 2015a; BRILLANTE *et al.*, 2015b). Variability in physical-mechanical parameters across cultivars can also be related to the climatic conditions of the ripening period, as shown in ROLLE et al., 2011b. That study showed that while the differences between cultivars for some texture parameters are, at least qualitatively, stable across vintages (an example is F_{sk}), others can also be affected by the climate (an example is W_{sk}).
This work carefully treats the BW effect on physical-mechanical parameters. When the BW effect is excluded from the analysis, results for other physical-mechanical characteristics are more reliable. In details, the study evidences an increase in SW with ripening. Although some authors showed that the percentage of skin cell wall material decreases during ripening (HERNÁNDEZ-HIERRO *et al.*, 2014) probably due to the cell walls become thinner (ORTEGA-REGULES *et al.*, 2006), others reported that cell wall material slightly but continuously increases as ripening progresses before decreasing (VICENS *et al.*, 2009). This increase in SW, even if modest in absolute terms, can become significant when considered as a ratio of the SW with BW, and is equal to 10%. This is a huge variation, especially if we consider that the accumulation of sugars in berries between the two ripening classes (approx. 34 g) accounts for just the 3% in average of BW.
Among the mechanical parameters measured by TA, and therefore excluding SW, whole berry characteristics, such as S, G, and Ch, were better related to berry ripeness than skin properties. Among all tested skin-related properties, Sp_{sk} was the only one showing a little effect. Since the texture properties of the whole berry depend on different characteristics, such as cell wall composition, cell structure and pulp turgescence (GOULAO and OLIVEIRA, 2008), and fruit softening occurring during ripening (Nunan 1998), it is not surprising to find larger evidence in the modification of the mechanical properties of the whole berry, as also reported in ZOUID *et al.* (2013).
In future, it could be interesting to couple TA analysis to the determination of pectins in grape berries. This will probably allow to better understand the direct relations between the physiological activity in grape berries during ripening, molecular structure and the macroscopic modifications of texture.

5. CONCLUSIONS

This work highlights that changes in physical-mechanical properties of grape berries are cultivar-specific in the final ripening stages. However, among all tested physical-mechanical parameters, a general behaviour was shown by skin weight. This parameter showed larger variation with ripening than the others considered. The observed increase in SW is particularly evident once considered its ratio over berry weight, which is equal to 10%. Proportionally, between early harvested and full ripe berries, berry weight changed more because of increased skin weight than because of sugar accumulation. Differences in sugar content between the two ripening classes accounted for just 3% in average of BW.

REFERENCES

Battista F., Tomasi D., Porro D., Caicci F., Giacosa S. and Rolle L. 2015. Winegrape berry skin thickness determination: comparison between histological observation and texture analysis determination. Ital. J. Food Sci. 27:136-141.

Bindon K.A., Bacic A. and Kennedy J.A. 2012. Tissue-specific and developmental modifications of grape cell walls influence the adsorption of proanthocyanidins. J. Agric. Food Chem. 60:9249-9260.

Brillante L., Gaiotti F., Lovat L., Vincenzi S., Giacosa S., Torchio F., Río Segade S., Rolle L. and Tomasi D. 2015a. Investigating the use of gradient boosting machine, random forest and their ensemble to predict skin flavonoid content from berry physical-mechanical characteristics in wine grapes. Comput. Electron. Agr. 117:186-193.

Brillante L.; Tomasi D., Gaiotti F., Giacosa S., Torchio F., Río Segade S., Siret R., Zouid I. and Rolle L. 2015b. Relationships between skin flavonoid content and berry physical-mechanical properties in four red wine grape cultivars (*Vitis vinifera* L.). Sci. Hort. 197:272-279.

Conde C., Silva P., Fontes N., Dias A.C.P., Tavares R.M., Sousa M.J., Agasse A., Delrot S. and Gerós H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food. 1, 1–22. Global Science Books Ltd, UK.

Coombe B.G. 1992. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43:101-110.

Deytieux-Belleau C., Vallet A., Donèche B. and Geny L. 2008. Pectin methylesterase and polygalacturonase in the developing grape skin. Plant Physiol. Biochem. 46:638-646.

Goulao L.F. and Oliveira C.M. 2008. Cell wall modifications during fruit ripening: When a fruit is not the fruit. Trends Food Sci. Technol. 19:4-25.

Hernández-Hierro J.M., Quijada-Morín N., Martínez-Lapuente L., Guadalupe Z., Ayestarán B., Rivas-Gonzalo J.C. and Escribano-Bailón M.T. 2014. Relationship between skin cell wall composition and anthocyanin extractability of Vitis vinifera L. cv. Tempranillo at different grape ripeness degree. Food Chem. 146:41-47.

Letaief H., Rolle L. and Gerbi V. 2008. Mechanical behavior of wine grapes under compression tests. Am. J. Enol. Vitic. 59:323-329.

Maury C., Madieta E., Le Moigne M., Mehinagic E., Siret R. and Jourjon F. 2009. Development of a mechanical texture test to evaluate the ripening process of Cabernet Franc grapes. J. Texture Stud. 40:511-535.

McLeod A.I. and Xu C. 2014. bestglm: best subset GLM. R package version 0.34. Available at: http://CRAN.R-project.org/package=bestglm

Nunan K.J. Changes in cell wall composition during ripening of grape berries. 1998. Plant Physiol. 118:783-792.

Nunan K.J., Davies C., Robinson S.P. and Fincher G.B. 2001. Expression patterns of cell wall-modifying enzymes during grape berry development. Planta. 214:257-264.

Ortega-Regules A., Romero-Cascales I., Ros-García J.M., López-Roca J.M. and Gómez-Plaza E. 2006. A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. Anal. Chim. Acta. 563:26–32.

Ortega-Regules A,; Ros-García J.M., Bautista-Ortín A.B., López-Roca J.M. and Gómez-Plaza, E. 2008. Differences in morphology and composition of skin and pulp cell walls from grapes (*Vitis vinifera* L.): technological implications. Eur. Food Res. Technol. 227:223–231.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

Río Segade S., Rolle L., Gerbi V. and Orriols I. 2008. Phenolic ripeness assessment of grape skin by texture analysis. J. Food Comp. Anal. 21:644-649.

Río Segade S., Soto Vázquez E., Orriols I., Giacosa S. and Rolle L. 2011a. Possible use of texture characteristics of winegrapes as markers for zoning and their relationship with anthocyanin extractability index. Int. J. Food Sci. Tech. 46:386-394.

Río Segade S.; Giacosa S.; Gerbi V. and Rolle L. 2011b. Berry skin thickness as main texture parameter to predict anthocyanin extractability in winegrapes. LWT-Food Sci. Technol. 44:392-398.

Río Segade S., Orriols I.; Giacosa S. and Rolle L. 2011c. Instrumental texture analysis parameters as winegrapes varietal markers and ripeness predictors. Int. J. Food Prop. 14:1318-1329.

Rolle L., Torchio F., Zeppa G. and Gerbi V. 2008. Anthocyanin extractability assessment of grape skins by texture analysis. J. Int. Sci. Vigne Vin, 42(3):157-162.

Rolle L., Río Segade S., Torchio F., Giacosa S., Cagnasso E., Marengo F. and Gerbi V. 2011a. Influence of grape density and harvest date on changes in phenolic composition, phenol extractability indices, and instrumental texture properties during ripening. J. Agric. Food Chem. 59:8796-8805.

Rolle L., Gerbi V., Schneider A., Spanna F., and Río Segade S. 2011b. Varietal relationship between instrumental skin hardness and climate for grapevines (*Vitis vinifera* L.). J. Agric. Food Chem. 2011, 59:10624-10634

Rolle L., Siret R., Río Segade S., Maury C., Gerbi V. and Jourjon F. 2012. Instrumental texture analysis parameters as markers of table-grapes and winegrape quality: a review. Am. J. Enol. Vitic. 63:11-28.

Santini D., Rolle L., Cascio P. and Mannini F. 2011. Modifications in chemical, physical and mechanical properties of Nebbiolo (*Vitis vinifera* L.) grape berries induced by mixed virus infection. S. Afr. J. Enol. Vitic. 32:183-189.

Todorov V. and Filzmoser P. 2009. An object-oriented framework for robust multivariate analysis. J. Stat. Softw. 32(3):1-47.

Vicens A., Fournand D., Williams P., Sidhoum L., Moutounet M. and Doco T. 2009. Changes in polysaccharide and protein composition of cell walls in grape berry skin (Cv. Shiraz) during ripening and over-ripening. J. Agric. Food Chem. 57:2955-2960.

Zouid I., Siret R., Mehinagic E., Maury C., Chevalier M. and Jourjon F. 2010. Evolution of grape berries during ripening: Investigations into the links between their mechanical properties and the extractability of their skin anthocyanins. J. Int. Sci. Vigne Vin. 44:87-99.

Zouid I., Siret R., Jourjon F., Mehinagic E. and Rolle L. 2013. Impact of grapes heterogeneity according to sugar level on both physical and mechanical berries properties and their anthocyanins extractability at harvest. J. Texture Stud. 44:95-103.

Paper Received September 25, 2016 Accepted November 3, 2016

PAPER

EFFECT OF LACTIC ACID AND BIOACTIVE COMPONENT MIXTURES ON THE QUALITY OF MINCED PORK MEAT

A. ROKAITYTE*[1], G. ZABORSKIENE[1,2], V. BALIUKONIENE[1], I. MACIONIENE[2] and A. STIMBIRYS[1]

[1]Lithuanian University of Health Sciences, Veterinary Academy, Department of Food Safety and Quality, Tilzes st. 18, LT-47181, Kaunas, Lithuania

[2]Food Institute, Kaunas University of Technology, Radvilenu st. 19, Kaunas LT-51180, Lithuania

*Corresponding author: anita.rokaityte@lsmuni.lt

ABSTRACT

The objective of this study was to investigate the effects of mixtures of lactic acid (LA), thymol (TH), linalool (LN) and dihydroquercetin (DHQ) on the quality of minced pork meat during 7 days of storage at +4 °C temperature. DHQ+LA+LN, DHQ+LA and LA exhibited the greatest antibacterial effect on the agar well diffusion assay and resulted in the best sensory evaluation. Samples treated with DHQ+LA had a statistically significant effect on the total bacterial count and showed the best antibacterial effect on the *E. coli* count. However, the reducing effect on the total amount of biogenic amines was not significant in all cases of treatment.

Keywords: dihydroquercetin, lactic acid, linalool, minced meat

1. INTRODUCTION

The production of safe and high quality meat and meat products along with recent consumer demand for all-natural and clean-label products is challenging. A significant level of meat product spoilage takes place every year at different levels of the production chain including preparation, storage, and distribution (DINESH and JAYASENA, 2013).
Many synthetic additives have been used over the years for preserving fresh meat and meat products and to extend the period of refrigerated storage (SOMOLINOS *et al*., 2010). Synthetic additives have been accused of having some carcinogenic and toxic properties. However, untreated products and natural foods may be more susceptible to the growth of food-borne pathogens than conventional food versions (JAY *et al*., 2005). The most important food-borne pathogenic bacteria that have survived and grow in these products include *Escherichia coli, Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus* spp. (OROOJALIAN *et al*., 2010). These bacteria cause a great proportion of food-borne outbreaks in different foods (WARRINER and NAMVAR, 2009). In this context, natural alternatives are attracting interest as food preservatives in order to ensure the safety of food (MARIUTTI *et al*., 2011).
One of the traditional ways of controlling microbial growth in these products, thus improving safety and delaying spoilage, is the addition of essential oils (EOs) (DINESH and JAYASENA, 2013). The composition and structure, as well as the functional groups of the oils, play an important role in determining their antimicrobial activity. Usually, compounds with phenolic groups are the most effective. EOs may be applied as part of a hurdle system to achieve preservative action (MASTROMATTEO *et al*., 2009). As an example, lower levels of EOs can be combined with existing and novel preservation technologies including low temperature and acidity, modified atmosphere packaging (MAP), high hydrostatic pressure, preservatives (e.g., nitrite, nisin, etc.) and low-dose irradiation. A series of preservative hurdles is established by these combined processes, which in turn improves the microbial stability and sensory quality of meat and meat products (AL-REZA, 2010; SKANDAMIS and NYCHAS, 2001; ZHOU *et al*., 2010). Although good antimicrobial activities were observed for many EOs, some limitations have also been identified in the application of EOs in meat and meat products. The interaction of some EOs with food ingredients and structure may decrease their effectiveness (SKANDAMIS and NYCHAS, 2000). Additionally, the markedly reduced activity of EOs may result in food systems such as meat and meat products when compared to *in vitro* results. This may be attributed to the presence of fats, carbohydrates, proteins, and salts in such systems. It is difficult to maintain consistent quality because the composition of an individual EO can vary due to several factors including the time of harvesting, variety, the part of the plant used, and method of extraction (HYLDGAARD *et al*., 2012). In addition, HYLDGAARD *et al*. (2012) reported that the antimicrobial potency of EO constituents depends on pH, temperature (RATTANACHAIKUNSOPON and PHUMKHACHORN, 2010), and the level of microbial contamination (SOMOLINOS *et al*., 2010). Further, the use of EOs as preservatives in food has been limited, as they are required in high concentrations in order to achieve the sufficient antimicrobial activity (HYLDGAARD *et al*., 2012). Lower concentrations of EOs can be combined with other antimicrobial compounds and/or other preservative technologies to obtain a synergistic effect without compromising antimicrobial activities (NGUEFACK *et al*., 2012).
Weak organic acids are one of the several primary agents used to control microorganisms in both fermented and nonfermented foods (BUCHANAN *et al*., 2002). For example, lactic acid, citric acid and acetic acid are either naturally produced or added to food or marinades to achieve food safety and meet quality requirements (MANI-LÓPEZ *et al*., 2012). Lactic acid has shown antimicrobial activities against many pathogenic organisms

because of it is abilities to reduce pH level, exert feedback inhibition and interfere with proton transfer across cell membranes (DAVIDSON *et al*., 2005).
Dihydroquercetin (also known as taxifolin) is a member of a group of flavonoids (VLADIMIROV et al., 2009). Satisfactorily pure dihydroquercetin may be extracted from Siberian larch *(Larix sibirica Ledeb).* It is also found in the açaí palm, in milk thistle seeds and in small quantities in red onion. Dihydroquercetin has a positive effect on human health, as it prevents the accumulation of free radicals (TROUILLAS *et al*., 2004), influences the physical properties of lipids in biological membranes, ameliorates cerebral ischemia-reperfusion injury (WANG *et al*., 2006) and activates the formation of collagen fibres (TARAHOVSKY *et al*., 2007). The application of dihydroquercetin is quite widely distributed in the production of different categories of products. In general, dihydroquercetin can be used as a natural antioxidant and antimicrobial activities additive in the food industry (WANG *et al*., 2011).
The objective of the study was to investigate the antimicrobial effects of bioactive components (lactic acid, linalool, dihydroquercetin) used in combination on microorganisms mostly found in pork minced meat and on the sensory properties and formation of biogenic amines.

2. MATERIALS AND METHODS

2.1. Preparation of bioactive component solutions for the antibacterial properties analyses

Powdered concentrate of DHQ (99.4%), extracted from Siberian larch *(Larix sibirica Ledeb)* and produced by the company Flavit Ltd, Pushtino (Russia) was used. DHQ was diluted into 35 °C distilled water to make 10 mL of 0.024% (w/v) DHQ aqueous solution.
LA (50.0%), TH (99.5%) and LN (97.0%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and kept at 4 °C. All of the solutions (each of them 10 mL) were made on the day of the research: 0.5% (w/v) LA aqueous solution, 0.003% (w/v) TH aqueous solution and 0.003% (w/v) LN aqueous solution.

2.2. Antimicrobial assay of bioactive components

The agar well diffusion method was used to determine the antimicrobial activity of LA (0.5%) and bioactive components (LN 0.03%, TH 0.03% and DHQ 0.024%). Reference strain cultures of conditionally pathogenic *Esherichia coli* ATCC 25922 and pathogenic bacteria such as *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 13076, *Bacillus cereus* ATCC 11778, and *Listeria monocytogenes* ATCC 19111 were used in this experiment. Bacteria cultures were kept in a "Viabank" (*Medical Wire & Equipment,* JK) system at minus 72 °C. During the tests, the examined cultures were pre-cultivated on the plate count agar (PCA, *Liofilchem*, Italy) slants for 18-24 h under the optimal temperature (30-37 °C). After cultivation, the bacterial culture was washed with a sterile physiological solution and cell suspensions were prepared according to the procedure of McFarland No 0.5 (approx. 1.5×10^8 cfu/mL). One millilitre of bacterial cell suspension was added to 100 mL of the melted PCA cooled to 45 °C. The prepared mixture of bacteria cell suspension and the medium was mixed and 10 mL was poured into each of 90 mm Petri dish. Fifty microlitres of the tested bioactive components was poured into wells of 8 mm diameter made in the hardened agar. The antimicrobial activity was assessed following 24 h of incubation at 30 ºC or 37 ºC by measuring the diameter of the inhibition zone around the wells (mm). The

strains were considered as exhibiting no antimicrobial activity if clear zones around the wells were not revealed.

2.3. Meat samples

Meat of pork carcasses from 1-year-old pig, after 48 h since postmortem were purchased from a local establishment in Kedainiai, Lithuania. The meat was trimmed of all exterior fat and connective tissue. The samples were transported to the laboratory while being kept at 4 °C and minced with a sterilized meat mincer to 3 mm in size. The minced meat samples were divided into 4 groups (4x0.5 kg) considering different treatments with bioactive substances. The minced meat samples were weighed and packed using a Multivac R230 model 542 packaging machine (Multivac, Wolfertschwenden, Germany). The samples were packaged under atmospheric air without the use of any gas composition. The samples were stored in the dark under refrigeration conditions (+ 4 °C) for 7 days. The samples were named as follows: (I) DHQ (0.024%) + LA (0.5%) + LN (0.003%), (II) DHQ (0.024%) + LA (0.5%), (III) untreated control group. Analyses of microorganisms, pH and biogenic amines were carried out on the 1st, 3rd, 5th and 7th day of storage. The whole experiment was replicated three times and the results are displayed as the mean values.

2.4. Microbial analysis

2.4.1 Detection of total aerobic bacterial count

Samples of 10 g were taken at random for each sample and aseptically weighed into a sterile stomacher bag with 90 mL of sterile 0.1% (w/v) Buffered peptone water (REF 611014, Liofilchem, Italy) and homogenized for 1 min in a model 400 Stomacher (Seward Medical, London, UK). 1.0 mL was seeded onto Plate count agar (REF 610040, Liofilchem, Italy) and incubated at 30 ºC for 72 hours.

2.4.2 Detection of *Escherichia coli*

Samples of 10 g were homogenized with 90 mL of sterile Buffered peptone water 0.1% (w/v). 0.1 mL of solution was plated using the pour plate method on Tryptone Bile X-Glucuronide Medium agar (REF 4021562, Biolife, Italy) and incubated at 37 ºC for 24 hours.

2.4.3 Detection of Salmonella

Samples of 25 g were homogenized with 225 mL of Buffered peptone water 0.1% (w/v) and incubated at 37 °C for 24 h. After incubation, 1.0 mL of the pre-enrichment culture was transferred into 9.0 mL of tetrathionate broth and incubated at 42 °C for 24 h. The enrichment culture was streaked onto XLT4 (Difco) agar plates and incubated for 24 h at 37 °C. Presumptive *Salmonella* colonies were confirmed by using API 20E (bioMérieux 20100). Agglutination tests were done with *Salmonella* polyvalent O and H antisera (Mast Diagnostics, UK).

2.4.4 Detection of Listeria monocytogenes

Samples of 25 g were homogenized with 225 mL of *Listeria* enrichment broth (Merck), incubated at 30 °C for 24 h. After incubation, 0.1 mL was plated in duplicate on PALCAM

Selective Listeria Agar (Merck). The presence of *L. monocytogenes* was determined after incubation of the plates at 37 °C for 24 h. Up to five colonies were selected for serological and biochemical confirmation using a *Listeria* latex test (Oxoid) and an API assay (BioMérieux sa, Marcy I' Etoile, France), respectively.

2.4.5 Detection of *Staphylococcus aureus*

Samples of 25 g were homogenized with 225 mL of buffered peptone water and seeded onto Baird-Parker RPF agar (Biolife, Milan, Italy) and incubated aerobically at 35 °C for 24 and 48 h.
Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g).

2.5. Determination of biogenic amines

A reversed-phase high-performance liquid chromatography method was used for the quantitative analysis of the biogenic amines – tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine. Biogenic amines were extracted from a homogenized sample with 0.4 mol/l perchloric acid. The derivatization of samples was carried out using the modificated methodology of Ben-Gigirey *et al*. (2000). The extract was derivatised for 45 min by a dansyl chloride (5-dimethylaminonaphtalene-1-sulfonylchloride) solution in acetone at 40 °C. The samples were filtered through a 0.45 μm membrane filter (Millipore Co., Bedford, MA, USA) and 10 μl was injected into a chromatographic system (Aligent 1200 Series, Germany). An analysis was performed using a LiChro column CART® 95 125-4. Carrier phase – eluents: B – acetonitrile, A – ammonium acetate 0.1mol/l. The analysis lasted 28 min, changing the content of eluents during the first 19 min from 50% of B to 90% of B (from 50% of A to 10% of A respectively), then leaving the content constant for 1 min – 90% of B (10% 99 of A); later, to ensure the isolation of materials for another analysis, eluent with a composition of 50% of B and 50% of A was added to the chamber for 8 minutes. A flow rate of 0.9 mL/min was maintained during analysis, with the column set at 40°C. UV detection was observed at 254 nm. Biogenic amines were identified by comparing the retention time of each amine in the chamber with the retention time of the respective reference material. The internal standard method of calculating the peak area for the defined amount of reference material was used to perform quantitative analysis. The limit of detection is between 0.02-0.1 μg/mL for different biogenic amines.

2.6. pH value

The pH of all samples was measured according to the standard method for determination of meat pH: LST ISO 2917:2002. The average pH of the sample was determined. pH measurements were carried out using a PP-15 pH-meter (Sartorius Professional meter for pH Measurement, Germany).

2.7. Acceptability evaluation

A ten-member panel was used to evaluate sensory taste, odour, and the overall acceptability attributes of the minced pork meat treated with LA and bioactive components. Before evaluation, the treated minced pork samples were wrapped in aluminium foil individually and cooked in a steam-cooker (MultiGourmet FS20, Braun, Germany) for 30 min. Each sample was served warm in dishes coded with 3-digit random

numbers and presented in individual booths to each panellist for evaluation. The panellists were required to rinse with water before tasting each sample. A 9-point hedonic scale was used to score the sensory attributes, where 1=dislike extremely, 9=like extremely, while the limit of acceptability was 5=neither like nor dislike. The sensory evaluation was accomplished at 2 day intervals up to the end of refrigerated storage at +4 °C.

2.8. Statistical analysis of the data

Data were statistically analysed using SPSS 20.0 software (SPSS Inc., Chicago, Illinois, USA). Differences between dates were evaluated by the analysis of variance method (one-way ANOVA) with a significant level of $p \leq 0.05$ (DRAPER and SMITH, 1998). Multiple comparisons were estimated by the Fishers Least Significant Difference method and the Dunnett's test was applied when the control group was present. The Student's t-test was used to determine the average values of indicators, standard deviations (SD) and linear correlations. The correlation was considered reliable when $p<0.05$.

3. RESULTS AND DISCUSSION

The antimicrobial activity of the bioactive components was determined against common food-borne pathogenic bacteria (*Escherichia coli, Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus* spp.). The evaluation of antibacterial activity was done using the agar well diffusion method. The results suggest that the bioactive components exhibited different antimicrobial activity. LA (0.5%) and it is mixtures with other bioactive components (LN 0.03%, TH 0.03% and DHQ 0.024%) had an antimicrobial effect against all tested bacteria. The resulting inhibition zone diameters ranged from 10.5±0.2 mm to 21.5±0.2 mm. A significantly higher inhibitory effect on the *E. coli* reference strain was reported with the DHQ+LA mixture (the inhibition zone diameter was 14.3±0.2 mm) ($p \leq 0.05$). On the *S. aureus* reference strain a significantly higher inhibitory effect was reported with the LA solution (the inhibition zone diameter was 15.8±0.2) ($p \leq 0.05$). The DHQ+LA+LN mixture showed a higher antimicrobial activity on the *S. typhimurium* reference strain compared to the other bioactive components (the inhibition zone diameter was 13.0±0.0 mm) ($p \geq 0.05$). The LN and TH solution did not affect all the microbes examined except *B. cereus* (the inhibition zone diameter was 9.0±0.0 mm) ($p \geq 0.05$). Moreover, the DHQ solution (0.024%) did not inhibit the growth of all the bacteria tested (Table 1).

The acceptability evaluation scores of the minced pork meat treated with bioactive components during the 3 days of refrigerated storage at +4 °C are shown in Fig. 1. The odour of the minced pork meat treated with bioactive components DHQ+LA+LN, DHQ+LA and LN, assessed by the panellists, was significantly higher ($p \leq 0.05$) compared to the control. The taste and overall acceptability of the minced pork meat treated with DHQ+LA+LN were scored significantly higher ($p \leq 0.05$) than the control. The scores of taste, odour and the overall acceptability of the minced pork meat treated with DHQ+LA+TH as compared to the control were significantly lower ($p \leq 0.05$). Therefore, further studies have been carried out with DHQ+LA+TH, TH, LN and DHQ because these bioactive components have lower acceptability scores for minced pork meat and antimicrobial activity using the agar well diffusion method.

The bioactive components that exhibited the greatest antibacterial effect in the agar well diffusion assay and had the best acceptability evaluation (DHQ+LA+LN, DHQ+LA and LA) were further tested for their microbiological and chemical attributes.

Significant differences were observed between the pH values of the control meat and all treated samples after 5 and 7 days of storage ($p \leq 0.05$). Besides, there were significant differences in pH among LA and DHQ+LA+LN, DHQ+LA treated samples after 5 days of storage ($p \leq 0.05$) (Fig. 2).

Table 1. Antimicrobial activity of bioactive components against the reference strains.

Target Microorganisms	Zone of inhibition, mm Bioactive components						
	LA	**LN**	**TH**	**DHQ**	**DHQ+LA+LN**	**DHQ+LA+TH**	**DHQ+LA**
E. coli ATCC 25922	12.5±0.1	0.0±0.0	0.0±0.0	0.0±0.0	12.5±0.0	12.8±0.2	14.3±0.2*
S. aureus ATCC 25923	15.8±0.2*	0.0±0.0	0.0±0.0	0.0±0.0	15.5±0.1	10.5±0.2	12.5±0.2
S. typhimurium ATCC 13076	12.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	13.0±0.0	12.0±0.1	12.5±0.2
L. monocytogenes ATCC 19111	20.5±0.1	0.0±0.0	0.0±0.0	0.0±0.0	20.5±0.2	19.3±0.2	21.0±0.1
B. cereus ATCC 11778	15.5±0.1	9.00±0.0	9.0±0.0	0.0±0.0	15.5±0.1	15.5±0.1	21.5±0.2

* The mean difference is significant at the 0.05 level.
LA – lactic acid 0.5%; LN – linalool 0.03%; TH – thymol 0.03%; DHQ – dihydroquercetin 0.024%

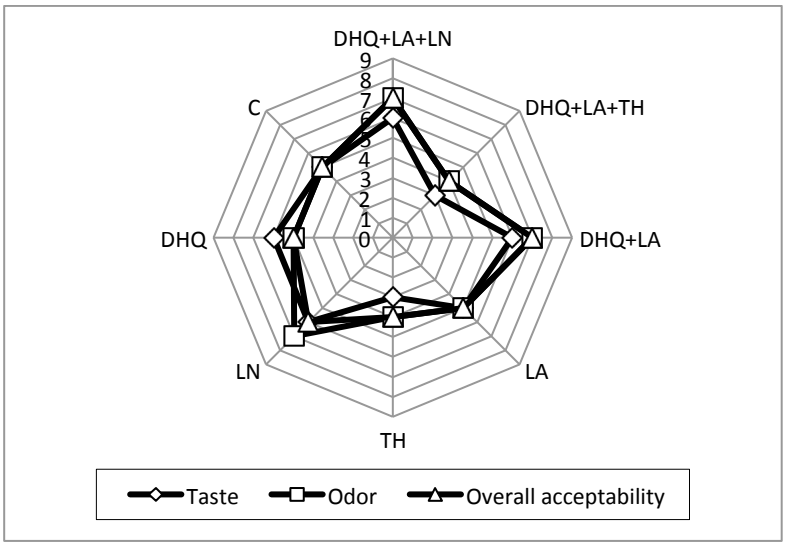


Figure 1. Acceptability evaluation of minced pork meat treated with bioactive components during 3 days of storage at +4 °C.

The results presented in this study showed the effect of DHQ and LA on the total aerobic bacterial count (Fig. 3). All combinations had effects on the total aerobic bacterial count compared to the control sample after 7 days of storage ($p \leq 0.05$).

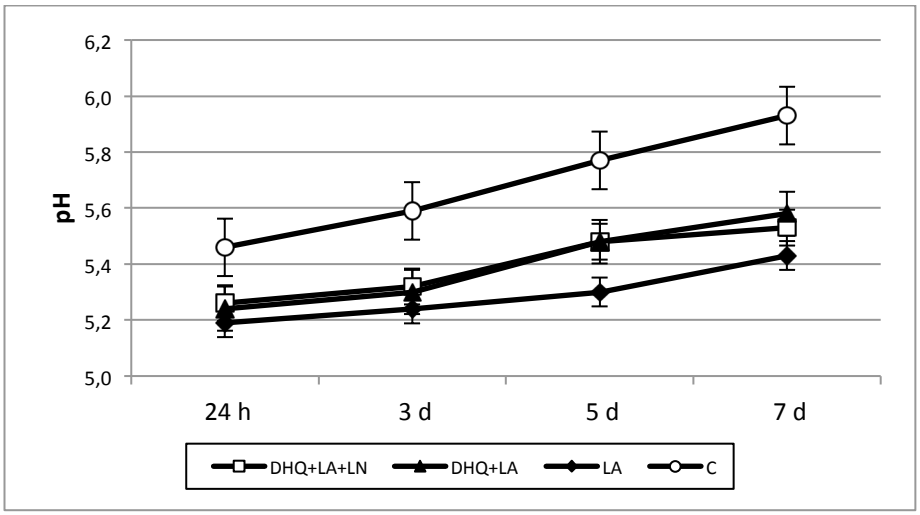


Figure 2. Variation of the mean pH values of minced pork meat during 7 days of storage at +4 °C.

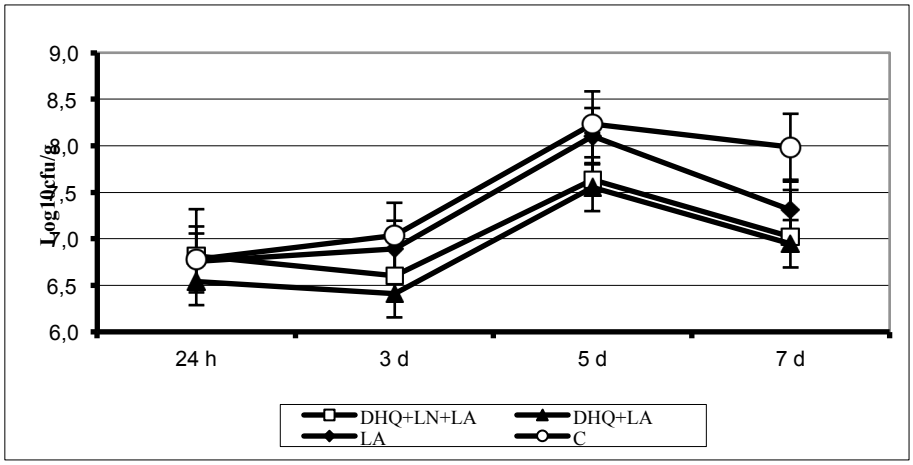


Figure 3. Variation of the total aerobic bacterial count in minced pork meat during 7 days of storage at +4 °C.

Salmonella spp., *L. monocytogenes* and *St. aureus* were not detected in any of the minced pork meat samples during the 7 days period. Therefore, we could not assess the antibacterial effect of the mixtures.

LA, used in a mixture with LN and DHQ, statistically significantly reduced the *E. coli* count. In addition, the mixture of LA and DHQ was distinguished by a strong bactericidal activity against *E. coli*. DIMITRIEVIĆ *et al.* (2007) noted that the anti-listerial effect of essential oils (*Thymus vulgaris* and *Rosmarinus officinalis*) was noticeably increased using it with LA. The same synergistic effect was reported by NAVEENA *et al*. (2006), who found that the combination of *Syzygium aromaticum* essential oil and LA provided a decrease in the psychrotrophic and coliform counts of buffalo meat.

Studies on the antibacterial mechanism of the phenolic compounds found in essential oils focused on their effects on the cellular membrane, changing it is structure and permeability. LIN *et al*. (2004) state that the damage to the cell membrane might explain the observed effects, since phenolics could cause sublethal injury to cell membranes, causing disruption of the proton motive force due to loss of H^+-ATPase. This could make bacteria more susceptible to an acidic environment. Moreover, at low pH, the

hydrophobicity of an essential oil increases, enabling it to more easily dissolve in the lipids of the cell membranes of target bacteria.

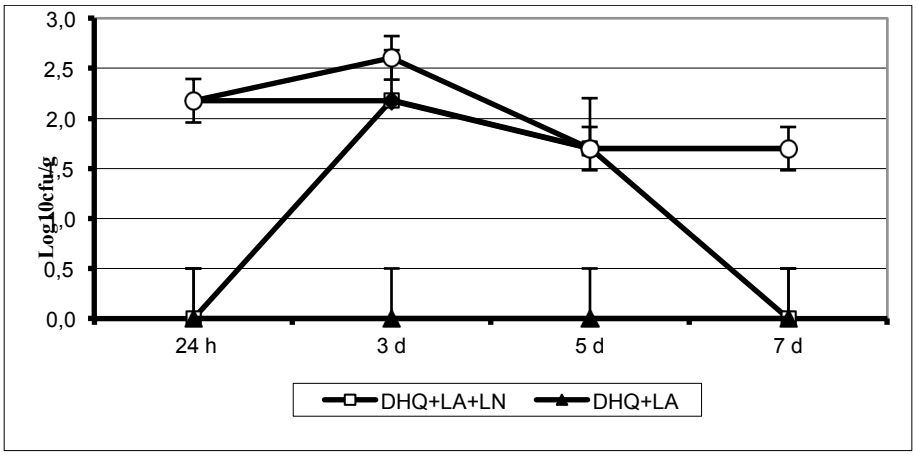


Figure 4. Variation of the *E. coli* count in minced pork meat during 7 days of storage at +4 °C.

The large correlation between the pH and total bacterial count was observed in samples treated with DHQ+LA+LN (R=0.648, p≤0.05) and DHQ+LA (R=0.692, p≤0.05) during the 7 day period (Table 2).

Table 2. Correlation coefficients R between the pH and average values of biogenic amines, *E. coli* counts and the total bacterial count during 7 days storage at +4 °C.

Samples		Correlation coefficients Parameters			
		pH	**TBC**	***E. coli***	**TBA**
DHQ+LA+LN	pH	1.00	0.648*	-0.070	-0.178
	TBC[1]	0.648*	1.00	0.078	-0.230
	E. coli	-0.070	0.078	1.00	0.149
	TBA[2]	-0.178	-0.230	0.149	1.00
DHQ+LA	pH	1.00	0.692*	-0.497*	-0.457
	TBC	0.692*	1.00	-0.474	-0.282
	E. coli	-0.497*	-0.474	1.00	0.484
	TBA	-0.457	-0.282	0.484	1.00
LA	pH	1.00	0.578	-0.033	-0.539*
	TBC	0.578	1.00	-0.539	-0.374
	E. coli	-0.033	-0.539	1.00	0.364
	TBA	-0.539*	-0.374	0.364	1.00
Control	pH	1.00	-0.348	-0.136	-0.326
	TBC	-0.348	1.00	-0.440	-0.336
	E. coli	-0.136	-0.440	1.00	-0.397
	TBA	-0.326	-0.336	-0.397	1.00

* The mean difference is significant at the 0.05 level.
1TBC - Total bacterial count; 2TBA - Total amount of biogenic amines.
LA – lactic acid 0.5%; LN – linalool 0.03%; TH – thymol 0.03%; DHQ – dihydroquercetin 0.024%

A medium negative correlation between the pH and *E. coli* count was found in samples treated with DHQ+LA (R=-0.497, p≤0.05). However, we did not find correlations between the biogenic amine contents and total bacterial count. The capability to form biogenic amines is generally considered a strain specific characteristic rather than a species property. It is thus difficult to find precise correlations between the biogenic amine contents and total bacterial count (STANDAROVÁ *et al*., 2008).
The formation of biogenic amines was intensive during the first 3 days of storage (Table 3).

Table 3. Variation of the biogenic amines (mg/kg) in minced pork meat during 7 days of storage at +4 °C.

Biogenic amines	Samples	Days of storage			
		24 h	3 d	5 d	7 d
Tryptamine	DHQ+LA+LN	14.39±0.86	13.71±2.41	6.47±0.52	3.47±0.39
	DHQ+LA	17.17±0.57	10.04±1.97	5.53±0.18	1.88±0.43
	LA	18.68±1.25	10.47±1.03	3.83±0.24	1.64±0.16
	Control	14.16±0.91	11.90±2.36	5.90±0.47	2.73±0.38
Phenylethylamine	DHQ+LA+LN	63.61±5.22	205.93±4.68	57.46±1.20	17.68±1.87
	DHQ+LA	57.85±4.72	148.36±7.42	6.23±0.73*	2.94±0.61*
	LA	78.27±2.18	159.28±8.13	38.42±1.54	16.23±1.13
	Control	113.65±4.56	168.64±5.61	37.38±0.93	16.66±1.58
Putrescine	DHQ+LA+LN	11.64±1.39	34.58±3.64	45.78±1.58	65.57±2.34
	DHQ+LA	16.52±1.94	29.43±2.18	38.54±1.12	43.18±3.50
	LA	17.60±2.40	23.09±3.05	35.67±1.39	44.61±1.92
	Control	16.52±1.28	27.89±4.24	36.85±0.91	42.10±2.48
Cadaverine	DHQ+LA+LN	23.05±3.72	40.92±3.29	51.99±4.38	77.71±4.66
	DHQ+LA	19.21±2.15	32.24±2.65	45.52±2.57	51.56±2.51
	LA	22.45±1.39	39.86±3.07	48.63±3.11	60.34±3.68
	Control	34.76±3.60	44.45±2.91	46.06±2.98	48.78±3.04
Histamine	DHQ+LA+LN	23.54±1.49	18.17±1.38	6.61±0.76	2.47±0.54
	DHQ+LA	21.88±2.08	16.49±1.60	4.18±0.35	1.82±0.21
	LA	19.84±1.67	14.33±0.92	3.07±0.64	1.26±0.35
	Control	24.32±0.68	13.01±1.77	5.65±0.51	1.87±0.72
Tyramine	DHQ+LA+LN	47.55±1.24	55.81±7.38	93.54±3.44	132.43±5.79
	DHQ+LA	52.48±0.92	67.05±5.06	87.48±2.76	115.97±4.17
	LA	34.37±2.48	41.88±8.17	84.07±2.95	145.41±6.82
	Control	38.64±1.61	49.35±6.98	66.65±1.73	108.24±3.48
Spermidine	DHQ+LA+LN	38.71±4.37	22.30±1.26	12.54±1.65	10.09±0.24
	DHQ+LA	48.96±1. 31	35.79±2.71	10.85±1.89	7.26±0.84
	LA	32.58±3.64	28.14±1.93	9.68±0.71	9.61±0.37
	Control	48.76±2.52	38.80±2.09	12.91±1.38	7.48±0.21
Spermine	DHQ+LA+LN	41.16±1.81	40.49±1.57	21.00±0.94	7.71±0.97
	DHQ+LA	54.03±3.77	48.06±1.30	18.04±2.48	5.43±0.60
	LA	51.38±2.26	42.18±2.61	17.68±1.57	5.89±2.34
	Control	48.19±3.49	48.67±1.55	18.85±1.90	3.97±0.91
Total biogenic amines	DHQ+LA+LN	263.65±6.58	431.91±5.46	295.39±4.82	317.13±5.07
	DHQ+LA	288.10±4.61	387.46±7.14	216.37±5.66	230.04±4.60
	LA	275.17±5.14	359.23±6.62	241.05±4.92	284.99±4.75
	Control	339.00±4.62	402.71±5.57	230.25±3.24	231.83±5.68

* The mean difference is significant at the 0.05 level
LA – lactic acid 0.5%; LN – linalool 0.03%; TH – thymol 0.03%; DHQ – dihydroquercetin 0.024%

The total amount of biogenic amines decreased from the 3rd to 5th days of the experiment in all cases of treatments. A significant lower amounts of phenylethylamine was found between DHQ+LA and all cases of samples between 5 and 7 days of storage ($p \leq 0.05$). After 5 days of storage, the control meat sample was fully unacceptable and the degration of amines began, with a noticeable smell of ammonia. In the cases of the other treatments, the BA increased after 5 days of storage.
Moreover, in a lower pH environment, bacteria are strongly encouraged to produce the amino acid decarboxylase as a part of their defence mechanism against acidity (KAROVIČOVÁ and KOHAJDOVÁ, 2005; TEODOROVIC *et al*., 1994). Therefore, the acidic conditions caused here by the addition of organic acids to minced meat may have not only reduced but also encouraged the production of BA. Besides this, the same raw material can lead to different amine levels depending on the presence of decarboxylating microorganisms, either derived from environmental contamination and the conditions supporting their growth and activity (STADNIK and DOLATOWSKI, 2010). The type and amount of BA detected in the minced pork meat was far below the level that can cause a health risk during the 7 days of storage.

4. CONCLUSIONS

The mixture of 0.024% DHQ and 0.5% LA solutions exhibited the greatest antibacterial effect on minced pork meat. A significant negative correlation between the pH and *E. coli* count was only found in samples treated with DHQ+LA ($R=-0.497$, $p \leq 0.05$). However, the DHQ+LA and DHQ+LA+LN mixtures could be used by the food industry as a natural barrier to control the growth of pathogens and natural spoilage microflora, while reducing the formation of biogenic amines in minced pork, thus providing a balance between sensory acceptability and antimicrobial efficacy. Our results encourage further research, focused on the application of LA and bioactive component mixtures in low doses to control the growth of the microorganisms mostly found in selected foods, particularly meat products.

REFERENCES

Al-Reza S.M., Rahman A., Lee J., & Kang S.C. 2010. Potential roles of essential oil and organic extracts of *Zizyphus jujuba* in inhibiting food-borne pathogens. Food Chem. 119: 981-986.

Ben-Gigirey B., Vieites Baptista de Sousa J., Villa T., Barros-Velázquez J. 2000. Characterization of biogenic amine-producing *Stenotrophomonas maltophilia* strains isolated from white muscle of fresh and frozen albacore tuna. Int. J. Food Microbiol. 57:19-31.

Buchanan R.L., Whiting R.C., Golden M.H. 2002. Modeling acid inactivation of foodborne microorganisms. In: Juneja, V.K., Sofos, J.N. (Eds.), Control of Foodborne Microorganisms. Marcel Dekker. 23:461-478.

Davidson P.M., Sofos J.N. and Branen A.L. 2005. Antimicrobials in Food, 3rd Ed., CRC Press, Boca Raton, FL. 3:460-469.

Dinesh D., Jayasena A. 2013. Essential oils as potential antimicrobial agents in meats and meat products. A review. Food Sci. Technol. 34:96-108.

Draper N. and Smith H. 1998. Applied Regression Analysis (3rd ed.). New York: Wiley.

Hyldgaard M., Mygind T., & Meyer R. L. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3:1-24.

Jay J.M., Loessner M.J. and Golden D.A. 2005. Modern Food Microbiology, Springer Science+Business Media (7th ed.). New York, USA.

Karovičová J., Kohajdová Z. 2005. Biogenic amines in food. Chemistry Paper. 59:70-79.

Lin Y.T., Labbe R.G., Shetty K. 2004. Inhibition of *Listeria monocytogenes* in fish and meat system by use of oregano and cranberry phytochemical synergies. Appl. Environ. Microbiol. 70:5672-5678.

Mah J.H. and Hwang H.J. 2009. Effects of food additives on biogenic amine formation in Myeolchi-jeot, a salted and fermented anchovy (*Engraulis japonicus*). Food Chem. 114:168-73.

Mani-López E., García H.S., López-Malo A. 2012. Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. Food Res. Int. 45:713-721.

Mariutti L.R.B., Nogueira G.C., & Bragagnolo N. 2011. Lipid and cholesterol oxidation in chicken meat are inhibited by sage but not by garlic. J. Food Sci. 76: 909-915.

Mastromatteo, M., Lucera, A., Sinigaglia, M., & Corbo, M.R. 2009. Combined effects of thymol, carvacrol and temperature on the quality of non conventional poultry patties. Meat Sci. 83:246-254.

Naveena B.M., Muthukumar M., Sen A.R., Babji Y., Murthy T.R.K. 2006. Improvement of shelf-life of buffalo meat using lactic acid, cove oil and vitamin C during retail display. Meat Sci. 74:409-415.

Nguefack J., Tamgue O., Dongmo J.B.L., Dakole C.D., Leth V., Vismer H.F., Zollo P.H.A., Nkengfack A.E. 2012. Synergistic action between fractions of essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against Penicillium expansum. Food Control. 23:377-383.

Oroojalian F., Kasra-Kermanshahi R., Azizi M., and Bassami M. R. 2010. Phytochemical composition of the essential oils from three *Apiaceae* species and their antibacterial effects on food-borne pathogens. Food Chemistry. 120:765-770.

Rattanachaikunsopon P., & Phumkhachorn P. 2010. Assessment of factors influencing antimicrobial activity of carvacrol and cymene against Vibrio cholerae in food. J. Biosci. Bioeng. 110:614-619.

Skandamis P.N., & Nychas G.J. 2001. Effect of oregano essential oil on microbiological and physicochemical attributes of minced meat stored in air and modified atmospheres. J. Appl. Microbiol. 91:1011-1022.

Skandamis P. N., & Nychas G.J. 2000. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs, and oregano essential oil concentrations. Appl. Environ. Microbiol. 66:1646-1653.

Solomakos N., Govaris A., Koidis P., & Botsoglou N. 2008. The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157:H7 in minced beef during refrigerated storage. Meat Sci. 80:159-166.

Somolinos M., Garcia D., Condon S., Mackey B., & Pagan R. 2010. Inactivation of *Escherichia coli* by citral. Appl. Environ. Microbiol. 108:1928-1939.

Stadnik J. and Dolatowski Z. J. 2010. Biogenic amines in meat and fermented meat products. Acta Sci. Pol., Technol. Aliment. 9:251-263.

Standarová E., Borkovcová I., Vorlová L. 2008. The occurrence of biogenic amines in dairy products on the Czech market. Act. Scient Pol. Med. Vet. **7**:35-42.

Tarahovsky Y.S., Selezneva I.I., Vasilieva N.A., Egorochkin M.A., Kim Y.A. 2007. Acceleration of fibril formation and thermal stabilization of collagen fibrils in the presence of taxifolin (dihydroquercetin). Bull. Exp.l Biol.Med. 144:791-794.

Teodorovic V., Buncic S. and Smiljanic D. 1994. A study of factors influencing histamine production in meat. Fleisch-wirtschaft. 74:170-172.

Trouillas P., Fagnère C., Lazzaroni R., Calliste C., Marfak A., Duroux J. 2004. A theoretical study of the conformational behavior and electronic structure of taxifolin correlated with the free radical-scavenging activity. Food Chemi. 88:571-582.

Vladimirov Y.A., Proskurnina E.V., Demin E.M., Matveeva N.S., Lubitskiy O.B., Novikov A.A., Izmailov D.Y., Osipov A.N., Tikhonov V.P., Kagan V.E. 2009. Dihydroquercetin (Taxifolin) and Other Flavonoids as Inhibitors of Free Radical Formation at Key Stages of Apoptosis. Biochemi. 74:301-307.

Wang Y., Wang W., Chang C., Liou K., Sung Y., Liao J., Chen C., Chang S., Hou Y., Chou Y., Shen Y. 2006. Taxifolin ameliorates cerebral ischemia-reperfusion injury in rats through its anti-oxidative effect and modulation of NF-kappa B activation. J. Biomed. Sci. 13:127-141.

Wang Y., Zu Y., Long J., Fu Y., Li S., Zhang D., Li J., Wink M., Efferth T. 2011. Enzymatic water extraction of taxifolin from wood sawdust of *Larix gmelini* (Rupr.) Rupr. and evaluation of its antioxidant activity. Food Chem. 126:1178-1185.

Warriner K. and Namvar A. 2009. What is the hysteria with *Listeria*? Trends in Food Science & Technology. 20: 245-254.
Zhou G. H., Xu X. L., & Liu Y. 2010. Preservation technologies for fresh meat a review. Meat Sci. 86:119-128.

Paper Received October 11, 2015 Accepted December 9, 2016

PAPER

SEASONAL VARIATION OF FATTY ACID AND AMINO ACID COMPOSITIONS IN THE MUSCLE TISSUE OF ZANDER (*SANDER LUCIOPERCA* LINNAEUS, 1758) AND THE EVALUATION OF IMPORTANT INDEXES RELATED TO HUMAN HEALTH

EMRE ÇAĞLAK* and BARIŞ KARSLI
Recep Tayyip Erdoğan University, Faculty of Fisheries, Department of Processing Technology, 53100 Rize, Turkey
*Corresponding author. Tel.: +90464 2233385; fax: +90464 2234118
E-mail address: emre.caglak@erdogan.edu.tr

ABSTRACT

In this study, the seasonal fatty acid and amino acid amounts in the muscles of the zander from Beyşehir Lake, Turkey, and their important indices for human health were evaluated. It was found that aspartic acid, glutamic acid and lysine levels in zander were dominant among the amino acids. The ratio of essential amino acids (EAA) to non-essential amino acids (NEAA) was between 0.69 and 0.78. In all seasons, the polyunsaturated fatty acid (PUFA, 89.85-109.11 mg/100g) amount in zanders was higher than saturated fatty acids (SFA, 55.08-81.89 mg/100g) and monounsaturated fatty acids (MUFA, 29.16-78.89 mg/100g). It was determined that EPA, DHA and omega-3 rates were high. The fatty acid quality indices (AI, TI, FLQ, w6/w3, h/H) were found at proper levels for human health. Summing up the results, it was found that seasons influenced both the compositions of amino acids and fatty acids of zander.

Keywords: amino acids, Beyşehir Lake, fatty acids, seasons, zander

1. INTRODUCTION

Seafood includes healthy nutrients being rich in protein, unsaturated essential fatty acids, minerals and vitamins they contain (SIDHU, 2003). Fish and shellfish play important roles for human health because of their fatty acid and amino acid varieties (GULER *et al*., 2008). Fish, as a source of food, have protein with high biological value. The building stones of the proteins consist of amino acids (WHO, 2007). Amino acids exist in seafood in important amounts and are classified as essential, non-essential and semi-essential amino acids according to their biological status (WU, 2010). These amino acids are the start-up material of many important substances for organisms, and have an important role, especially in energy metabolism. In addition, fish contains essential amino acids (threonine, valine, leucine, isoleucine, lysine, methionine, phenylalanine, tryptophan, histidine, and arginine) in proper amounts in their bodies (POLAT, 1999; VARLIK *et al*., 2011).

The polyunsaturated fatty acids in fish oil are a vital importance for human health. Consuming fish and fish oil decreases the risk of coronary heart diseases. The nutritional importance of the fish consumption is closely related to the ω-3 fatty acid content of each species. These health benefits are also in a close relationship with ω-3 PUFAs. Long-chain ω-3 PUFAs cannot be synthesized by humans, and therefore they have to be taken with food. It was demonstrated with clinical and epidemiological studies that the major source of EPA and DHA, which constitute most ω-3 PUFAs, is the seafood (GULER *et al*., 2008; CENGIZ *et al*., 2012).

DHA and EPA fatty acids are significant for the body because they could prevent coronary artery diseases (CONNOR, 2000; MOZAFFARIAN *et al*., 2005). Since DHA is the main component of the brain, eye retina and heart muscles, its importance for human health is undeniable. It was reported that EPA is beneficial in brain diseases and cancer treatment (CENGIZ *et al*., 2012). Fish are a good source of EPA and DHA. Some countries (Canada, Sweden, United Kingdom, Australia, Japan), World Health Organization (WHO) and North Atlantic Treaty Organization (NATO) declared the daily ω-3 need as 1.1.-1.6 g; and suggested that the intake should be as 0.3-0.5 g EPA+DHA and 0.8-1.1 g α-linoleic acid (ERKAN, 2013). When compared to sea fish, freshwater fish have higher C18 PUFA and lower EPA and DHA levels. Freshwater fish are generally characterized with high n6 PUFA (especially linoleic acid (18:2n6) and arachidonic acid (20:4n6)). For this reason, freshwater fish contain lower n3 PUFA and n3/n6 levels than sea fish (ÖZOĞUL *et al*., 2007; ÇELIK *et al*., 2005). In addition, the criteria such as atherogenic index (AI), thrombogenic index (TI), flesh lipid quality (FLQ) and hypocholesterolemic/hypercholesterolemic ratio (h/H) provide information on the lipid quality of fish (ULBRICHT and SOUTHGATE, 1991; ABRAMI *et al*., 1992; SANTOS-SILVA *et al*., 2002).

The fish oil and fatty acid compounds show biochemical changes depending on ecological factors and the physiological status of the fish. Even among the same species, the fatty acid component may vary according to the nutrition, region, season, gender and environmental conditions (UYSAL, 2004; ÖZOĞUL *et al*., 2007; GULER *et al*., 2007). The rich amino acid and fatty acid contents in the bodies of freshwater fish make them nutritious, and therefore they are used as animal protein source all over the world (STEFFENS, 2006).

The zander is a predatory freshwater species from the Percidae family, and is an important nutrient because of its high protein, low lipid rate, and essential ω-3 fatty acids. The zander is a lean carnivorous fish with high economic value spreading in inland waters in Turkey (UYSAL, 2004; ÇELIK *et al*., 2005).

The aim of this study was to determine the seasonal fatty acids and amino acid amounts in the muscles of the zander, and evaluate their important indices (AI, TI, FLQ, w6/w3, h/H) for human health.

2. MATERIALS AND METHODS

2.1. The study area and period

This study was conducted in 2012-2013 seasonally, in Beyşehir Lake located within the borders of Konya and Isparta in 37°47′0″N, 31°33′0″E coordinates.

2.2. Fish material

The zander (*Sander lucioperca* Linnaeus, 1758) whose height was between 34.85±1.33 cm and weight was between 395±44.76 g in 2-3 years of age were obtained from the fishermen in the Beyşehir side of the lake. The fishermen stated that they used stretching nets for fishing. A total of 32 fishes were examined throughout the study. The head, tail, fins, and viscera of zander were removed and muscle tissues of zander were kept at -70 °C until analysis of fatty acid and amino acid composition.

2.3. Amino acid analysis

Zander samples were sent to The Scientific and Technological Research Council of Turkey (TUBITAK) Marmara Research Center (MAM) Food Institute for analysis of amino acid. In amino acid analysis, an in-house method was created by modifying those of DIMOVA (2003) and GHESHLAGHI *et al*. (2008), and the sample analysis was carried out. The analysis process was performed using a UFLC (Ultra-Fast Liquid Chromatography) device and a UV detector. The amino acid analyses were conducted in triplicate.

2.4. Analysis of fatty acid methyl esters

Analysis of fatty acid methyl esters (FAME%) was carried out according to TUFAN *et al*. (2013). Lipid extraction of the samples was carried out in triplicate based on the method of BLIGH and DYER (1959), using chloroform:methanol (2:1, v/v). Methyl esters were prepared by transesterification using 2M potassium hydroxide (KOH; Merck, Darmstadt, Germany) in methanol and n-hexane (Sigma-Aldrich, Steinhein, Germany) according to the method described by ICHIHARA *et al*. (1996) with minor modification; 10 mg of extracted oil were dissolved in 2 mL n-hexane, followed by 4 mL of 2M methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4,000 rpm for 10 min, the hexane layer was taken for gas chromatography (GC) - Mass Spectrometry analyses.

2.5. Gas chromatography-mass spectrometry conditions

The identification of fatty acids was conducted on gas chromatography-mass spectrometry (GC-MS) device (QP2010 Ultra with AOC-20i+s model auto sampler) using a mass selective detector (GC-MS QP 2010 PLUS) equipped with GC/MS solutions software (Shimadzu, Kyoto, Japan). FAME mix standards were separated on a Restek RT-2560 column (USA Cat no: 13199 Serial no: 47623-07; 100 m × 0.25 mm internal diameter, Thickness: 0,20μm) with helium (1.0 mL/min) as the carrier gas. The injection temperature

was 240 ºC, and split ratio 50 injection mode was used. The oven temperature was programmed as follows: Column oven temperature was started as 140 ºC, then at 4th min, the temperature increased to 240 ºC and held at this temperature for 20 min, and then held at this temperature for further 50 min starting at 25th min. The MS was scanned from m/z of 45 to 550. The ion source and interface temperatures were 200 ºC and 240 ºC, respectively.
Fatty acids were identified by comparing the retention times of FAME with Supelco (tm) 37 component FAME mixture (Cat. No. 47885-U) and the results were confirmed by using WILEY/NIST 2011 library. Quantification of FAME was carried out using the area normalization method. According to the area value of each compound, area compositions were detected and results were shown as FAME%. The fatty acid content in the zander was calculated according to WEIHRAUCH *et al*. (1977).

2.6. Lipid quality indices

Lipid quality indices as atherogenicity index (AI), thrombogenicity index (TI), fish lipid quality (FLQ) and hypocholesterolemic/hypercholesterolemic ratio (h/H) were calculated with the formulas below (ULBRICHT and SOUTHGATE, 1991; ABRAMI *et al*. 1992; FERNÁNDEZ *et al*., 2007).

AI= [(12:0+(4x14:0)+16:0)]/[(n-6 PUFA+n-3 PUFA)+∑ MUFA]
IT= [14:0+16:0+18:0]/[(0.5x∑ MUFA)+0.5(n-6 PUFA)+3(n-3 PUFA)+(n-3 PUFA/n-6 PUFA)]
FLQ= (EPA+DHA)/total lipids
h/H=(C18:1+C18:2+C18:3+C20:3+C20:4+C20:5+C22:4+C22:5+C22:6)/(C14:0+C16:0

2.7. Statistical analysis

Statistical analysis was performed using the JMP 5.0.1 (SAS) package program. Analysis of variance (ANOVA) was used to compare the results the among seasons, and the Tukey's test was applied to the groups demonstrating difference ($P<0.05$) (SOKAL and ROHLF, 1987).

3. RESULTS AND DISCUSSIONS

The amino acid contents of the zander in seasonal periods are shown in Table 1. The protein contents of the zander are the lowest in spring (17.75%) and the highest in autumn (19.35%). While the protein amount in summer and winter seasons showed statistical similarities to each other ($p>0.05$), they were found to be different from the other seasons ($p<0.05$). When the amount of the amino acids of the zander, which contain 16 types of amino acids, are examined, the methionine, which is one of the essential amino acids, had the lowest value with 465.5 mg/100g and aspartic acid, which is one of the non-essential amino acids had the highest value with 3202.5 mg/100g. It was determined that ten of the amino acids (phenylalanine, lysine, valine, leucine, isoleucine, tyrosine, glycine, proline, arginine and alanine) were at maximum level in autumn; five of them (histidine, threonine, aspartic acid, glutamic acid and serine) were at maximum level in summer; and one of them (methionine) was highest in winter. It was also seen that in spring, when the zander reproduce, all the amino acids except for lysine were at the lowest level. Lysine, leucine, threonine and valine were dominant in EAA. The aspartic acids, glutamic acid, alanine and serine were at the highest level in NEAA. Similarly, MOHANTY *et al*. (2012)

found that in giant river-catfish (*Sperata seenghala*) histidine, threonine and leucine from EAA; and glutamic acid, aspartic acid and serine amino acids from NEAA were dominant. It was observed that the highest values of the total essential amino acids (ΣEAA) were in autumn; the highest values of the total non-essential amino acids (ΣNEAA) were in summer. The ΣEAA/ΣNEAA ratio is an index that shows the protein quality (SWENDSEID *et al*., 1963). The ΣEAA/ΣNEAA ratio, which was obtained seasonally, changed between 0.69-0.78. IWASAKI and HARADA (1985) reported the EAA/NEAA ratio as being 0.70 in average in seawater fish species. MOHANTY *et al*. (2012) noted the ΣEAA/ΣNEAA ratio of giant river-catfish as being 0.89. In another study, the ΣEAA/ΣNEAA ratio (1.08) of *Puntius sophore*, which are among freshwater fish, was found to be higher than those reported in other studies (MAHANTY *et al*., 2014). It is considered that the differences between the ΣEAA/ΣNEAA ratios are resulted from the fish species, seasons, nutrition and location.

Table 1. Seasonal amino acid contents of zander samples.

Amino acids (mg/100g)	Spring	Summer	Autumn	Winter
Protein (%)	17.75±0.01^{a}	18.75±0.11^{b}	19.35±0.05^{c}	18.73±0.26^{b}
Methionine*	465.5±7.78^{a}	509±1.41^{b}	499±0.00^{b}	513±5.66^{b}
Phenylalanine*	661±9.90^{a}	687.5±6.36^{b}	812±1.41^{c}	749±2.83^{d}
Lysine*	2052.5±17.68^{a}	1975±2.83^{b}	2544.5±12.02^{c}	1969.5±6.36^{b}
Histidine*	492±12.73^{a}	643±1.41^{b}	628±28.28^{b}	632.5±26.16^{b}
Valine*	790.5±13.44^{a}	861±1.41^{b}	928±7.07^{c}	887±11.31^{d}
Leucine*	1119.5±19.09^{a}	1181±5.66^{b}	1317.5±7.78^{c}	1198±9.90^{b}
Isoleucine*	701.5±12.02^{a}	744±2.83^{b}	839.5±3.54^{c}	809±5.66^{d}
Threonine*	808±12.73^{a}	933±1.41^{b}	878.5±0.71^{c}	836.5±2.12^{d}
Tyrosine	579±0.00^{a}	603±1.41^{b}	682.5±2.12^{c}	630±1.41^{d}
Glycine	613±8.49^{a}	706.5±4.95^{b}	764±1.41^{c}	682±2.83^{d}
Proline	496±8.49^{a}	543±24.04^{ab}	604.5±0.71^{c}	579.5±2.12^{bc}
Arginine	724.5±10.61^{a}	744±1.41^{a}	851.5±0.71^{b}	806±2.83^{c}
Alanine	1034.5±16.26^{a}	1116±31.11^{b}	1194±1.41^{c}	1125±2.83^{bc}
Aspartic Acid	2779.5±47.38^{a}	3202.5±31.82^{b}	2783.5±3.54^{a}	3079±16.97^{c}
Glutamic Acid	2781±42.43^{a}	3174±2.83^{b}	3097.5±4.95^{b}	2985±4.24^{c}
Serine	809±14.14^{a}	899±2.83^{b}	851.5±4.95^{c}	866±7.07^{bc}
ΣEAA	7090.5±70.00^{a}	7533.5±23.33^{b}	8447±9.90^{c}	7594.5±4.95^{b}
ΣNEAA	9816.5±147.79^{a}	10988±79.20^{b}	10829±7.07^{b}	10752.5±14.85^{b}
ΣAA	16907±217.79^{a}	18521.5±55.86^{b}	19276±2.83^{c}	18347±9.90^{b}
ΣEAA/ΣNEAA	0.72	0.69	0.78	0.71

EAA*: Essential amino acid, NEAA: Non-essential amino acid, AA: Amino acid. Different letters (a,b,c,d) in the same line indicates statistical differences among seasons ($p<0.05$).

The World Health Organization recommended methionine, phenylalanine, lysine, histidine, valine, leucine, isoleucine and threonine requirements for adults of 10, 25, 30, 10, 26, 39, 20, respectively and 15 mg amino acid/kg body weight per day (WHO, 2007). When the results of our study are evaluated according to the reports of WHO (2007), it is clear that if a person whose weight is 70 kg consumes 200 g meat of zander, he or she receives methionine, lysine, histidine, isoleucine and threonine amino acids; and if one consumes 300 g zander, all his or her amino acid needs could be met.
The fatty acid contents of the zander in each season are shown in Table 2.

Table 2. Seasonal fatty acid contents of zander samples.

Fatty Acids mg/100g	Spring	Summer	Autumn	Winter
C14:0	1.79±0.32^{a}	2.38±0.13^{a}	3.10±0.25^{a}	5.80±0.79^{b}
C15:0	0.74±0.01^{a}	0.72±0.12^{a}	0.87±0.19^{a}	1.65±0.14^{b}
C16:0	38.05±0.60^{a}	51.87±0.93^{b}	54.05±0.90^{b}	52.99±3.70^{b}
C17:0	0.96±0.09^{a}	1.14±0.09^{ab}	1.14±0.02^{ab}	1.76±0.34^{b}
C18:0	10.65±0.18^{a}	18.99±0.25^{b}	15.35±0.10^{bc}	11.83±1.96^{ac}
C20:0	0.22±0.03^{a}	0.31±0.01^{a}	0.51±0.02^{b}	0.56±0.06^{b}
C21:0	1.84±0.10^{a}	2.03±0.15^{a}	3.49±0.36^{b}	5.67±0.45^{c}
C24:0	0.84±0.04^{a}	0.80±0.00^{a}	0.77±0.07^{a}	1.65±0.10^{b}
ΣSFA	55.08±0.93^{a}	78.24±1.42^{b}	79.28±0.14^{b}	81.89±3.95^{b}
C16:1	7.51±0.16^{a}	5.50±0.27^{a}	12.65±1.02^{a}	25.31±3.95^{b}
C17:1	0.75±0.00^{a}	0.47±0.00^{a}	0.81±0.10^{a}	2.02±0.22^{b}
C18:1n9t	0.13±0.00^{a}	0.24±0.01^{ab}	0.31±0.00^{b}	0.57±0.08^{c}
C18:1n9c	20.51±0.53^{a}	22.42±0.99^{a}	36.17±2.00^{b}	49.48±0.77^{c}
C20:1	0.59±0.10^{a}	0.53±0.00^{a}	0.63±0.12^{a}	1.50±0.18^{b}
ΣMUFA	29.49±0.58^{a}	29.16±1.27^{a}	50.58±3.24^{b}	78.89±5.20^{c}
C18:2n6c	4.68±0.05^{a}	5.01±0.45^{ab}	8.46±0.47^{b}	12.05±0.57^{c}
C18:3n6	0.30±0.19^{a}	0.55±0.00^{a}	0.57±0.17^{a}	1.26±0.00^{b}
C20:2n6	0.17±0.00^{a}	0.42±0.00^{b}	0.69±0.03^{c}	0.63±0.00^{c}
C20:3n6	0.60±0.01^{a}	0.42±0.03^{a}	0.55±0.07^{a}	0.90±0.06^{b}
C20:3n3	0.28±0.00^{ab}	0.13±0.03^{a}	0.34±0.07^{b}	0.86±0.04^{c}
C20:4n6	18.33±0.30^{ab}	19.01±0.16^{a}	15.56±0.80^{b}	18.06±1.06^{ab}
C20:5n3	9.19±0.22^{a}	11.08±0.15^{a}	23.04±1.32^{b}	15.47±1.19^{c}
22:5n3	8.14±0.38^{bc}	5.35±0.03^{a}	6.69±0.02^{ab}	9.29±0.63^{c}
C22:6n3	48.17±1.23^{ab}	53.28±1.72^{a}	42.76±3.29^{b}	50.60±1.58^{ab}
ΣPUFA	89.85±1.06^{a}	95.26±1.47^{ab}	98.66±4.81^{ab}	109.11±4.71^{b}

Different letters (a,b,c,d) in the same line indicates statistical differences among seasons ($p<0.05$).

It was observed that in all seasons, the PUFAs are the highest (ave. 43.32%), MUFA's are the lowest (ave. 19.64%). The palmitic acid (C16:0) has the highest amount among saturated fatty acids. The 16:0 amount was detected between 18.52%-24.52%. Similarly, in a study conducted by JANKOWSKA *et al*. (2003), C16:0 was determined as 19.91% in wild zander, 20.24% in cultured zander (fed artificial feed) and 20.33% in cultured zander (fed natural food), respectively. The total saturated fatty acid (∑SFA) were found in spring, summer, autumn and winter as 55.08 mg/100g, 78.24 mg/100g, 79.28 mg/100g and 81.89 mg/100g, respectively; which shows the values in spring were different from the other seasons ($p<0.05$). Among the monounsaturated fatty acids (MUFA), palmitoleic acid (C16:1), cis-10-heptadecanoic acid (C17:1) and cis-11-eicosenoic acid (C20:1) values were observed minimum in summer; while the lowest values of elaidic acid (C18:1n9t) and oleic acid (C18:1n9c) were found in spring. The values of C16:1, C17:1, C20:1, C18:1n9t and C18:1n9c in winter was found different from other seasons ($p<0.05$). The ∑MUFA amounts were determined between 29.49-78.89 mg/100g and there were significant differences among the seasons ($p<0.05$).

Polyunsaturated fatty acids (∑PUFA) showed constant increase from spring till winter. The majority of ∑PUFA consisted of docosahexaenoic acid (C22:6n3) DHA, arachidonic acid (C20:4n6) and eicosapentaenoic acid (C20:5n3) EPA. In all seasons, the DHA amount was found higher than the other PUFA amounts at a significant level ($p<0.05$) and was determined as 48.70 mg/kg in average.

The SFA (28.62%-30.01%), MUFA (13.79%-27.57%) and PUFA (38.19%-48.95%) amounts of zander in this study were found similar to the results reported in zander as 31.8%, 13.8% and 42.4%, respectively in the study conducted on seawater and freshwater fish species of Turkey by ÖZOĞUL *et al*. (2007). In another study, the SFA, MUFA and PUFA contents of the zander were determined in Seyhan Lake as 32.9%, 28.0%, 20.8%, in Eğirdir Lake as 30.5%, 20.3%, 30.5% (ÇELIK *et al*., 2005).

The fatty acid contents of the zander examined in this study were more consistent with the results of the zander of Eğirdir Lake than those from Seyhan Lake. However, the PUFA rates were found to be lower than our results. It is considered that this difference occurs from the undetected % fatty acid rates being high in the study conducted by ÇELIK *et al*. (2005). In another study, the SFA%, MUFA% and PUFA% rates of the wild zander were found as 27.84%, 21.36% and 50.80, respectively (JANKOWSKA *et al*., 2003).

The PUFA/SFA ratio must be minimum 0.45 (JUSTI *et al*., 2003; TUFAN *et al*., 2011). The data of this study were 1.22-1.63, which is consistent with the values recommended in terms of health (Table 3). The PUFA/SFA ratio determined in spring (1.63) was statistically different from those determined in other seasons ($p<0.05$). ÖZOĞUL *et al*. (2007) reported that the PUFA/SFA ratio changed between 0.78-1.56 is freshwater fish, and in zander this ratio was 1.33. This PUFA/SFA ratio was found to be similar with our results especially recorded in winter.

It is reported that the atherogenic (AI) and thrombogenic (TI) indices that are higher than (>1.0) is harmful for human health (OURAJI *et al*., 2009). If this value gets lower, the risk of coronary heart diseases decreases (CUTRIGNELLI *et al*., 2008). The AI (0.38-0.49) and TI (0.22-0.31) values obtained in this study were found lower than this value in all seasons (Table 3), and it was also determined that there were no risks for human health.

SOUSA BENTES *et al*. (2009) reported that the h/H ratio of fatty acids is the indicator of whether the fat in the product is nutritionally adequate. The h/H ratio was found between 2.17-2.77 in this study, and no differences between spring and winter, also between summer and autumn were determined ($p<0.05$).

Table 3. Fatty acid ratios and lipid quality indexes.

Fatty acid	Spring	Summer	Autumn	Winter
∑PUFA/∑SFA	1.63±0.05^{a}	1.22±0.04^{b}	1.24±0.06^{b}	1.33±0.01^{b}
∑PUFA/∑MUFA	3.05±0.10^{a}	3.27±0.19^{a}	1.96±0.22^{b}	1.39±0.15^{b}
EPA+DHA	57.36±1.01^{a}	64.36±1.57^{a}	65.8±4.61^{a}	66.06±2.77^{a}
∑n3 PUFA	65.78±0.64^{a}	69.84±1.57^{a}	72.83±4.56^{a}	76.21±4.57^{a}
∑n6 PUFA	24.07±0.43^{a}	25.42±0.1^{b}	25.83±0.25^{b}	32.9±0.14^{c}
∑n3/∑n6	2.73±0.02^{ab}	2.75±0.07^{a}	2.82±0.15^{a}	2.32±0.13^{b}
∑n6/∑n3	0.37±0.00^{a}	0.36±0.01^{a}	0.36±0.02^{a}	0.43±0.02^{b}
AI	0.38±0.02^{a}	0.49±0.01^{b}	0.45±0.01^{bc}	0.41±0.00^{ac}
TI	0.22±0.01^{a}	0.31±0.01^{b}	0.28±0.01^{bc}	0.25±0.01^{ac}
FLQ	32.89±0.67^{a}	31.76±0.97^{a}	28.79±1.80^{ab}	24.47±0.71^{b}
h/H	2.77±0.08^{a}	2.17±0.05^{b}	2.35±0.02^{b}	2.70±0.07^{a}

AI: atherogenic index, TI, thrombogenic index, FLQ: flesh-lipid quality, h/H: hypocholesterolemic/hypercholesterolemic ratio. Different letters (a,b,c,d) in the same line indicates statistical differences among seasons ($p<0.05$).

If the FLQ value is high, this indicates that there are nutrient lipids with good quality (ABRAMI *et al*., 1992). The highest FLQ values were seen in spring (32.89), and the lowest in winter (24.47). The difference between them was significant ($p<0.05$) (Table 3). The FLQ values in winter were found to be similar only to those in autumn ($p>0.05$). The EPA+DHA amounts in zander were determined between 57.36-66.06 mg/100g (Table 3). There were differences between the seasons ($p>0.05$). The EPA+DHA amounts in the zander caught in the same lake were determined as 29.23%, 21.32%, 28.27% and 24.24% for spring, summer, autumn and winter, respectively (GULER *et al*., 2007). These results show similarity with this study except for the summer season. The n6/n3 ratio is recommended max. 4 by the UK Department of Health (JUSTI *et al*., 2003). The ∑n6/∑n3 ratios in this study were found in the recommended limit value. The ∑n6/∑n3 ratio in winter (0.43) was found higher ($p<0.05$) than the other seasons (0.36-0.37) (Table 3). JANKOWSKA *et al*. (2003) found the ∑n6/∑n3 ratio of the wild zander as 0.31. ÖZOĞUL *et al*. (2007) determined that the n6/n3 ratio of zander is 0.46 similarly with our winter data. On the other hand, GULER *et al*. (2007) reported that n6/n3 ratio of zander is between 0.67-1.39 in all seasons.

4. CONCLUSIONS

As a conclusion, the zander, which are also called freshwater seabass, have an important fatty acid composition. It was determined that the EPA, DHA and omega-3 rates of the zander, which played an important role in human nutrition are high. In addition, the fatty acids quality indexes were found at proper levels for human health. Moreover, it was observed that the zander have high protein and rich amino acid content although the rate of amino acids changes according to the seasons. The lowest amino acid rates were found in spring, the period of reproduction. At the end of the seasonal examination of amino

acid and fatty acid contents of the zander, it has been observed that these contents have a nutrient function for a healthy and balanced diet.

REFERENCES

Abrami G., Natiello F., Bronzi P., McKenzie D., Bolis L. and Aggrade E. 1992. A comparison of highly unsaturated fatty acid levels in wild and farmed eels (*Anguilla anguilla*). Comp. Biochem. Physiol. 101:79.

Bligh E.G. and Dyer W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Phys. 37:911.

Cengiz E.İ., Ünlü E., Bashan M., Satar A. and Uysal E. 2012. Effects of seasonal variations on the fatty acid composition of total lipid, phospholipid and triacylglicerol in the dorsal muscle of mesopotamian catfish (*Silurus triostegus* Heckel, 1843) in Tigris River (Turkey). Turk. J. Fish. Aquat. Sc. 12:33.

Connor W.E. 2000. Importance of n−3 fatty acids in health and disease. The American J. Clin. Nutr. 71:171.

Cutrignelli M.I., Calabrò S., Bovera F., Tudisco R., D'Urso S., Marchiello M., Piccolo V. and Infascelli F. 2008. Effects of two protein sources and energy level of diet on the performance of young Marchigiana bulls. 2. Meat quality. Ital. J. Anim. Sci. 7:271.

Çelik M., Diler A. and Küçükgülmez A. 2005. A comparison of the proximate compositions and fatty acid profiles of zander (*Sander lucioperca*) from two different regions and climatic conditions. Food Chem. 92:637.

Dimova N. 2003. RP-HPLC analysis of amino acids with UV-Detection. Bulg. Acad. Sci. 56:75.

Erkan N. 2013. Evaluation of omega 3 (ω-3) fatty acid profile of seafood products consumed in Turkey. Journal of FisheriesSciences.com 7:194.

Fernández M., Ordóñez J.A., Cambero I., Santos C., Pin C. and de la Hoz L. 2007. Fatty acid compositions of selected varieties of Spanish ham related to their nutritional implications. Food Chem. 101:107.

Gheshlaghi R., Scharer J.M., Moo-Young M. and Douglas P.L. 2008. Application of statistical design for the optimization of amino acid separation by reverse-phase HPLC. Anal. Biochem. 383:93.

Guler G.O., Aktumsek A., Citil O.B., Arslan A. and Torlak E. 2007. Seasonal variations on total fatty acid composition of fillets of zander (*Sander lucioperca*) in Beysehir Lake (Turkey). Food Chem. 103:1241.

Guler G.O., Kiztanır B., Aktumsek A., Citil O.B. and Ozparlak H. 2008. Determination of the seasonal changes on total fatty acid composition and ω3/ω6 ratios of carp (*Cyprinus carpio* L.) muscle lipids in Beysehir Lake (Turkey). Food Chem. 108:689.

Ichihara K., Shibahara A., Yamamoto K. and Nakayama T. 1996. An improved method for rapid analysis of the fatty acids of glycerolipids. Lipids 31:535.

Iwasaki M. and Harada R. 1985. Proximate and amino acid composition of the roe and muscle of selected marine species. J. Food Sci. 50:1585.

Jankowska B., Zakęś Z., Żmijewski T. and Szczepkowski M. 2003. Fatty acid profile and meat utility of wild and cultured zander, *Sander lucioperca* (L.). *Electronic Journal of* Polish Agricultural Universities, Fisheries 6: http://www.ejpau.media.pl/articles/volume6/issue1/fisheries/art-02.pdf.

Justi K.C., Hayashi C., Visentainer J.V., De Souza N.E. and Matsushita M. 2003. The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids. Food Chem. 80:489.

Mahanty A., Ganguly S., Verma A., Sahoo S., Mitra P., Paria P., Sharma A.P., Singh B.K. and Mohanty B.P. 2014. Nutrient profile of small indigenous fish *Puntius sophore*: proximate composition, amino acid, fatty acid and micronutrient profiles. Natl. Acad. Sci. Lett. 37:39.

Mohanty B.P., Paria P., Das D., Ganguly S., Mitra P., Verma A., Sahoo S., Mahanty A., Aftabuddin M., Behera B.K., Sankar T.V. and Sharma A.P. 2012. Nutrient profile of giant river-catfish *Sperata seenghala* (Sykes). Natl. Acad. Sci. Lett. 35:155.

Mozaffarian D., Bryson C.L., Lemaitre R.N., Burke G.L. and Siscovick D.S. 2005. Fish intake and risk of incident heart failure. J. Am. Coll. Cardiol. 45:2015.

Ouraji H., Shabanpur B., Abediankenari A., Shabani A., Nezami A., Sudagar M. and Faghani S. 2009. Total lipid, fatty acid composition and lipid oxidation of Indian white shrimp (*Fenneropenaeus indicus*) fed diets containing different lipid sources. J. Sci. Food Agr. 89:993.

Özoğul Y., Özoğul F. and Alagoz S. 2007. Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: A comparative study. Food Chem. 103:217.

Polat A. 1999. Changes in the total and free amino acid composition in African catfish (*Clarias gariepinus* Burchell, 1822) larvae. Turk. J. Zool. 23:309.

Santos-Silva J., Bessa R.J.B. and Santos-Silva F. 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. Livest. Prod. Sci. 77:187.

Sidhu K.S. 2003. Health benefits and potential risks related to consumption of fish or fish oil. Regul. Toxicol. Pharm. 38:336.

Sokal R.R. and Rohlf F.J. 1987. Introduction to Biostatistics. 2nd ed, Freeman. New York, WH, USA.

Sousa Bentes A., Souza H.A.L., Simões M.G. and Mendonça X.M.F.D. 2009. Physical and chemical characterization and lipid profile of three amazon fish species. R. bras. Tecnol. Agroindustr. 3:97.

Steffens W. 2006. Freshwater fish–wholesome foodstuffs. Bulg. J. Agric. Sci. 12:320.

Swendseid M.E., Villalobos J. and Friedrich B. 1963. Ratios of essential-to-nonessential amino acids in plasma from rats fed different kinds and amounts of proteins and amino acids. J. Nutr. 80:99.

Tufan B., Koral S. and Köse S. 2011. Changes during fishing season in the fat content and fatty acid profile of edible muscle, liver and gonads of anchovy (*Engraulis encrasicolus*) caught in the Turkish Black Sea. Int. J. Food Sci. Tech. 46:800.

Tufan B., Koral S. and Köse S. 2013. The variations in proximate chemical composition and fatty acid profile in different parts of the thornback ray (*Raja clavata*) caught from Black Sea, Turkey. J. Aquat. Food Prod. T. 22:83.

Ulbricht T. and Southgate D. 1991. Coronary heart disease: seven dietary factors. Lancet 338:985.

Uysal K. 2004. Gonad olgunlaşmasi esnasinda sudak (*Sander lucioperca*) balığının ovaryum ve testislerinin yağ asidi bileşimindeki değişimler. Dumlupınar Üniversitesi Fen Bilimleri Enstitüsü Dergisi 7:91.

Varlık C., Erkan N., Özden Ö., Mol S. and Baygar T. 2011. Su Ürünleri İşleme Teknolojisi. 2nd ed., İstanbul Üniversitesi Yayınları, İstanbul, Turkey.

Weihrauch J.L., Posati L.P., Anderson B.A. and Exler J. 1977. Lipid conversion factors for calculating fatty acids contents in foods. J. Am. Oil. Chem. Soc. 54:36.

WHO 2007. Protein and amino acid requirements in human nutrition. WHO Technical Report series 935, World Health Organization, Geneva, Switzerland.

Wu G. 2010. Functional amino acids in growth, reproduction, and health. Adv. Nutr. 1:31.

Paper Received August 8, 2016 Accepted December 15, 2016

PAPER

KINETICS PARAMETERS OF REFINED AND COLD-PRESSED RAPESEED OILS AFTER OXIDATION BY RANCIMAT

E. SYMONIUK*, K. RATUSZ and K. KRYGIER

[a]Department of Food Technology, Warsaw University of Life Sciences (SGGW), Nowoursynowska Street 166, 02-787 Warsaw, Poland

*Corresponding author. Tel.: +48 225937525; fax: +48 225937527

E-mail address: edyta_popis@sggw.pl

ABSTRACT

This works summarizes results of quality assessment of five labelled as refined and five labelled as cold-pressed rapeseed oils. The analyzed oils were characterized by a good quality that meet requirements of the Codex Alimentarius standard. Oxidative stability of the oils was determined by Rancimat test at five temperatures (90, 100, 110, 120, 130 °C). The oxidation rate constant (k) was observed to increase along with increasing process temperature. The Arrhenius equation and active complex theory were used to compute the exponential factor (Z), activation energy (Ea), reaction rate constant at various temperatures (k) as well as enthalpy ($\Delta H^{\leftrightarrow}$) and entropy ($\Delta S^{\leftrightarrow}$) of the oxidation reaction of the analyzed oils. The Ea, $\Delta H^{\leftrightarrow}$and $\Delta S^{\leftrightarrow}$ values ranged from 76.43 to 82.44 kJ/mol, from 72.12 to 79.26 kJ/mol and from –48.35 to –68.45 J/molK for the refined oils as well from 74.24 to 77.56 kJ/mol, from 71.04 to 75.47 kJ/mol and from –56.40 to –69.99 J/molK for the cold-pressed oils, respectively.

Keywords: oxidation kinetics, oxidative stability, Rancimat, rapeseed oil

1. INTRODUCTION

Today, rapeseed (*Brassica napsus L.*) oil is produced mainly from double zero "00" cultivar. Double zero cultivar compared to first rapeseed cultivars is characterised by low content of eruic acid (0-2%), whose decomposition products are harmful to human health and glucosinolate (8-15 μM/g of seeds), which did not allow for full use of the protein contained in the seeds. Rapeseed are used mainly for edible oil production, but also as an important feedstock for biodiesel production. Rapeseed may be manufactured with the cold- or hot-pressing method or via extraction and purified by refining. The refined oil, also referred to as universal cooking oil, is the most popular on the market and used for various culinary purposes. However, in last years has been observed a growing interest in the cold-pressed oil. Compared to crude oil, the refined oil contains less free fatty acids, phospho- and glycolipids and metal ions, it has also a brighter color and higher oxidative stability. A negative effect of refining is an increase in the number of trans isomers and a decrease in the content of natural antioxidants, e.g. tocopherols (ČMOLIK *et al*., 2000; TASAN and DEMIRCI, 2003; KARABULUT *et al*., 2005). One of the key reactions proceeding in edible oils during storage and heat treatment is oxidation. The oxidation products formed contribute to the development of unpleasant odor, to deterioration of oil quality and nutritive value, and may also induce toxic effects on a human body (JACOBSEN and NIELSEN, 2008; MATTHÄUS *et al*., 2010; SHAHIDI and ZHONG, 2010).
The process of oxidation is linked with the notion of oxidative stability, namely resistance to oxidation under specified conditions. Lipid oxidation may be analyzed with various methods. The most reliable one is a shelf test that consists in placing a sample of oil at a room temperature and periodical measurements of the peroxide and anisidine values (RATUSZ *et al*., 2002; VELASCO *et al*., 2003; FARHOOSH, 2007; KOWALSKA *et al*., 2014). Owing to a long duration of this test (a few months), an increasing interest is expressed today in the so-called accelerated tests: they are significantly faster, more precise and unbiased (OSTROWSKA – LIGĘZA *et al*., 2010).
Today, the following accelerated tests are found applicable for the evaluation of the oxidative stability of fresh edible oils: electron spin resonance (ESR), magnetic spectroscopy (NMR), Furier transform (FT), active oxygen method (AOM) and differential scanning calorimetry (DSC) (WANASUNDARA *et al*., 1995; ANDERSEN and SKIBSTED, 2002; ROHN and KROHN, 2005). However, the most popular method of edible and nonedible oil stability assessment in the Metrohm Rancimat (PAWAR *et al*., 2013).
The method of oxidative stability evaluation in the Rancimat test consists in the measurement of the concentration of volatile products of oxidation, e.g. short-chain fatty acids formed as a result of accelerated oxidation of the sample. The accelerated oxidation of fat in a reaction vessel is induced by sample heating and blowing through with air. The volatile products of oxidation formed in this process are blown away to water in a measuring vessel, the electrode is immersed in. The electrode allows measuring conductivity of water with oxidation reaction products dissolving in it. A rapid increase of conductivity is tantamount to the formation of secondary products of oxidation that are well soluble in water. Results of conductivity measurements in time are presented in the form of a graph. The time preceding the process of accelerated oxidation, the moment of sudden increase of conductivity, is a measure of sample resistance to oxidation and is referred to as inductive time, induction time or oxidative stability index (OSI) (MATTHÄUS, 1996; ANWAR *et al*., 2003; JAIN and SHARMA, 2011).
Many kinetic parameters of the oxidation process may be determined based on results of the Rancimat test. They may be applied to differentiate the origin of oils and to identify differences or similarities between them. These parameters may be very useful in predicting oxidative stability of oils during their heat treatment, storage and distribution

(TAN *et al*., 2001). Many studies were conducted to determine kinetic parameters of edible oils, however they mainly concerned refined oils, whereas investigations are missing that would address a comparison of oxidation kinetics of oils produced with various methods. Considering the above, the objective of this study was to determine and compare kinetic parameters of refined and cold-pressed rapeseed oils.

2. MATERIALS AND METHODS

2.1. Chemical reagents

All the solvents and chemical reagents used in the study were of analytical grade and were purchased from POCH S.A. (Gliwice, Poland). The Supelco 37 Component Fatty Acid Methyl Esters (FAME) mix was from Sigma - Aldrich Gmbh (Schnelldorf, Germany). Deionized water (0.05 μS) was obtained using an HLP Smart 2000 apparatus (Hydrolab, Poland).

2.2. Material

The experimental material included ten oils labelled as rapeseed oils: five refined (R) and five cold-pressed (C). The oils were purchased on the local market within their shelf-life. The cold-pressed oils were packed in bottles made of dark-green glass, whereas the refined ones were in ethylene polyterephthalate (PET) bottles. Once purchased, the oils were transported to the laboratory and subjected to analyses.

2.3. Chemical analysis

The physicochemical quality of oils was determined based on the lipid values. The extent of hydrolytic changes in the analyzed oils, expressed as the acid value (AV), was determined according to the AOCS Cd 3d-63 (AOCS, 2000). The content of peroxides, expressed as the peroxide value (PV), was determined according to the AOCS 965.33 (AOCS, 1999). The extent of oxidative changes, expressed as the anisidine value (AnV), was determined according to the AOCS Cd 18-90 (AOCS, 2002). The total oxidation index (TOTOX) was computed based on results achieved for the peroxide value and anisidine value (TOTOX = 2PV + AnV).
Fatty acid composition of the analyzed oils was determined using gas chromatography according to the AOCS Ce 1h-05 (AOCS, 2005) with small modifications. Methyl esters of fatty acids were prepared according to the AOCS 2-66 (AOCS, 1997). Determinations were conducted in a Hewlett – Packard model 5890 II gas chromatograph (Agilent Technologies, Avondale, PA, USA) with a flame ionization detector, equipped in a Supelcowax 10 column (30 m x 0.25 mm x 0.25 μm). Injection temperature was 240 °C, and injection was at 1:25. Assay conditions were as follows: helium flow rate 1mL/min and oven temperature 240 °C. Detector's temperature was set at 240 °C. Peaks were identified by comparing their retention times with that of FEME mix standard.

2.4. Rancimat Test - oxidative stability

Oxidative stability of the oils was determined using a Rancimat 743 Metrohm apparatus (Herisau, Switzerland). The Rancimat test was conducted on 2.50 ± 0.01 g samples of oils that were oxidized at five temperatures: 90 °C, 100 °C, 110 °C, 120 °C and 130 °C, at a

constant air flow of 20 L/h. The volatile products formed from the oxidation reaction were soluble in 0.06 L of deionized water.
Eight samples of oils were placed in the apparatus and analyzed simultaneously. The samples were placed at random. The induction times were recorded automatically by the apparatus' software and taken as the break point of the plotted curves with the accuracy of 0.01 h.

2.5. Kinetic analysis

Based on the results obtained for the oxidative stability of the oils assayed at different temperatures, graphs were plotted and regression lines were determined according to the following equations:

$$\ln(\tau\text{Rancimat}) = a(T) + b \quad (1)$$

$$\ln(\tau\text{Rancimat}) = A(1/T) + B \quad (2)$$

Activation energy - *Ea* (kJ/mol) of the oxidation reaction, exponential factor *Z* (h^{-1}) and oxidation reaction rate constants at different temperatures were determined for individual rapeseed oils using the Arrhenius equation, according to a method by KOWALSKI *et al*. (2004):

$$k = Ze^{-Ea/RT} \quad (3)$$

Values of reaction enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) were calculated based on the equation derived from the activated complex theory:

$$\ln(k/T) = \ln(k_B/h) + (\Delta S/R) - (\Delta H/RT) \quad (4)$$

2.6. Statistical analysis

All Rancimat measurements were conducted in three replications for each oil sample, then results were averaged and presented in tables. Data were subject to analysis of variance (one-way ANOVA). Statistical differences were determined with the Tukey's multiple comparison test at a significance level of $p = 0.05$. Pearson's linear correlations were calculated at the $p<0.05$ level. All statistical analyses were performed using the Statistica 10.0 software (2010, StatSoft, Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

Results of the evaluation of the initial quality of the analyzed oils were collected and presented in Table 1. The refined oils were characterized by lower acid value (0.09 – 0.27 mg KOH/ g oil) and peroxide value (0.97 – 2.02 mEq O_2/ kg oil) compared to the cold-pressed oils. In turn, they showed higher anisidine values (1.10 – 2.42), which may be due to high temperatures applied during one of the stages of the refining process – deodorization, that cause the formation of secondary products of oxidation (ČMOLIK and POKORNÝ, 2000). Similar values of the above indices were reported for refined rapeseed oils by RATUSZ *et al*. (2005).

The cold-pressed oils were characterized by higher acid value (1.03 – 1.47 mg KOH/ g oil) and peroxide value (2.12 – 8.28 mEq O_2/ kg oil). In addition, they exhibited a higher degree of oxidation expressed as TOTOX index (4.75 – 17.25) compared to the refined oils (3.04 – 5.56). The peroxide values of the cold-pressed oils differed statistically significantly. Great differences in the value of this index were also determined by MATTHÄUS and BRÜHL (2003). Higher values of these quality indices are linked with the fact that the cold-pressed oils are subjected only to mechanical treatment (filtration, centrifugation) and not to the complete refining process (degumming, deacidification, bleaching, deodorization) as in the case of the refined oils. Higher contents of free fatty acids and primary oxidation products depend on the quality of raw materials used for oil manufacturing. The anisidine values of the cold-pressed oils ranged from 0.18 to 0.69.
Values of particular lipid indices of the analyzed refined and cold-pressed rapeseed oils did not exceed the values recommended in Codex Alimentarius standard (2013). According to this standard, the threshold value of the acid value reaches 0.6 for refined oils and 4.0 mg KOH/ g oil for cold-pressed oils, whereas that of the peroxide value reaches 10 and 15 mEq O_2/ kg oil, respectively.

Table 1. Characteristics of the initial quality of the analyzed rapeseed oils.

Oil	AV (mg KOH/g)	PV (mEq O_2/kg)	AnV (absorbance x 1000)	TOTOX
R1	0.13±0.01^{ab}	2.02±0.03^{b}	1.32±0.05^{e}	5.36±0.16^{d}
R2	0.27±0.03^{c}	0.98±0.02^{a}	1.61±0.04^{f}	3.57±0.10^{b}
R3	0.25±0.02^{bc}	1.02±0.03^{a}	1.54±0.05^{f}	3.58±0.16^{b}
R4	0.09±0.03^{a}	0.97±0.04^{a}	1.10±0.03^{d}	3.04±0.07^{a}
R5	0.19±0.01^{abc}	1.57±0.05^{c}	2.42±0.02^{g}	5.56±0.11^{d}
CA*	**0.6**	**10.0**	**-**	**-**
C1	1.25±0.02^{e}	4.38±0.02^{e}	0.31±0.05^{ab}	9.07±0.02^{f}
C2	1.03±0.03^{d}	5.85±0.02^{f}	0.18±0.04a^{b}	11.88±0.02^{g}
C3	1.09±0.04^{d}	2.12±0.03^{b}	0.51±0.02^{bc}	4.75±0.11^{c}
C4	1.47±0.04^{f}	3.24±0.03^{d}	0.57±0.03^{b}	7.05±0.04^{e}
C5	1.27±0.01^{e}	8.28±0.03^{g}	0.69±0.02^{b}	17.25±0.05^{h}
CA**	**4.0**	**15.0**	**-**	**-**

^{a,b,c,d,e,f,g,h}mean values in columns are statistically significantly different at a significance level of p=0.05
*threshold values for refined oils according to Codex Alimentarius (2013)
**threshold values for cold-pressed oils according to Codex Alimentarius (2013)

The composition of selected fatty acids of the analyzed oil was summarized in Table 2. The investigated rapeseed oils were characterized by a low content of saturated fatty acids (6.49 – 8.48 %). As indicated by literature data, the fatty acid composition of rapeseed oils is predominated by monounsaturated fatty acids, including mainly oleic acid, which was also confirmed in our study (59.31 – 63.22 %). The analyzed oils were characterized by ca. 30% content of polyunsaturated fatty acids. The fatty acid composition of the analyzed oils was typical of rapeseed oil (ROSZKOWSKA *et al*., 2014).
Differences in fatty acid composition of refined and cold-pressed oils were small and depended, most of all, on the raw materials used to produce the oils (RAMOS *et al*., 2009).
Oxidative stability of the investigated oils was examined with Rancimat test at different temperatures and respective results were presented in Table 3.

Table 2. Fatty acid composition of the analyzed rapeseed oils [%].

Fatty acid	Oil sample									
	R1	R2	R3	R4	R5	C1	C2	C3	C4	C5
Palmitic (16:0)	4.85±0.03^{b}	4.54±0.02^{a}	4.95±0.03^{b}	4.61±0.02^{a}	5.89±0.01^{e}	5.00±0.01^{cd}	5.07±0.01^{d}	5.89±0.02^{e}	5.85±0.02^{e}	4.98±0.02^{cd}
Palmitoleic (16:1)	0.23±0.01^{ab}	0.24±0.01^{ab}	0.22±0.01^{a}	0.25±0.02^{ab}	0.28±0.01^{ab}	0.28±0.01^{ab}	0.30±0.01^{b}	0.28±0.02^{ab}	0.26±0.01^{ab}	0.29±0.01^{ab}
Stearic (18:0)	1.65±0.02^{c}	1.53±0.02^{ab}	1.45±0.01^{a}	1.52±0.00^{ab}	2.13±0.00^{e}	1.68±0.01^{c}	1.51±0.01^{ab}	1.54±0.00^{b}	1.92±0.02^{d}	1.56±0.01^{b}
Oleic (18:1)	62.14±0.04^{b}	62.87±0.05^{c}	63.22±0.08^{c}	63.21±0.06^{c}	59.34±0.08^{a}	61.15±0.05^{b}	62.16±0.07^{b}	61.26±0.05^{b}	59.31±0.04^{a}	62.21±0.09^{b}
Linoleic n-6 (18:2)	19.72±0.02^{c}	19.26±0.02^{a}	19.10±0.04^{a}	19.15±0.03^{a}	19.15±0.02^{a}	19.69±0.04^{c}	19.54±0.04^{b}	19.14±0.03^{a}	19.22±0.03^{a}	19.58±0.03^{bcd}
Linolenic n-3 (18:3)	9.12±0.01^{a}	9.56±0.02^{b}	9.23±0.03^{a}	9.43±0.04^{b}	10.92±0.02^{d}	9.75±0.03^{c}	9.57±0.03^{b}	10.15±0.02^{d}	11.25±0.03^{e}	9.73±0.02^{c}
Arachidonic (20:0)	0.48±0.01^{de}	0.42±0.00^{b}	0.52±0.00^{f}	0.50±0.00^{ef}	0.46±0.00^{cd}	0.37±0.00^{a}	0.45±0.00^{c}	0.45±0.01^{c}	0.47±0.00^{cd}	0.39±0.00^{a}
Eicosenoic (20:1)	1.21±0.01^{cd}	1.26±0.02^{ce}	1.31±0.01^{e}	1.27±0.01^{ce}	1.02±0.00^{ab}	1.06±0.01^{b}	1.18±0.02^{c}	0.96±0.01^{a}	0.98±0.00^{a}	1.05±0.01^{b}
Other	0.60^{bc}	0.32^{ab}	0.10^{a}	0.16^{a}	0.81^{cd}	1.02^{d}	0.22^{a}	0.32^{ab}	0.74^{c}	0.21^{a}
Σ SFA	6.98^{b}	6.49^{a}	6.92^{b}	6.63^{a}	8.48^{d}	7.05^{b}	7.03^{b}	7.89^{c}	8.24^{d}	6.93^{b}
Σ MUFA	63.58^{c}	64.37^{d}	64.65^{d}	64.63^{d}	60.64^{a}	62.49^{b}	63.64^{c}	62.50^{b}	60.55^{a}	63.55^{c}
Σ PUFA	28.84^{c}	28.82^{c}	28.33^{a}	28.58^{b}	30.07^{f}	29.44^{e}	29.11^{d}	29.29^{de}	30.47^{g}	29.31^{de}

^{a,b,c,d,e,f,g}mean values in columns are statistically significantly different at a significance level of p=0.05.

Table 3. Induction time [h] of the analyzed rapeseed oils at 90, 100, 110, 120, 130 °C in Rancimat test.

Temperature		Oil sample									
T[K]	T[°C]	R1	R2	R3	R4	R5	C1	C2	C3	C4	C5
		Induction time [h]									
403	130	3.02±0.15^{b}	2.65±0.17^{ab}	2.42±0.11^{ab}	2.63±0.16^{ab}	2.95±0.12^{b}	1.93±0.10^{a}	1.92±0.12^{a}	2.28±0.15^{ab}	2.09±0.16^{a}	1.89±0.10^{a}
393	120	5.72±0.20^{g}	5.08±0.13^{efg}	4.56±0.14^{cde}	4.98±0.12^{def}	5.65±0.14^{fg}	3.77±0.06^{ab}	3.54±0.12^{b}	4.27±0.011^{bcd}	3.87±0.10^{abc}	3.46±0.11^{a}
383	110	11.36±0.21^{e}	10.48±0.19^{cde}	9.97±0.16^{c}	10.35±0.22^{cd}	11.25±0.18^{de}	7.28±0.11^{ab}	6.66±0.15^{a}	7.94±0.18^{b}	7.57±0.21^{ab}	7.19±0.14^{ab}
373	100	22.21±0.31^{e}	21.32±0.24^{de}	20.76±0.25^{d}	21.26±0.21^{de}	21.63±0.30^{de}	13.56±0.24^{ab}	12.99±0.22^{a}	16.03±0.27^{c}	14.80±0.25^{bc}	13.56±0.23^{ab}
363	90	42.55±0.28^{f}	40.89±0.22^{e}	40.78±0.22^{e}	40.78±0.22^{e}	41.12±0.25^{e}	26.21±0.27^{b}	25.18±0.25^{b}	33.46±0.22^{d}	28.57±0.21^{c}	23.67±0.26^{a}

^{a,b,c,d,e,f,g}mean values in columns are statistically significantly different at a significance level of p=0.05.

The induction time of rapeseed oil oxidation recorded by Rancimat depends on process temperature. Stability of the oils was decreasing along with temperature increase. The highest oxidative stability of the analyzed rapeseed oils was determined at a temperature of 90 °C, whereas the lowest one – at 130 °C. Compared to the cold-pressed oils, the refined oils were characterized by longer induction times in the Rancimat test at all temperatures.

Rancimat is the most commonly used method to determinate the oxidative stability of oils for edible and nonedible purpose (ANWAR *et al*., 2007; CASAL, 2010; FARHOOSH, 2010; GIUFFRÈ, 2016; GIUFFRÈ, 2016a, GIUFFRÈ, 2016b). The requirements for "rapeseed fuel for Diesel engines" were published in the official DIN standard where oxidation stability at 110 °C is one of the most important parameters and the value has to be less than 6h (DIN 51605, 2010).Our paper shows that the oxidative stability of analyzed rapeseed oils at 110°C varied, and ranged from 9.97– 11.36 h for refined oils, and from 6.66 to 7.94 for cold-pressed oils. In the case of edible oils the most commonly used temperature of measuring the oxidative stability for refined oil is 120 °C, but in the case of cold-pressed oils is 100 °C. The induction time of analyzed refined rapeseed oils at 120 °C were between 4.56 to 5.72 h. On the other hand, cold-pressed rapeseed oils were characterized by induction time at 100 °C between 12.99 - 16.03 h. The induction times were higher than those presented by SZTERK *et al*. (2010) in their study of cold-pressed rapeseed – (7.07 h), camelina (6.12 h), primrose (6.14 h), amaranth (6.14 h). The obtained induction times were also lower than those for hazelnut oil (22.44 h) in CIEMNIEWSKA-ŻYTKIEWICZ *et al*. (2014).

Oil oxidation at oxygen (air) excess is the first order reaction. Therefore, kinetic analysis may be carried out for the oil oxidative transformation constant (KOWALSKI *et al*., 2004). Based on the determined induction times (Table 3) and Arrhenius equation (3), the following kinetic parameters were calculated acc. to the method by KOWALSKI *et al*. (2004): activation energy of oxidation reaction - *Ea*, pre-exponential factor - *Z*, reaction rate constants - *k* for particular temperatures of the Rancimat test, as well as enthalpy $\Delta H^{\neq}$ and entropy $\Delta S^{\neq}$ of oxidation activation.

Correlations between logarithm induction time $\tau Rancimat$ and temperature and the reverse of temperature were determined for each type of oil (Figs. 1 and 2). The character of these correlations was linear with a correlation coefficient of R>0.99 and they may be determined using equations 1 and 2.

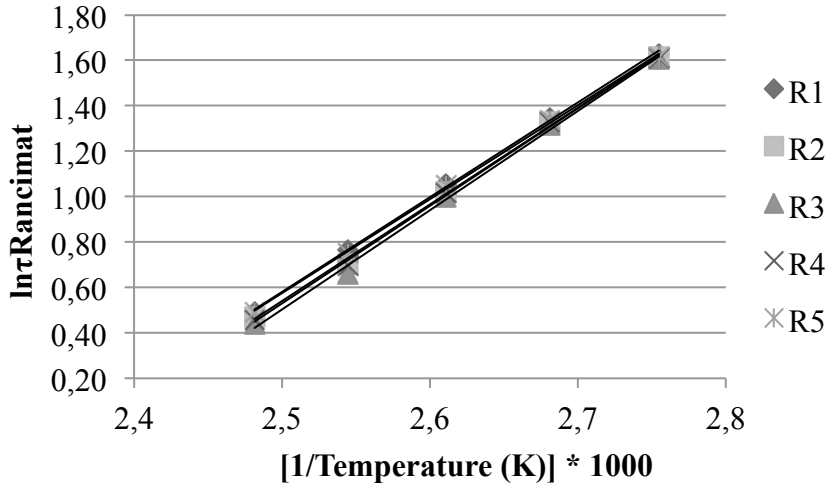


Figure 1. Semi-logarithmic correlation between lnτRancimat and (1/T) for oxidation of refined rapeseed oils.

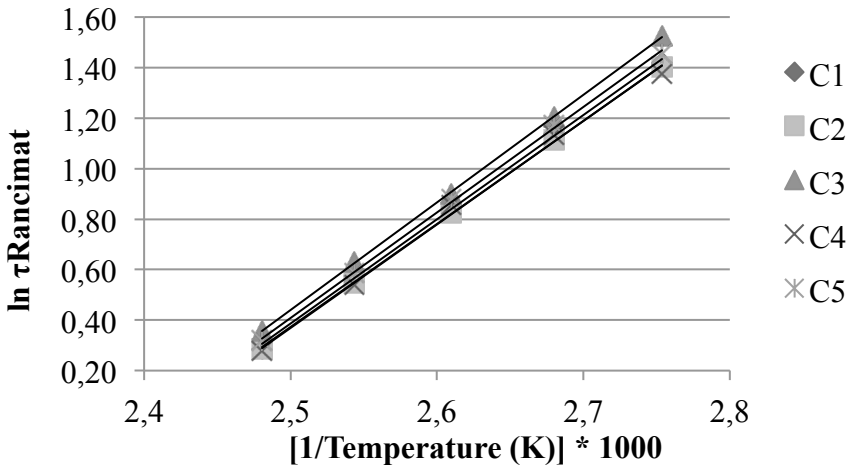


Figure 2. Semi-logarithmic correlation between lnτRancimat and (1/T) for oxidation of cold-pressed rapeseed oils.

Table 4 presents values of particular kinetic parameters of rapeseed oil oxidation. The refined rapeseed oils were characterized by generally higher values of activation energy (*Ea*) – a minimal energy of molecules needed to initiate the oxidation reaction, compared to the cold-pressed oils. The *Ea* values of oxidation reaction ranged from 76.43 to 82.44 kJ/mol for the refined oils as well as from 74.24 to 77.56 kJ/mol for the cold-pressed oils. The smaller load of energy required to initiate the oxidation reaction in the case of the cold-pressed oils may depend on many factors like, e.g., fatty acid composition, presence of endogenous antioxidants and prooxidants, e.g. primary and secondary products of oxidation, and metal ions. The cold-pressed oils were characterized by a higher TOTOX indicator than the refined ones (Table 1), which may affect a reduction in activation energy. The *Ea* values obtained for the refined oils in this study were lower than the values reported by KOWALSKI *et al*. (2004) – 85.3 kJ/mol and by FARHOOSH *et al*. (2008) – 89.94 kJ/mol, and similar to the value achieved by CIEMIENIEWSKA–ŻYTKIEWICZ *et al*. (2014) – 80.99 kJ/mol which fitted within the range obtained in our study. Differences in the values of this kinetic parameter between investigations of various authors may result from the biological variability of the raw material (ripening stage of a variety) (ADHVARYU *et al*., 2000).

In the case of the analyzed rapeseed oils, the value of a reaction rate constant *k* was increasing along with increasing temperature of oxidation. The rate of oxidation reaction was the highest at a temperature of 130 °C. The cold-pressed oils were characterized by a higher value of oxidation reaction rate constant at all temperatures compared to the refined oils. The same dependency was noted by KOWALSKI *et al*. (2004) and FARHOOSH *et al*. (2008) for rapeseed oils as well as by OSTROWSKA–LIGĘZA *et al*. (2010) for olive oil and by CIEMIENIEWSKA-ŻYTKIEWICZ *et al*. (2014) for hazel nuts. The oxidation rate constant of the cold-pressed oil was 2-3-fold higher than that of the refined oils despite small differences in the values of activation energy. It may be due to the fact that the cold-pressed oils contain more pro-oxidants that are removed from the refined oils in the refining process. The prediction of oil oxidation rate at low temperatures is limited based on data obtained. The course of oxidation process of oils differs at low and high temperatures (TAN *et al*., 2001).

Table 4. Kinetics parameters of refined and cold-pressed rapeseed oils oxidation.

Parameter	Rancimat calculation based on induction time									
	R1	R2	R3	R4	R5	C1	C2	C3	C4	C5
Equation 1										
a	0.0289	0.0300	0.0310	0.0301	0.0287	0.0282	0.028	0.0291	0.0285	0.0279
b	4.2293	4.3176	4.3990	4.3265	4.2030	3.9593	3.9145	4.122	4.022	3.9033
R^2	0.9999	0.9996	0.9989	0.9994	0.9999	0.9999	0.9997	0.9985	0.9998	0.9981
Equation 2										
A	4.2210	4.3855	4.5279	4.5279	4.1975	4.1283	4.0998	4.26	4.1778	4.0775
B	9.9824	10.4480	10.8460	10.8460	9.9301	9.9344	9.8809	10.21	10.036	9.8208
R^2	0.9990	0.9985	0.9979	0.9979	0.9985	0.9984	0.9998	0.9999	0.9995	0.9956
Ea [kJ/mol]	76.85	79.85	82.44	82.44	76.43	75.17	74.65	77.56	76.07	74.24
$Z^{\dagger}$	$9.6x10^9$	$2.81x10^{10}$	$7.01x10^{10}$	$7.01x10^{10}$	$8.51x10^9$	$8.60x10^9$	$7.60x10^9$	$1.61x10^{10}$	$1.09x10^{10}$	$6.62x10^9$
$k^{\dagger}$ at 130 °C	0.7611	0.5538	0.5438	0.5438	0.7233	1.5670	1.6167	1.0435	1.5178	1.5896
$k^{\dagger}$ at 120 °C	0.4246	0.3020	0.2908	0.2908	0.4048	0.8858	0.7828	0.7962	0.8522	0.9049
$k^{\dagger}$ at 110 °C	0.2297	0.1596	0.1505	0.1505	0.2198	0.4861	0.4295	0.4286	0.4642	0.5002
$k^{\dagger}$ at 100 °C	0.1203	0.0815	0.0752	0.0752	0.1155	0.2583	0.2282	0.2232	0.2448	0.2679
$k^{\dagger}$ at 90 °C	0.0608	0.0401	0.0361	0.0361	0.0586	0.1325	0.1171	0.1121	0.1246	0.1386
$\Delta H^{\dagger\dagger}$ [kJ/mol]	73.67	76.67	79.23	79.26	72.12	71.98	71.46	74.38	75.47	71.04
$\Delta S^{\dagger\dagger}$[J/molK]	-64.42	-55.84	-48.43	-48.35	-68.45	-67.47	-69.60	-62.33	-56.40	-69.99

†Z and k from Rancimat in h^{-1}

In addition, temperature increase is linked with increased solubility of oxygen – an increase in temperature by 10°C causes oxygen solubility increase by 25% (ROBERTSON, 2000).
Enthalpy and entropy were computed based on the active complex theory and results of linear regression from Tables 4. A high correlation ($R > 0.99$) is indicative of a very good fit and characterizes the effect of temperature on lipid oxidation using the active complex theory. The $\Delta H^{\neq}$ value determined for the analyzed refined rapeseed oils ranged from 72.12 to 79.26 kJ/mol, and that for the cold pressed oils from 71.04 to 75.47 kJ/mol. In turn, the $\Delta S^{\neq}$ value ranged from -48.35 to -68.45 J/molK for refined oils and from -56.40 to -69.99 J/molK for cold-pressed oils. KOWALSKI *et al*. (2004) obtained $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values at 82.0 kJ/mol and – 52.7J/molK for refined rapeseed oil, whereas FARHOOSH *et al*. (2008) at 86.79 kJ/mol and 112.99 J/molK, respectively. The negative $\Delta S^{\neq}$ values indicate that the active complexes are more ordered than molecules of reagents. Higher negative values point to a lesser probability of the formation of an active complex and to a slower rate of lipid oxidation (FARHOOSH *et al*., 2008). Results of our study confirm that the above statement is true for the samples of both refined and cold-pressed rapeseed oils.

4. CONCLUSIONS

In summary, the oxidative stability of the refined rapeseed oils determined with the Rancimat test is higher than that of the cold-pressed rapeseed oils. In turn, the cold-pressed oils are characterized by higher values of oxidation rate constant than the refined oils. In addition, they show slightly lower values of activation energy – *Ea*, which is linked, among other things, with a higher extent of oxidation of a fresh sample (a higher content of primary oxidation products) that accelerates the process of oxidation. Results obtained in this study seem to be consistent with findings and conclusions reported by other authors. The evaluation of oils stability at high temperatures based on this method should lead to similar conclusions and recommendations.

REFERENCES

Andersen L.M. and Skibsted L.H. 2002. Detection of early events in lipid oxidation by electron spin resonance spectroscopy. Eur. J. Lipid Sci. Technol. 104:65.

Anwar F., Bhanger M.I. and Kazi T.G. 2003. Relationship between Rancimat and Active Oxygen Method values at varying temperatures for several oils and fats. J. Am. Oil Chem. Soc. 80:151.

AOCS. 1997. Official Method Ce 2-66, Preparations of methyl esters of fatty acids.

AOCS. 1998. "Official Methods and Recommended Practice" 5th ed. Association of Official Analytical Chemists, Washington, DC.

AOCS. 1999 Official Method 965.33, Peroxide value of oils and fats.

AOCS. 2000. Official Method Cd 3d-63, Acid value.

AOCS. 2002. Official Method Cd 18-20, p-Anisidine Value.

AOCS. 2005. Official Method Ce 1h-05, Determination of cis-, trans-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC.

Casal S., Malheiro R., Sendas A., Olivieira B.P.P. and Pereira J.A. 2010. Olive oil stability under deep-frying conditions. Food Chem Toxicol. 48:2972.

Ciemieniewska-Żytkiewicz H., Ratusz K., Bryś J., Reder M. and Koczoń P. 2014. Determination of the oxidative stability of hazelnut oils by PDSC and Rancimat methods. J. Therm. Anal. Calorim. 118:875.

Čmolik J., Pokorńy J. 2000. Physical refining of edible oils. Eur. J. Lipid Sci. Technol. 102:472.

Čmolik J., Schwarz W., Svoboda Z., Pokorny J., Réblová Z., Dolezal M. and Valentová H. 2000. Effects of plant-scale alkali refining and physical refining on the quality of rapeseed oil. Eur. J. Lipid Sci. Technol. 102:15.

Codex Stan 2013. Codex Standard for Named Vegetable Oils. FAO/WHO, Rome (CODEX STAN 210-1999).

DIN 51605. 2010. Fuels for vegetable oil compatible combustion engines - fuel from rapeseed oil – requirements and test methods. Berlin:Deutsches Institut fur Normung.

Farhoosh R. 2007. The effect of operational parameters of the Rancimat method on the determination of the oxidative stability measures and shelf-life prediction of soybean oil. J. Am. Oil Chem. Soc. 84:205.

Farhoosh R. and Hoseini-Yazdi S.Z. 2014. Evolution of oxidative values during kinetic studies on olive oil oxidation in the Rancimat test. J. Am. Oil Chem. Soc. 91:281

Farhoosh R., Niazmand R., Rezaei M. and Sarabi M. 2008. Kinetic parameter determination of vegetable oil oxidation under Rancimat test conditions. Eur. J. Lipid. Sci. Technol. 110:587.

Giuffrè A.M., Capocasale M., Zappia C., Sicari V., Pellicanò T.M., Poiana M. and Panzera G. 2016. Tomato seed oil for biodiesel production. Eur. J. Lipid Sci. Technol. 118:640.

Giuffrè A.M., Tellah S., Capocasale M., Zappia C., Latati M., Badiani M. and Ounane S.M. 2016a. Seed oil from ten Algerian peanut landraces for edible use and biodiesel production. J. Oleo Sci. 65:9.

Giuffrè A.M. and Capocasale M. 2016b. Physicochemical composition of tomatoseed oil for an edible use:the effect of cultivar. Int. Food Res. J. 23:538.

Jacobsen C. and Nielsen N.S. 2008. Optimization of oxidative stability of omega – 3 enriched foods. In H. Brevik (Ed.). Longchain omega – 3 specialty oils (pp. 197 – 217). Bridgewater:The Oily Press Ltd.

Jain S. and Sharma M.P. 2011. Thermal stability of biodiesel and its blends:A review. Renew. Sust. Energ. Rev. 151:438.

Karabulut I., Topcu A., Yorulmaz A., Tekin A. and Sivri Ozay D. 2005. Effects of the industrial refining process on some properties of hazelnut oil. Eur. J. Lipid Sci. Technol. 107:476.

Kowalska D., Kostecka M., Tarnowska K. and Kowalski B. 2014. Oxidative stabilities of enzymatically interesterified goose fat and rapeseed oil blend by Rancimat and PDSC. J. Therm. Anal. Calorim. 115:2063.

Kowalski B., Ratusz K., Kowalska D. and Bekas W. 2004. Determination of the oxidative stability of vegetable oils by Differential Scanning Calorimetry and Rancimat measurements. Eur. J. Lipid Sci. Technol. 106:165.

Manzoor M., Anwar F., Igbal T. and Bhanger M.I. 2007. Physico-chemical characterization of Moringa concanensis seeds and seed oil. J. Am. Oil Chem. Soc. 84:413.

Matthäus B. and Brühl L. 2003. Quality of cold-pressed edible rapeseed oil in Germany. Nahrung/Food. 47:413.

Matthäus B., Guillaume D., Gharby S., Haddad A., Harhar H. and Charrouf Z. 2010. Effect of processing on the quality of edible argan oil. Food Chem. 120:425.

Matthäus B.W. 1996. Determination of the Oxidative Stability of Vegetable Oils by Rancimat and Conductivity and Chemiluminescence Measurements. J. Am. Oil Chem. Soc. 73:1039.

Ostrowska – Ligeza E., Bekas W., Kowalska D., Lobacz M., Wroniak M. and Kowalski B. 2010. Kinetics of commercial olive oil oxidation:Dynamic differentia scanning calorimetry and Rancimat studies. Eur. J. Lipid. Sci. Technol. 112:268.

Pawar N., Purohit A., Gandhi K., Arora S. and Singh R.R.B. 2014. Effect of operational parameters on determination of oxidative stability measured by Rancimat method. Int. J. Food Prop. 17:2082.

Ramos M.J., Fernández C.M., Casas A. and Rodríguez L.A. 2009. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresource Technol. 100:261.

Ratusz K., Kowalski B., Bekas W. and Wirkowska M. 2005. Monitorowanie autooksydacji oleju rzepakowego i słonecznikowego. Rośl. Oleiste. 26:211.

Ratusz K., Kowalski B., Gruczyńska E., Tarowska K., Bekas W. and Kowalska M. 2002. Evaluation of antioxidants activity in vegetable oils and triacylglycerol matrices by Rancimat measurements. Riv. Ital. Sostanze Gr. 79:251.

Rohn S. and Krohn L.W. 2005. Electron spin resonance – A spectroscopic method for determining the antioxidative activity. Mol. Nutr. Food Res. 49:898.

Roszkowska B., Tańska M., Czapliski S. and Konopka I. 2014. Variation in the composition and oxidative stability of commercial rapeseed oils during their shelf life. Eur. J. Lipid Sci. Technol. 117:673.

Shahidi F. and Zhong Y. 2010. Lipid oxidation and improving the oxidative stability. Chem. Soc. Rev. 39:4067.
Tan C.P., Che Man Y.B., Selemat J.M., Yusoff S.A. 2001. Application of Arrhenious kinetics to evaluate oxidative stability in vegetable oils by isothermal differential scanning calorimetry. J. Am. Oil. Chem. Soc. 78:1113.

Tasan M. and Demirci M. 2003. Trans fatty acids in sunflower oil at different steps of refining. J. Am. Oil Chem. Soc. 80:825.

Velasco J., Dobarganes C. and Márquez-Ruiz G. 2003. Variables affecting lipid oxidation in dried microencapsulated oils. Grasas Aceites. 54:304.

Wanasundara U.N., Shahidi F. and Jabłoński Ch.R. 1995. Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils. Food Chem. 52:249.

Paper Received November 26, 2015 Accepted August 25, 2016

MODELLING OF HYDRATION OF BEAN (*PHASEOLUS VULGARIS L.*): EFFECT OF THE LOW-FREQUENCY ULTRASOUND

L.R. LÓPEZ LÓPEZ[a], J.A. ULLOA*[a,b], P. ROSAS ULLOA[b], J.C. RAMÍREZ RAMÍREZ[c], Y. SILVA CARRILLO[d] and A. QUINTERO RAMOS[e]

[a]Posgrado en Ciencias Biológico Agropecuarias. Unidad Académica de Agricultura, Universidad Autónoma de Nayarit, Carretera Tepic-Compostela Km 9, CP 63780 Xalisco, Nayarit, México

[b]Centro de Tecnología de Alimentos, Universidad Autónoma de Nayarit, Ciudad de la Cultura Amado Nervo, C.P. 63155 Tepic, Nayarit, México

[c]Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nayarit, Carretera a Chapalilla Km 3.5, CP 63700 Compostela, Nayarit, México

[d]Unidad Académica de Agricultura, Universidad Autónoma de Nayarit, Carretera Tepic-Compostela Km 9, CP 63780 Xalisco, Nayarit, México

[e]Departamento de Investigación y Posgrado, Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, Circuito Universitario s/n, Campus Universitario No. 2, CP 31240 Chihuahua, Chihuahua, México

*Corresponding author. Tel. +52 3112118851; Fax +52 3112118861

E-mail address: arulloa5@gmail.com

ABSTRACT

Beans of six varieties were soaked in distilled water at 30 °C and exposed to ultrasound at powers of 5, 12 or 19 W, in addition to treatment control without ultrasound to attain the equilibrium moisture. From four model studied, the Weibull model presented the best fit (R^2 0.986-0.999) for the experimental data of the hydration kinetics. Soaking time was reduced from 52.6 % to 77.2 %, while the effective diffusivity was increased from 2.25 times to 3.50 times at 19 W, depending on the bean variety. The ultrasound power improved the hydration capability being suitable for possible use in industrial applications.

Keywords: beans, hydration, mathematical modelling, ultrasound, water diffusivity, Weibull´s model

1. INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) are a grain legumes that belong to the family of *Fabaceae*. They provide an affordable source of protein (16-33%), almost two to three times that of cereals), and also are a rich source of dietary fibre, starch, minerals and vitamins (MKANDA e*t al.*, 2007). Bioactive compounds present in common bean have been associated with the prevention and/or regulation of chronic degenerative diseases such as obesity, diabetes, coronary heart disease and cancer (CAMPOS-VEGA *et al*., 2010; PLANS *et al*., 2013).

On the other hand, the World Health Organization has issued several recommendations for reducing overweight, obesity and cardiovascular diseases that also are likely to reduce the risk of diet-related diseases, such as type 2 diabetes and obesity. These recommendations include the achievement of adequate intakes of non-starch polysaccharides through regular consumption of wholegrain cereals, legumes, fruits and vegetables (WHO/FAO, 2003). Of the leguminous plants consumed by humans, one of those of greatest importance on a worldwide level is the common bean (OLMEDILLA-ALONSO *et al*., 2013). Cooked dry beans along with whole grains have been emphasized as the primary nutritional shifts in food intake patterns to a plant-based diet by the 2010 Dietary Guidelines Advisory Committee (CAMPOS-VEGA *et al*., 2012).

Before the cooking step, beans are hydrated in water until maximum weight is reached (ULLOA *et al*., 2016). Hydration capacity is dependent on the ease of water absorption through the seed coat to the cotyledons (MKANDA *et al*., 2007). However, the soaking process is a time-consuming step, requiring approximately 12 h at room temperature (PIERGIOVANNI, 2011), and many attempts have been directed towards shortening it (Abu-Ghannam and McKenna, 1997).

Ultrasound power is a novel technology in the food industry, and research on its application is a rapidly growing field. Ultrasonic waves can cause a rapid series of alternative compressions and expansions similar to a sponge when it is squeezed and released repeatedly, a phenomenon known as cavitation (CHEMAT *et al*., 2011). Ultrasound cavitation results in the occurrence of microstreaming, which enhances heat and mass transfer (CÁRCEL *et al*., 2012).

Ultrasound applications were reported to enhances the hydration of chickpeas (YILDIRIM *et al*., 2011), sorghum grains (PATERO and AUGUSTO, 2015), and navy beans (GHAFOOR *et al*., 2014). However, as soaking conditions vary depending on the particular legume or food material, it is necessary for practical applications to characterise and optimise these conditions.

Therefore, the objective of the present study was to evaluate the effect of low-frequency ultrasound on the kinetics and modeling of the hydration of the six varieties of the most consumed beans in Mexico.

2. MATERIALS AND METHODS

2.1. Material

Common bean seeds from six varieties, which are highly consumed in Mexico (RODRÍGUEZ-LICEA *et al*., 2010) and classified as most preferred (Azufrado, Mayacoba, Pinto, Peruano Bola, Flor de Mayo and Negro Jamapa), were used for this study and were obtained from the Mercado de Abastos, located in Tepic, Nayarit, Mexico. These food legumes were separated from broken, small and split. Seeds were cleaned and size-graded

manually. Samples were stored in hermetically sealed bags inside closed plastic jars at room temperature (25 °C) in a dark room.

2.2. Chemical and morphological characterization of the bean seeds

The chemical composition (moisture, protein, fat, ash, and carbohydrates) of the beans was determined following the official methods of the AOAC (2002). For the morphological characterization, 100 beans of each variety were selected, and the weight (*we*), length (*l*), width (*w*) and depth (*d*) were measured to determine the geometric mean diameter (G_m), arithmetic mean diameter (A_m), square mean diameter (S_m) and the radius of an equivalent sphere (*r*) according to the methods reported by GAFHOOR *et al*. (2014).

2.3. Seed coat content

To determine the percentage of the coat of the seeds, the TIZAZU and EMIRE (2010) method with some modifications was used. Thirty bean seeds were soaked in distilled water for 8 h, and the coat was removed manually, separating it from the cotyledon. The seed coats collected were dried in an oven at 60° C for 24 h, followed cooling in a desiccator. It was then weighed and the percentage of seed coat was calculated.

2.4. Ultrasound soaking treatments

A sample of 5 g of beans was used in each one of the three replicates. The bean samples were soaked in a 100-mL beaker with 50 mL of distilled water and exposed to ultrasound at powers of 5, 12 or 19 W (20 kHz, 30 °C), using a GE-130 ultrasonic processor (Cole Parmer, Vernon Hills, Connecticut, Illinois) provided of a titanium probe of 6 mm. At specific time intervals (10 min for ultrasound treatments and 30 min for control, until stabilization), the seeds were removed from the water, drained, superficially blotted with absorbent paper, weighed and returned to the water.

2.5. Modeling the kinetics of hydration

For fitting the moisture uptake of soaked bean, four models were used to estimate the parameters associated with each model. The list of the models, and the respective equations used in this study, is presented in Table 1. The best fitted model was determined by the highest coefficient of determination (R^2) and the lowest values of the root mean square error (RMSE) and chi-square (χ^2) amongst the predicted and experimental results (COX *et al*., 2012).

2.6. Effective diffusivity (D_{eff})

D_{eff} was determined according to the method reported by KAPTSO *et al*. (2008) with the following equation:

$$c = \frac{\pi^2 D_{eff}}{r^2} \qquad \text{Eq. 1}$$

Where D_{eff} (m^2/s) is the effective diffusivity, c (s^{-1}) is the rate constant of the water absorption of the mathematical model with the best fit and r (m) is the value of radius of the equivalent sphere.

Table 1. Models used to describe moisture content and rate of moisture uptake by effect of ultrasound.

Model and reference	Equation
Peleg (PELEG, 1988)	$M_t = M_0 + \left[\frac{t}{c_1 + c_2 t}\right]$ (2)
Sigmoid (LEAL-OLIVEIRA *et al.*, 2013)	$M_t = \frac{M_e}{1+\exp[-k(t-\tau)]}$ (3)
First order (GHAFOOR *et al.*, 2014)	$MR = 1 - \exp(-k_1 t)$ (4)
Weibull (ZURA *et al.*, 2013)	$MR = 1 - \exp\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right]$ (5)

MR is the rate of moisture uptake, and is given by the equation: $MR = \frac{M_0 - M_t}{M_0 - M_e}$
where M_0 is the initial moisture content of the bean, M_t is the moisture content of bean at time *t*, and M_e is the final moisture content at equilibrium.
t is the hydration duration (in min), and the variables c_1, c_2, k_1, k, τ, α and β are the coefficients used in nonlinear regression analysis with the various models.

2.7. Statistical analysis

One-way analysis of variance and Tukey tests were performed to determine the difference between the values of seed morphological traits (*we, l, w, d*, G_m, A_m, S_m *and r*), chemical composition (moisture, proteins, fats, ashes and total carbohydrates), *%Cs* and the kinetic parameters of the soaking treatments (M_e and D_{eff}) of the different varieties of beans, using Statgraphics Plus 5.0 statistical package (Statistical Graphics Corp., MD, USA). Significance level were tested at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Chemical and morphological characterization of the bean seeds

Table 2 shows the results of *we*, *l*, *w*, *d*, G_m, A_m, S_m and *r* of the six bean varieties studied. According to the obtained results, the Azufrado bean variety presented the lowest values in the majority of the morphological characteristics studied, and the Mayocoba and Flor de Mayo varieties presented the highest values. Although Flor de Mayo and Azufrado beans had the highest *r* (0.41 cm) and the lowest *r* (0.30 cm), respectively, the majority of the varieties of bean used in this study presented a higher *r* value in comparison with the Navy bean (GHAFOOR *et al*., 2014).
Table 3 shows the chemical composition of the six varieties of beans studied. The values of the proximal composition of the six bean varieties of this study are similar to those reported by WANI *et al*. (2015) for other bean varieties, with the exception of the moisture content for the Mayocoba variety, which was the highest, and the fat content for the Pinto variety, which was the lowest.

3.2. Kinetics of water absorption

The water absorption kinetics of the different varieties of bean studied are shown in Fig. 1. The time to attain the *Me* ranged from 330 min to 660 min depending of the bean variety, being the shortest time for Mayacoba and longest time for Negro Jamapa. According to this finding, exposure to ultrasound reduced the soaking time of the bean; however, at a higher power of ultrasound, the time lapse was even lower. From the six different varieties of bean studied, after of the ultrasound treatments at 5W, 10 W and 19 W, the shortest and longest soaking times were 173 min and 280 min, 108 and 360 min, and 123 min and 313

min, respectively, corresponding to Mayacoba and Peruano Bola, Mayacoba and Negro Jamapa, and Pinto and Negro Jamapa.

Table 2. Characteristics of the six varieties of common bean *(Phaseolus vulgaris)* seeds.

Parameters	Bean varieties					
	Mayocoba	Azufrado	Pinto	Peruano Bola	Flor de Mayo	Negro Jamapa
we (g)	0.39±0.03^{a}	0.25±0.02^{d}	0.36±0.05^{b}	0.32±0.04^{c}	0.35±0.06^{b}	0.24±0.04^{d}
l (cm)	1.29±0.06^{a}	1.08±0.06^{c}	1.25±0.08^{b}	1.03±0.06^{d}	1.25±0.01^{b}	0.96±0.07^{e}
w (cm)	0.70±0.04^{cd}	0.66±0.03^{e}	0.73±0.05^{b}	0.72±0.03^{bc}	0.76±0.05^{a}	0.69±0.08^{d}
d (cm)	0.59±0.05^{a}	0.47±0.04^{d}	0.56±0.05^{b}	0.61±0.03^{a}	0.52±0.04^{c}	0.53±0.04^{c}
G_m (cm)	0.82±0.04^{a}	0.70±0.03^{d}	0.79±.0.04^{ab}	0.76±0.03^{c}	0.79±0.06^{b}	0.71±0.04^{d}
A_m (cm)	0.86±0.04^{a}	0.74±0.03^{d}	0.85±0.05^{ab}	0.78±0.03^{c}	0.84±0.06^{b}	0.73±0.04^{b}
S_m (cm)	0.41±0.01^{d}	0.37±0.01^{e}	0.41±0.01^{d}	0.84±0.02^{b}	0.87±0.04^{a}	0.80±0.03^{c}
r (cm)	0.35±0.01^{d}	0.30±0.00^{e}	0.34±0.02^{d}	0.39±0.01^{b}	0.41±0.02^{a}	0.37±0.02^{c}

Values are given as means±standard deviation (n = 100). Different superscript letters in the same row indicate significant differences ($P < 0.05$).
we: weight; *l*: length; *w*: width; *d*: depth; G_m: geometric mean diameter; A_m: arithmetic mean diameter; S_m: square mean diameter; *r*: radius of an equivalent sphere.

Table 3. Seed proximate composition of the six varieties of common bean (*Phaseolus vulgaris*).

Component	Bean varieties					
	Mayocoba	Azufrado	Pinto	Peruano Bola	Flor de mayo	Negro Jamapa
Moisture (%)	12.8 ±0.1^{a}	11.2±0.1^{bc}	11.0±0.4^{bcd}	11.4±0.3^{b}	9.5±0.1^{e}	8.9±0.1^{f}
Fat (%)	1.6±0.2^{bc}	2.1±0.2^{b}	0.6±0.1^{d}	4.7±0.5^{a}	1.0±0.1^{cd}	1.5± 0.1^{bcd}
Ash (%)	4.5±0.1^{a}	4.8±0.2^{a}	4.1±0.1^{a}	4.5±0.1^{a}	4.0±0.2^{a}	4.6±0.1^{a}
Protein (%, N x 6.25)	23.5± 0.5^{a}	23.6±0.1^{a}	23.9±0.5^{a}	23.8±0.4^{a}	23.2±0.2^{a}	23.9±0.5^{a}
Total carbohydrates (%)	57.6±0.5	58.3±0.2	60.4±0.5	55.6±0.5	62.3±0.2	61.1±0.5

Values are given as means±standard deviation (n = 3). Different superscript letters in the same row indicate significant differences ($P < 0.05$).

On the other hand, the Azufrado and Peruano Bola bean varieties presented an initial lag phase or lateness (period with a low water absorption rate) for the 5 W ultrasound treatment and the control treatment, whereas the Pinto, Flor de Mayo and Negro Jamapa bean varieties presented initial lag phase for the ultrasound treatments of 5 W and 12 W, as well as the control treatment.
The behavior of the hydration kinetics of this study has been observed in the conventional soaking (25-55° C) of common bean by PIERGIOVANNI (2011), who classified the bean varieties into three groups as a function of the rate of hydration (fast, intermediate and slow); the fast and intermediate bean varieties did not present an initial lag phase, but the slow hydration bean varieties did have an initial lag phase. According to this classification, the Mayocoba bean variety can be considered to have a rapid hydration rate, in contrast to the rest of the bean varieties studied, even though with the application of the ultrasound power, especially at 19 W, the initial lag phase of the bean hydration of the varieties of

Azufrado, Pinto, Peruano Bola and Flor de Mayo was reduced. KAPTSO *et al*. (2008) reported that the application of ultrasound for soaking has an effect similar to increasing the soaking temperature, thus overcoming the defect of low water absorption observed in the seeds.

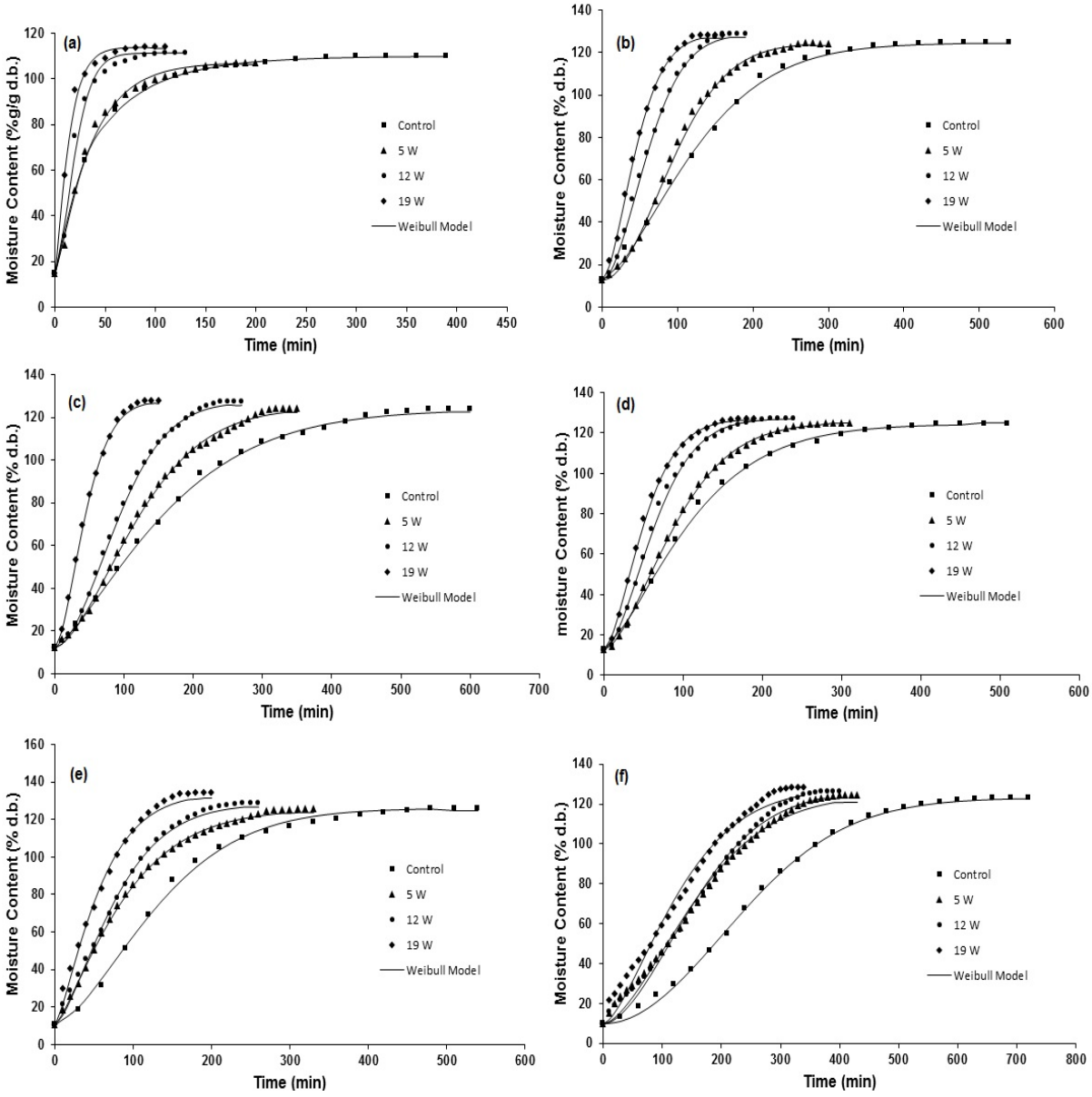


Figure 1. Water absorption kinetic of (a) Mayocoba, (b) Azufrado, (c) Pinto, (d) Peruano Bola, (e) Flor de Mayo and (f) Negro Jamapa bean varieties during soaking at different ultrasound powers. Solid lines represent the corresponding Weibull model fitted to the experimental data.

The statistical parameters generated from the application of the different mathematical models (Weibull, Peleg, First Order, and Sigmoid) describing the kinetics of water absorption for the bean varieties are presented in Table 4. In general, the Sigmoid model

presented higher values of R^2 (0.998) and lower χ^2 (0.000) and *RSME* (0.019) than the Peleg and First Order models (Table 4); in addition, Sigmoid model can describe the initial lag phase followed by a high rate absorption phase and, finally, a stationary phase (LEAL-OLIVEIRA *et al*., 2013). However, the Weibull model presented the best fit, giving the highest values of R^2 (0.986-0.999) and the lowest of χ^2 (0.000-0.002) and *RSME* (0.013-0.044), in accordance with MARABI *et al*. (2003), who found that such model is one of the best for describing the kinetics of food hydration.

Table 4. Statistical parameters of the mathematical models fitted to the kinetics of hydration of six bean varieties.

Model	Ultrasound power (W)	Mayocoba			Azufrado			Pinto			Peruano Bola			Flor de Mayo			Negro Jamapa		
		R^2	χ^2	RSME	R^2	χ^2	RSME	R^2	χ^2	RSME	R^2	χ^2	RSME	R^2	χ^2	RSME	R^2	χ^2	RSME
Sigmoidal	Control	0.961	0.003	0.050	0.997	0.001	0.023	0.997	0.001	0.027	0.989	0.002	0.035	0.991	0.002	0.036	0.993	0.001	0.016
	5	0.970	0.002	0.046	0.998	0.000	0.019	0.996	0.001	0.024	0.996	0.001	0.022	0.987	0.002	0.039	0.996	0.001	0.020
	12	0.983	0.002	0.040	0.996	0.001	0.024	0.998	0.000	0.019	0.994	0.001	0.029	0.997	0.001	0.020	0.995	0.001	0.025
	19	0.985	0.002	0.036	0.996	0.001	0.024	0.997	0.001	0.023	0.985	0.002	0.040	0.992	0.001	0.034	0.995	0.001	0.028
First Order	Control	0.989	0.001	0.026	0.971	0.006	0.070	0.991	0.002	0.046	0.961	0.006	0.070	0.959	0.006	0.076	0.906	0.016	0.123
	5	0.990	0.001	0.026	0.921	0.013	0.110	0.940	0.009	0.091	0.949	0.007	0.084	0.981	0.002	0.046	0.933	0.010	0.095
	12	0.966	0.004	0.056	0.929	0.013	0.108	0.922	0.014	0.112	0.943	0.009	0.092	0.966	0.005	0.069	0.911	0.014	0.113
	19	0.991	0.001	0.028	0.949	0.009	0.090	0.951	0.009	0.087	0.956	0.007	0.080	0.977	0.004	0.059	0.942	0.008	0.087
Peleg	Control	0.994	0.000	0.020	0.977	0.004	0.062	0.994	0.002	0.038	0.973	0.004	0.059	0.962	0.006	0.075	0.959	0.007	0.081
	5	0.979	0.002	0.038	0.961	0.007	0.077	0.979	0.003	0.053	0.972	0.004	0.062	0.986	0.002	0.039	0.988	0.002	0.039
	12	0.948	0.006	0.070	0.975	0.005	0.064	0.971	0.005	0.067	0.959	0.007	0.078	0.986	0.002	0.0433	0.987	0.002	0.041
	19	0.979	0.002	0.044	0.980	0.004	0.056	0.979	0.004	0.057	0.970	0.005	0.059	0.993	0.001	0.031	0.993	0.001	0.031
Weibull	Control	0.995	0.000	0.017	0.996	0.001	0.022	0.999	0.000	0.013	0.997	0.000	0.0171	0.995	0.001	0.025	0.998	0.001	0.018
	5	0.991	0.001	0.025	0.997	0.001	0.022	0.998	0.000	0.016	0.999	0.001	0.010	0.997	0.001	0.018	0.991	0.001	0.033
	12	0.986	0.002	0.036	0.998	0.000	0.019	0.999	0.000	0.014	0.996	0.001	0.0213	0.996	0.001	0.021	0.991	0.002	0.035
	19	0.993	0.001	0.025	0.999	0.000	0.015	0.999	0.000	0.013	0.997	0.000	0.0163	0.993	0.001	0.033	0.984	0.002	0.044

Figure 1 also shows the kinetics of bean hydration fitted to the Weibull model where α is a scale parameter and β a shape parameter. The scale parameter defines the rate of moisture uptake process (α is the reciprocal of the process rate constant) and represents the time needed to accomplish the approximately 63% of the moisture uptake process, while the shape parameter is a behavior index, which depends on the process mechanism.
The kinetic parameters generated from the application of the different mathematical models for evaluating the hydration kinetics of beans by effect of the ultrasound treatment are shown in Table 5. According to the results obtained, the constants of hydration rate, k and k_1, of the Sigmoidal and First Order Models, respectively, were increased for effect of ultrasound power from 1.49 to 4.04 times and from 1.93 to 3.56 times in comparison with the control treatment, depending of the bean variety. Regarding Peleg's model, the first constant, c_1, which has been shown to be linked to the hydration was reduced for all bean varieties by effect of ultrasound in comparison with the control treatment. Therefore, the application of ultrasound and its increasing of power level improved the hydration properties such as reflected in the rate constants of the Sigmoidal, First Order and Peleg models.
In relation to the α parameter of Weibull model, its value decreased with increasing ultrasound power for all the bean varieties (Table 5), in agreement with the reported results by GHAFOOR *et al*. (2014) for Navy bean.
On the other hand, the values of kinetic parameter of hydration β of Weibull model (Table 5) for the soaking treatments of the different varieties of beans were high (0.834-1.995). According to MACHADO *et al*. (1999), the Weibull model predicts the initial lag phase of the hydration kinetics when β is greater than 1, as it was observed for the different treatments and bean varieties in this study, except for the control treatment in the Mayocoba variety.

3.3. Seed coat content

The Flor de Mayo and Mayacoba bean varieties presented the highest *%Cs* (9.85±0.62 and 8.82±0.62, respectively), followed by the Negro Jamapa (8.65±0.02), Peruano Bola (7.92±0.36), Azufrado (7.56±0.19) and Pinto (7.48±0.50) varieties bean. In this study, the varieties of bean with the first and third highest *%Cs* (Flor de Mayo and Negro Jamapa) required a higher soaking lapse to attain *Me* in comparison with the fourth and fifth highest *%Cs* (Peruano Bola and Azufrado). However, the time to attain *Me* of the Pinto variety with a *%Cs* lower than the Mayacoba variety was longer (Fig. 1).
On the other hand, the varieties of bean with a higher *%Cs* presented a higher coat rupture by effect of the ultrasound (Table 6). In the varieties of Azufrado, Pinto, Peruano Bola, Flor de Mayo and Negro Jamapa, the percentage of seeds with a higher percentage of coat rupture increased as the ultrasound power was higher (5, 12 and 19 W). The Negro Jamapa and Flor de Mayo varieties presented coat rupture in all soaking treatments, including the control. The results suggest that the percentage of the coat rupture depended on bean variety and the ultrasound power used in the soaking process, agreeing with the report of PAN *et al*. (2010).

Table 5. Kinetic parameters for the models fitted to the hydration kinetics data of six bean varieties.

Model	Soaking treatment	Bean varieties					
		Mayocoba	**Azufrado**	**Pinto**	**Peruano Bola**	**Flor de Mayo**	**Negro Jamapa**
Sigmoidal	Control	$k = 5.351 \times 10^{-2}$ $\tau = 26.906$	$k = 1.740 \times 10^{-2}$ $\tau = 104.356$	$k = 1.261 \times 10^{-2}$ $\tau = 131.756$	$k = 2.034 \times 10^{-2}$ $\tau = 87.458$	$k = 1.803 \times 10^{-2}$ $\tau = 114.714$	$k = 1.111 \times 10^{-2}$ $\tau = 225.728$
	5 W	$k = 5.433 \times 10^{-2}$ $\tau = 24.634$	$k = 2.666 \times 10^{-2}$ $\tau = 84.062$	$k = 1.900 \times 10^{-2}$ $\tau = 104.513$	$k = 2.571 \times 10^{-2}$ $\tau = 76.631$	$k = 2.267 \times 10^{-2}$ $\tau = 71.294$	$k = 1.436 \times 10^{-2}$ $\tau = 139.061$
	12 W	$k = 11.354 \times 10^{-2}$ $\tau = 16.368$	$k = 3.920 \times 10^{-2}$ $\tau = 54.383$	$k = 2.681 \times 10^{-2}$ $\tau = 82.217$	$k = 3.739 \times 10^{-2}$ $\tau = 56.192$	$k = 2.754 \times 10^{-2}$ $\tau = 65.193$	$k = 1.595 \times 10^{-2}$ $\tau = 143.056$
	19 W	$k = 15.757 \times 10^{-2}$ $\tau = 10.395$	$k = 5.020 \times 10^{-2}$ $\tau = 39.387$	$k = 5.100 \times 10^{-2}$ $\tau = 38.667$	$k = 4.524 \times 10^{-2}$ $\tau = 43.394$	$k = 3.540 \times 10^{-2}$ $\tau = 46.346$	$k = 1.659 \times 10^{-2}$ $\tau = 110.453$
First Order	Control	$k_1 = 2.458 \times 10^{-2}$	$k_1 = 0.750 \times 10^{-2}$	$k_1 = 0.576 \times 10^{-2}$	$k_1 = 0.8445 \times 10^{-2}$	$k_1 = 0.694 \times 10^{-2}$	$k_1 = 3.678 \times 10^{-3}$
	5 W	$k_1 = 2.711 \times 10^{-2}$	$k_1 = 0.945 \times 10^{-2}$	$k_1 = 0.742 \times 10^{-2}$	$k_1 = 1.051 \times 10^{-2}$	$k_1 = 1.060 \times 10^{-2}$	$k_1 = 5.729 \times 10^{-3}$
	12 W	$k_1 = 4.425 \times 10^{-2}$	$k_1 = 1.460 \times 10^{-2}$	$k_1 = 0.965 \times 10^{-2}$	$k_1 = 1.422 \times 10^{-2}$	$k_1 = 1.219 \times 10^{-2}$	$k_1 = 5.575 \times 10^{-3}$
	19 W	$k_1 = 6.848 \times 10^{-2}$	$k_1 = 2.000 \times 10^{-2}$	$k_1 = 2.050 \times 10^{-2}$	$k_1 = 1.808 \times 10^{-2}$	$k_1 = 1.703 \times 10^{-2}$	$k_1 = 7.121 \times 10^{-3}$
Peleg	Control	$c_1 = 23.619$ $c_2 = 0.959$	$c_1 = 123.560$ $c_2 = 0.568$	$c_1 = 160.676$ $c_2 = 0.569$	$c_1 = 100.512$ $c_2 = 0.626$	$c_1 = 123.575$ $c_2 = 0.558$	$c_1 = 329.984$ $c_2 = 0.321$
	5 W	$c_1 = 30.503$ $c_2 = 0.884$	$c_1 = 123.423$ $c_2 = 0.374$	$c_1 = 156.812$ $c_2 = 0.3657$	$c_1 = 102.118$ $c_2 = 0.471$	$c_1 = 81.568$ $c_2 = 0.564$	$c_1 = 223.034$ $c_2 = 0.274$
	12 W	$c_1 = 18.995$ $c_2 = 0.818$	$c_1 = 79.673$ $c_2 = 0.328$	$c_1 = 123.713$ $c_2 = 0.3115$	$c_1 = 69.255$ $c_2 = 0.505$	$c_1 = 79.689$ $c_2 = 0.468$	$c_1 = 234.070$ $c_2 = 0.159$
	19 W	$c_1 = 10.078$ $c_2 = 0.868$	$c_1 = 54.001$ $c_2 = 0.402$	$c_1 = 51.482$ $c_2 = 0.4301$	$c_1 = 54.229$ $c_2 = 0.506$	$c_1 = 52.746$ $c_2 = 0.475$	$c_1 = 163.072$ $c_2 = 0.297$
Weibull	Control	$\alpha = 42.481$ $\beta = 0.834$	$\alpha = 141.833$ $\beta = 1.493$	$\alpha = 181.544$ $\beta = 1.336$	$\alpha = 124.212$ $\beta = 1.372$	$\alpha = 150.984$ $\beta = 1.534$	$\alpha = 286.092$ $\beta = 1.995$
	5 W	$\alpha = 37.404$ $\beta = 1.076$	$\alpha = 110.515$ $\beta = 1.838$	$\alpha = 139.957$ $\beta = 1.607$	$\alpha = 102.955$ $\beta = 1.593$	$\alpha = 96.963$ $\beta = 1.259$	$\alpha = 182.245$ $\beta = 1.6121$
	12 W	$\alpha = 23.207$ $\beta = 1.514$	$\alpha = 71.718$ $\beta = 1.732$	$\alpha = 107.840$ $\beta = 1.788$	$\alpha = 74.790$ $\beta = 1.660$	$\alpha = 86.939$ $\beta = 1.425$	$\alpha = 183.437$ $\beta = 1.880$
	19 W	$\alpha = 15.019$ $\beta = 1.159$	$\alpha = 52.644$ $\beta = 1.599$	$\alpha = 51.570$ $\beta = 1.588$	$\alpha = 58.377$ $\beta = 1.545$	$\alpha = 62.154$ $\beta = 1.287$	$\alpha = 146.326$ $\beta = 1.483$

3.4. Equilibrium moisture content (*Me*)

The varieties of beans studied presented a *Me* that oscillated from 107.18 to 133.99 % d.b. (Table 6). The *Me* in the soaking control treatments increased for all the bean varieties, except for Peruano Bola (Table 6). The effect caused by the ultrasound power in the bean hydration is similar to the effect caused by the increase of temperature for the conventional soaking of bean (SHAFAEI *et al*., 2014), sesame seeds (KHAZAEI and MOHAMMADI, 2009), rice (CHEEVITSOPON and NOOMHORM, 2011), and Botswana Bambara (JIDEANI and MPOTOKWANA, 2009).

Table 6. Effect of soaking treatment on the hydration properties of beans at 30 °C.

Variety/Property	Soaking treatments			
	Control	5 W	12 W	19 W
Mayocoba				
Me (% g/g d.b.)	110.08^{b}	107.18^{c}	111.46^{ab}	113.98^{a}
Rupture of coat (%)	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
D_{eff} (x 10^{-10} m^2/s)	4.845^{c}	5.501^{c}	8.852^{b}	13.717^{a}
Azufrado				
Me (% g/g d.b.)	124.47^{b}	124.32^{b}	128.60^{a}	128.38^{a}
Rupture of coat (%)	0.0^{b}	0.0^{b}	0.0^{b}	5.0^{a}
D_{eff} (x 10^{-10} m^2/s)	0.380^{d}	0.488^{c}	0.752^{b}	1.026^{a}
Pinto				
Me (% g/g d.b.)	123.72^{b}	124.46^{b}	127.24^{a}	127.69^{a}
Rupture of coat (%)	0.0^{b}	0.0^{b}	0.0^{b}	16.6^{a}
D_{eff} (x 10^{-10} m^2/s)	1.097^{d}	1.413^{c}	1.839^{b}	3.847^{a}
Peruano Bola				
Me (% g/g d.b.)	124.47^{a}	125.08^{a}	127.18^{a}	126.96^{a}
Rupture of coat (%)	0.0^{b}	0.0^{b}	0.0^{b}	1.5^{a}
D_{eff} (x 10^{-10} m^2/s)	2.135^{b}	2.616^{b}	4.168^{a}	4.633^{a}
Flor de mayo				
Me (% g/g d.b.)	125.96^{b}	125.59^{b}	128.39^{ab}	133.99^{a}
Rupture of coat (%)	15.5^{c}	29.0^{b}	40.0^{a}	49.0^{a}
D_{eff} (x 10^{-10} m^2/s)	1.967^{c}	3.056^{b}	3.414^{b}	5.025^{a}
Negro Jamapa				
Me (% g/g d.b.)	123.07^{c}	124.68^{bc}	126.22^{ab}	128.05^{a}
Rupture of coat (%)	1.6^{b}	10.0^{a}	13.3^{a}	21.6^{a}
D_{eff} (x 10^{-10} m^2/s)	0.711^{c}	1.287^{b}	1.275^{b}	1.60^{a}

Values are given as means±standard deviation (n = 3). Different superscript letters in the same row indicate significant differences ($P < 0.05$).

3.5. Effect of ultrasound on the effective diffusivity (D_{eff})

To calculate D_{eff} (Eq. 6), the inverse of α was used ($1/\alpha$) as the rate constant of water absorption. Table 6 shows the effects of the ultrasound treatments during the bean soaking process on D_{eff} for the distinct studied varieties.

The soaking treatment with ultrasound at 19 W obtained the highest values of D_{eff} for all bean varieties. The increase of D_{eff} observed in this study as the ultrasound power was increased is congruent with a study on chickpeas, where the value of D_{eff} of the soaking control treatment at 30 °C was 1.87 x 10^{10} m^2/s and it was increased with the application of ultrasound to values of 2.10 x 10^{10} and 2.62 x 10^{10} (m^2/s) for the soaking treatments at 25 kHz and 100 W and 25 kHz and 300 W, respectively (YILDIRIM *et al*., 2011).
The increase of the absorption of water during the assisted soaking process with ultrasound is due to the formation of microscopic channels in the grains, which reduce the internal resistance to mass transference (FUENTE-BLANCO *et al*., 2006).
In this study we observed that increasing the ultrasound power in the soaking treatments of the beans generated a proportional increase on the values of D_{eff} (Fig. 2), in similarity to the proportional increasing of the water absorption during soaking of bean by effect of increase of temperature (SHAFAEI *et al*., 2014).

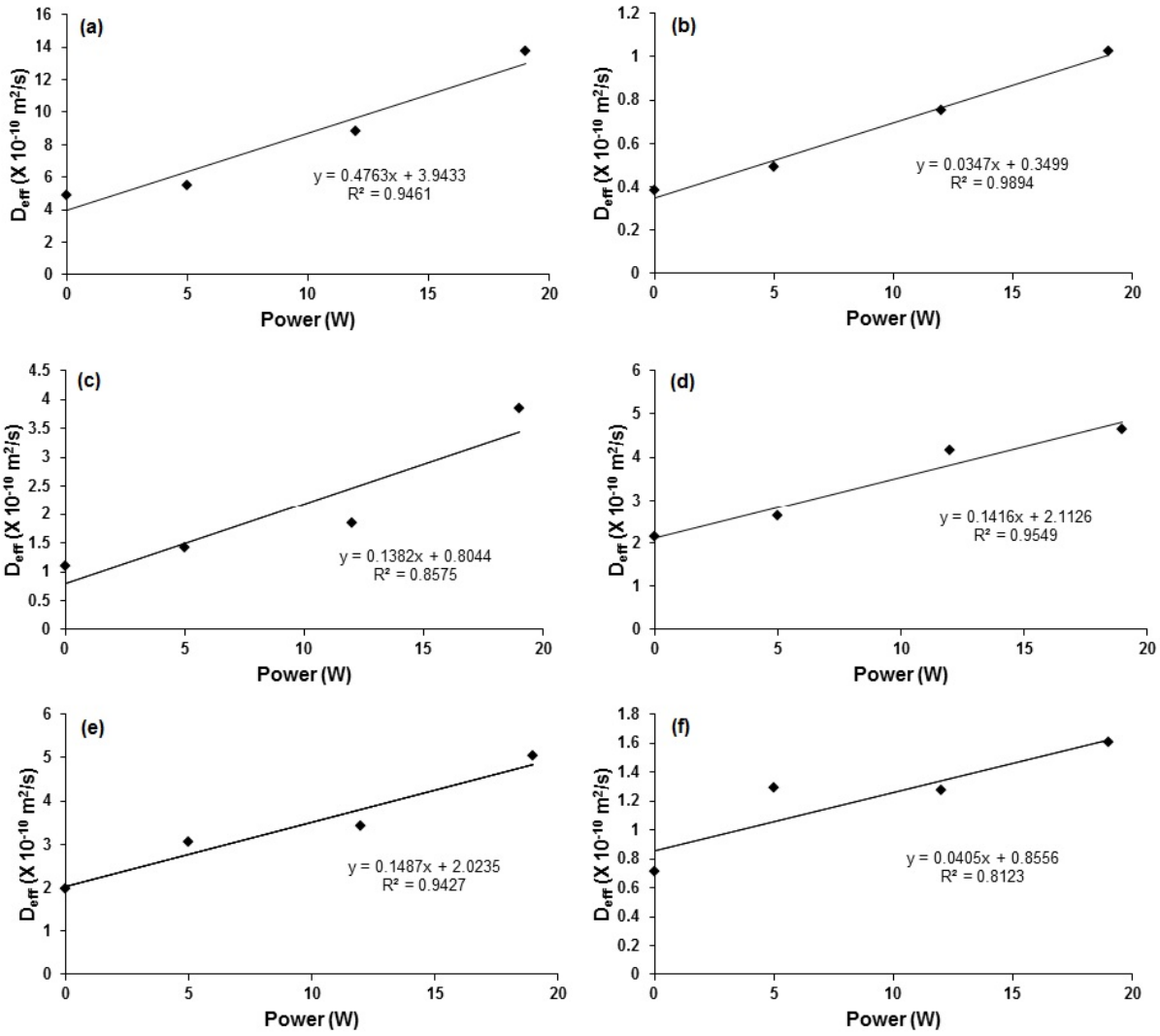


Figure 2. Effect of low frequency ultrasound power (20 kHz) on the D_{eff} in the soaking of the Mayocoba (a), Azufrado (b), Pinto (c), Peruano Bola (d), Flor de mayo (e) and Negro Jamapa (f) bean varieties.

4. CONCLUSIONS

The application of ultrasound reduced the lapse of soaking in the six varieties of beans studied, although the reduction depended of the bean variety and the ultrasound power. The soaking treatment at 19 W presented the highest values of D_{eff}, *Me* and the least time to attain *Me*. However, this ultrasound treatment also presented coat rupture of the beans, in different proportion, except for the Mayocoba variety. Of the models used, the Weibull model presented the best fit for the experimental data of the hydration kinetics, in the different soaking treatments and for the majority of the bean varieties.

ACKNOWLEDGEMENTS

During the devolvement of this study, financial support was obtained from the Consejo Nacional de Ciencia y Tecnología (CONACYT) with scholarship number 550780.

REFERENCES

Abu-Ghannam, N. and McKenna, B. 1997. Hydration kinetics of red kidney beans (*Phaseolus vulgaris* L.). J. Food Sci. 62, 520-523.

AOAC. 2002. "Official Methods of Analysis of AOAC International" Association of Official Analytical Chemists, Gaithersburg, MD.

Campos-Vega R., Loarca-Piña G. and Oomah B.D. 2010. Minor components of pulses and their potential impact on human health. Food Res Int**.** 43:461-482

Campos-Vega R., Vergara-Castañeda H.A. and Oomah B.D. 2012. In: "Beans: Nutrition, Consumption and Health: Functional Food Sources: Beans in Sight". E. Popoescu, I. Golubev (Ed.), p 1-56. Nova Science Publishers, New York.

Cárcel J.A., García-Pérez J.V., Benedito J. and Mulet A. 2012. Food process innovation through new technologies: Use of ultrasound. J. Food Eng. 110: 200-207.

Cheevitsopon E. and Noomhorm A. 2011. Kinetics of hydration and dimensional changes of brown rice. J. Food Process Pres. 35:840-849.

Chemat F., Huma Z. and Khan M.K. 2011. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrason Sonochem. 18:813-835.

Cox S., Gupta S. and Abu-Ghannam N. 2012. Effect of different rehydration temperatures on the moisture, content of phenolic compounds, antioxidant capacity and textural properties of edible Irish brown seaweed. LWT-Food Sci Technol. 47:300-307.

Fuente-Blanco, S.D.L., Riera-Franco de Sarabia E., Acosta-Aparicio V.M., Blanco-Blanco A. and Gallego-Juárez J.A. 2006. Food drying process by power ultrasound. Ultrasonics 44: e523-e527.

Ghafoor M., Misra N.N., Mahadevan K. and Tiwari B.K. 2014. Ultrasound assisted hydration of navy beans (*Phaseolus vulgaris*). Ultrason Sonochem. 21:409-414.

Jideani V.A. and Mpotokwana S.M. 2009. Modeling of water absorption of Botswana Bambara varieties using Peleg's equation. J. Food Eng 92:182-188.

Kaptso K.G., Njintang Y.N., Komnek A.E., Hounhouigan J., Scher J. and Mbolung C.M.F. 2008. Physical properties and rehydration kinetics of two varieties of cowpea (*Vigna unguiculata*) and bambara groundnuts (*Voandzeia subterrânea*) seeds. J. Food Eng. 86: 91-99.

Khazaei J. and Mohammadi N. 2009. Effect of temperature on hydration kinetics of sesame seeds (*Sesamum indicum* L.). J. Food Eng 91:542-552.

Leal-Oliveira A., Gouveia-Colnaghi B., Zucatti-da-Silva E., Romáo-Gouvêa I., Lopes-Vieira R. and Duarte-Augusto P.E. 2013. Modelling the effect of temperature on the hydration kinetic of Adzuki beans (*Vigna angularis*). J. Food Eng 118:417-420.

Machado M.F., Oliveira F.A. and Cunha L.M. 1999. Effect of milk fat and total solids concentration on the kinetics of moisture uptake by ready-to-eat breakfast cereal. Int. J. Food Sci. Tech. 34: 47-57.

Marabi A., Livings S., Jacobson M. and Saguy. I. 2003. Normalized Weibull distribution for modeling rehydration of food particulates. Eur. Food Res. Technol. 217: 311-318.

Mkanda A.V., Minnaar A. and Kock H.L. 2007. Relating consumer preferences to sensory and physicochemical properties of dry beans (*Phaseolus vulgaris*). J. Sci. Food Agric. 87:2868-2879.

Olmedilla-Alonso B., Pedrosa M.M., Cuadrado C., Brito M., Asensio-S-Manzanera C. and Asensio-Vegas, C. 2013. Composition of two Spanish common dry beans (*Phaseolus vulgaris)*, 'Almonga' and 'Curruquilla', and their postprandial effect in type 2 diabetics. J. Sci. Food Agric. 93:1076-1082.

Pan Z., Atungulu G.G., Wei L. and Haff R. 2010. Development of impact acoustic detection and density separations methods for production of high quality processed beans. J. Food Eng. 97:292-300.

Patero T. and Augusto P.E.D. 2015. Ultrasound (US) enhances the hydration of sorghum (*Sorghum bicolor*) grains. Ultrason. Sonochem 23:11-15.

Peleg, M. 1988. An empirical model for the description of moisture sorption curves. J. Food Sci. 53:1216-1217.

Piergiovanni A.R. 2011. Kinetic of water adsorption in common bean: considerations on the suitability of Peleg's model for describing bean hydration. J. Food Proc. Pres. 35:447-452.

Plans M., Simó J., Casañas F., Sabaté J. and Rodriguez-Saona L. 2013. Characterization of common beans (*Phaseolus vulgaris* L.) by infrared spectroscopy: comparison of MIR, FT-NIR and dispersive NIR using portable and benchtop instruments. Food Res. Int. 54:1643-1651.

Rodríguez-Licea G., García-Salazar J.A., Rebollar-Rebollar S. and Cruz-Contreras A.C. 2010. Preferencias del consumidor de frijol (*Phaseolus vulgaris* L.) en México: factores y características que influyen en la decisión de compra diferenciada por tipo y variedad. Paradigma Económico 2:121-145.

Shafaei S.M., Masoumi A.A. and Roshan H. 2014. Analysis of water absorption of bean and chickpea during soaking using Peleg model. Journal of the Saudi Society of Agricultural Sciences DOI: 10.1016/j.jssas.2014.08.003.

Tizazu H. and Emire S. 2010. Chemical composition, physicochemical and functional properties of lupin (*Lupinus albus*) seeds grown in Ethiopia. Afr. J. Food Agric. Nutr. Dev. 10: 3029-3046.

Wani I.A., Sogi D.S., Wani A.A. and Gill B.S. 2015. Physical and cooking characteristics of some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars. Journal of the Saudi Society of Agricultural Sciences DOI: 10.1016/j.jssas.2014.12.002.

WHO/FAO. 2003. Diet, Nutrition and the Prevention of Chronic Diseases. WHO Technical Report Series 916, Geneva.
Yildirim A., Öner M.D. and Bayram M. 2011. Fitting Fick's model to analyze water diffusion into chickpeas during soaking with ultrasound treatment. J. Food Eng. 104:134-142.

Zura L., Uribe E., Lemus-Mondaca R., Saavedra-Torrico, J., Vega-Gálvez, A. and Di Scala K. 2013. Rehydration capacity of Chilean papaya (*Vasconcellea pubescens*): Effect of process temperature on kinetic parameters and functional properties Food Bioprocess Technol. 6:844-850.

Paper Received November 17, 2016 Accepted December 31, 2016

PAPER

NEW FORMULATIONS OF OLIVE-BASED PÂTÉ: DEVELOPMENT AND QUALITY

L. COSMAI, F. CAPONIO*, C. SUMMO, V.M. PARADISO, A. CASSONE and A. PASQUALONE

*Department of Soil, Plant and Food Science (DISSPA), University of Bari Aldo Moro, Via Amendola, 165/a, I-70126 Bari, Italy

*Corresponding author. Fax: +39 0805443467

E-mail address: francesco.caponio@uniba.it

ABSTRACT

The aim of this research was the chemical, microbiological, and sensory characterization of new ingredient formulations of olive-based pâtés, since few studies are present in the literature regarding table olive-based processed products. Three ingredient formulations were selected by a consumer test on the basis of the scores of *odour* and *taste* descriptors, higher than 6 (linear scale 0-10 cm). The chemical and sensory analyses allowed to clearly differentiate all ingredient formulations on the basis of both composition and volatile profile. Microbiological data corresponded to levels usually detected for similar products and excluded the presence of pathogens. Finally, no off-flavour was perceveid in the tested formulations.

Keywords: characterization olive-based pâté, sensory analysis, table olives, volatile profile

1. INTRODUCTION

Table olives (*Olea europaea* L.) are one of the most important traditional fermented vegetables in the Western world (BLEVE *et al*., 2015) and, similarly to olive oil, one of the most characteristic components of the diet in Mediterranean countries. The world production of table olives has shown a steady increase for the last 20 years and is mainly concentrated in Spain, Turkey, Egypt, Syria, Morocco, Italy, and Greece. The International Olive Council (IOC, 2014) estimated that table olive production (2011/2012 season) was above 2.4 milliontons.

According to the Trade Standard Applying to Table Olives (IOC, 2004), table olives are prepared from sound olive fruits whose volume, shape, flesh-to-stone ratio, firmness, and taste make them particularly suitable for processing. Moreover, table olives have to be treated to remove bitterness by natural fermentation or by heat treatment, with or without the addition of preservatives.

Table olives have been attracting increasing interest, due to their postulated health benefits, that seem to be intrinsically linked to the high monounsaturated fatty acid content, as well as to their important antioxidant capacity, antimicrobial activity, and protection against mycotoxin effects due to minor constituents like tocopherols and phenolic compounds (MALHEIRO *et al*., 2011).

Table olives consumption is greatly varied: the fruits, conveniently processed, can be served as an appetizer or as a complement to salads, pasta, pizza, fish, and meat. Even bread can be prepared by adding green/black olives to the dough (SABATINI *et al*., 2009; LANZA, 2012).

As reported by IOC (2004), table olives consumption showed an increase primarly attributable to the marketing efforts by manufacturers essentially aimed at the introduction of new products, to satisfy the growing consumers' awareness of their health benefits. In this context, the table olives characterized by defects in terms of size and/or shape, unsuitable to be marketed as such, could represent the base for the preparation of new commercial products among which olive-based pâtés.

The term "pâté" indicates a processed product that has important gastronomic tradition and good sensory properties with a coarse texture (ÜNLÜSAYIN *et al*., 2007) in which the main ingredients are more or less finely ground and mixed with various ingredients, considered essential for their binding capacity.

With respect to other types of vegetable pâtés – for example tomato-based pâtés, characterized by a complex ingredient formulation (the starting vegetable is usually associated with other kinds of ingredients, such as mushrooms, eggplants, peppers, oil, spices, and herbs) – usually commercial olive-based pâtés contain only table olives and olive oil.

Table olives have been studied from microbiological (PEREIRA *et al*., 2008; TASSOU *et al*., 2002; HURTADO *et al*., 2008; ARROYO-LÓPEZ *et al*., 2008; PANAGOUA *et al*., 2008; CAMPANIELLO *et al*., 2005), chemical (MONTAŇO *et al*., 2003; APONTE *et al*., 2010; ROMEO *et al*., 2010; PASQUALONE *et al*., 2014), and sensory points of view (SABATINI and MARSILIO, 2008; PÉREZ *et al*., 2007). However, no studies are present in the literature regarding table olive-based processed products, except for a study carried out by ALVARENGA *et al*. (2012).

On the basis of these considerations, the aim of this study was the development of new formulations of olive-based pâtès to satisfy more dynamic, complex and differentiated consumer demands for traditional and functional foods. It is crucial to consider food safety as well as nutritional, sensory and commercial qualities. In this context sensory, microbiological, and chemical properties of olive-based pâtès were reported.

2. MATERIALS AND METHODS

2.1. Sampling

The trials were carried out utilizing cv. *Bella di Cerignola* table olives, debittered by the Spanish method (PASQUALONE *et al.*, 2014), purchased at local retailers without defects. The experimental plan required that the olives represent at least 50% of the total ingredient formulation, while the choice of the other ingredients was made through preliminary tasting sessions that were designed to assess which combinations were judged the best among different ingredients. Extra virgin olive oil (EVOO) of Coratina cultivar was used in the experimental trials.
A total of six formulations (F1, F2, F3, F4, F5, F6) were evaluated:

- F1, table olives (500 g kg^{-1}), EVOO (170 g kg^{-1}), sweet shelled almonds (165 g kg^{-1}), water (165 g kg^{-1});
- F2, table olives (640 g kg^{-1}), EVOO (100 g kg^{-1}), olive-oil tuna (130 g kg^{-1}), dried tomatoes (130 g kg^{-1});
- F3, table olives (770 g kg^{-1}), EVOO (155 g kg^{-1}), zucchini (75 g kg^{-1});
- F4, table olives (750 g kg^{-1}), EVOO (50 g kg^{-1}), dried tomatoes (50 g kg^{-1}), red peppers (50 g kg^{-1}) eggplants (50 g kg^{-1}), mushrooms (*Pleurotus eryngii*) (50 g kg^{-1}), capers (1 No.);
- F5, table olives (770 g kg^{-1}), EVOO (150 g kg^{-1}), salted anchovies (40 g kg^{-1}), red onion (40 g kg^{-1}), leaves of arugula (2-3 No.), drop balsamic vinegar (5 No.);
- F6, table olives (750 g kg^{-1}), EVOO (130 g kg^{-1}), salted anchovies (60 g kg^{-1}), red onion (60 g kg^{-1}).

Before the olive-based pâtés production, some raw materials were subjected to preliminary treatments: table olives were washed and pitted; dried tomatoes and mushrooms were blanched in boiling water or water/vinegar (1:1 v/v), respectively, for two minutes; zucchini, red peppers, and eggplants were roasted.
All ingredients were mixed by means of a homogenizer (Waring LB 20 ES, Rome, Italy) until obtaining a homogeneous creamy paste (about 5 minutes at 12,000 rpm). After mixing, the product was transferred and packed in plastic boxes having the capacity of 100 g.

2.2. Consumer test

A consumer test was conducted for the six different formulations. A total of 90 people (mean age 27 years, range 17-60 years, 45 males and 45 females) participated in the consumer test, which was performed within 4 hours from pâté production.
The participants were selected among people who regularly consumed vegetable pâtés (i.e. 1-4 times a month). An alpha-numeric code was assigned to each pâté, served in white plastic cups (about 10 g). After trying, several different palate cleansers to avoid carry-over effects and adaptation to sensory stimuli, mineral water and a 1-minute break between samples were chosen. Before starting the evaluation of products, the investigator read aloud the instructions to the participants. Each subject was asked to fill a sensory evaluation sheet composed of a first part regarding personal information (age and sex), and a second part that included a continuous 10 cm-line on which consumers expressed an acceptability judgment for *odour pleasant* and *taste pleasant* descriptors, respectively. Testing was performed at room temperature (20 °C) with artificial lighting, simulating daylight.

2.3. Sensory analysis

On the basis of the results of consumer test, the three most appreciated pâté were submitted to a descriptive sensory analysis by a trained panel of assessors. The first step has provided the identification of the sensory descriptors, in order to develop a common vocabulary for the description of the sensory attributes and to familiarize with scales and procedures. Each attribute term was extensively described and explained to avoid any doubt about the relevant meaning. On the basis of the citation frequency (>60%), eleven descriptors were selected and related to the rheological characteristics (consistency and phase separation), visual characteristics (brightness and colour homogeneity), taste (bitter, acid, sweet, salt), and flavour (olive flavour and off-flavour). Each descriptor was scored on a non-structured line scale of 10 cm.
A panel of eight assessors, aged between 25 and 50 years, was trained through preliminary sessions to ensure that each panelist interpret the same descriptor in the same way. The same product was also subjected several times, without their knowledge, to repeatedly tasting to assess the ability of panelists to detect the intensity of each descriptor always in the same way. The panel consisted of teachers and students, and all sensory tests were performed in the sensory laboratory of Food Science and Technology section of Bari University, which fulfills the requirements of the international standards (ISO, 1988).
During the evaluation phase, the panelists were seated in private booths equipped with air conditioning and under incandescent/fluorescent light. The sample presentation order was randomized for each panelist. The samples, identified by an alpha-numeric code, were served to each panelist in plastic cups at room temperature, and tap water was provided between samples to cleanse the palate.
Samples were analyzed in duplicate.

2.4. Microbiological analysis

Microbiological analysis included the determination of contaminating micro-organisms and pathogens (Coliforms, *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli*). All cultural media, supplements, and diagnostic kits were provided by Oxoid (Basingstoke, UK).
Coliforms were determined by count in plates of selective medium Violet Red Bile Glucose Agar (38.5 g/L in distilled water boiled for 2 min), seeding 1 mL of decimal dilutions for inclusion and incubating the plates at 30 °C for 24 h (ANON, 1996).
The counting was done considering only the characteristic colonies (dark red with diameter > 0.5 mm, with or without the surrounding precipitate), while the others were submitted to the confirmation. This was made inoculating 3-5 colonies with a loop in tubes of sterile broth of Brilliant Green Lactose then incubated at 30 °C for 24 h. All colonies developed in the culture broth with gas production were included in the count of coliform.
The monitoring of *L. monocytogenes* (ANON, 1996) consists of several stages: i) the primary selective enrichment: 25 g of sample were spiked with 225 mL of Half Fraser and the incubated at 30 °C for 24-48 h; ii) secondary selective enrichment: 0.1 mL of the primary enrichment culture were incubated in 10 mL of medium Fraser Broth at 37 °C for 24-48 h; iii) isolation: a loopful of enrichment culture was streaked on distinct plates of Oxford Agar and Palcam Agar, incubated at 37 °C for 24-48 h (in microarofilia conditions with 5-12% CO_2, 5-15% O_2, 75% N_2 only for Palcam agar plates); iv) confirmation and identification: by each isolation medium were taken at least 5 characteristic colonies to perform subculture by streaking on the Tryptone Soy Yeast Extract Agar, incubated at 37 °C for 24-48 h. The identification was performed with biochemical tests of catalase, Gram-

stain and hemolysis on plates of Blood Agar Bases supplemented with defibrinated sheep blood after 24-48 h of incubation at 37 °C.
For the determination of *E. coli*, 25 g of each pâté were homogenized in 225 mL of the solution of Tryptone Soy Broth and incubated at 37 °C for 18 h under stirring (150 rpm). From each crop, inocula of 0.1 mL were made through smear on MacConkey Sorbitol Agar enriched with Cefixime-Tellurite Supplement. The plates, produced in triplicates, were incubated at 35 °C for 20-22 h before counting the probable colonies of E. coli 0157:H7; identification was made with the use of E. coli O157 Test Kit (ABDUL-RAOUF *et al*., 1993). The results were expressed as cfu/g of sample.

2.5. Chemical analysis

The measurements of pH were conducted by using a Basic 20 pH meter (Crison Instruments S.A., Barcelona, Spain) provided with a conductivity penetration probe (Codito 50 10T, Crison Instruments S.A., Barcelona, Spain) and a temperature compensator.
Moisture content was estimated by drying the samples at 105±5 °C, and ash content by incineration, both until constant weight (AOAC, 2000). Fat content was determined by Soxhlet extraction with 40-60 °C diethyl ether for 6 hours, followed by evaporation of the solvent (IUPAC, 1979). Protein content was determined by Kjeldahl method (N × 6.25) (AOAC, 2000). Carbohydrate content was estimated by subtracting the weight of the other components to the total weight.

2.6. Headspace analyses

Volatile compounds were extracted by solid-phase micro-extraction (SPME) and analyzed by a gas-chromatographic system equipped with mass spectrometer (GC-MS). In particular, an aliquot of sample (1 g ± 0.05) was placed inside 12-mL glass vials, closed by silicone/PTFE septa and an aluminum seal. The pâté sample was homogenized for 2 min using a laboratory vortex shaker. Before extraction, stabilization of the headspace in the vial was achieved by equilibration for 5 min at 40 °C. The extraction was performed by exposing a 75-µm Polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR) fiber (Supelco, Bellefonte, Pa., USA) in the headspace of the sample at 40 °C for 15 min. When the extraction process was completed, the fiber was removed from the vial and desorbed in the injection port of the GC in a splitless mode. The GC-MS instrumentation included an Agilent 6850 gas-chromatograph (Milan, Italy) equipped with an Agilent 5975 single quadruple mass-spectrometer. Compounds were resolved on a HP-Innowax (20 m × 0.18 mm, 0.18 μm film thickness) polar capillary column (Agilent, Milan, Italy) under the following conditions: injector temperature, 220 °C; helium as the carrier gas at a flow rate of 15 mL/min for 7 min; oven temperature was at 40 °C/0.70 mL/min (linear speed of 36 cm/sec), then increased at 18 °C/min up to 180 °C, then increased at 20 °C/min up to 220 °C. The mass spectrometer was operated in the electron impact mode (electron energy = 70 eV) and the ion source temperature was 250 °C. The mass range was m/z 20-250. The volatile compounds were identified by comparison with the mass spectra present in the NIST and Wiley libraries, quantified and expressed in terms of integrated area.

2.7. Statistical analysis

The results were expressed as mean and standard deviation of three different trials and all the analytical determinations were carried out in triplicate. Analysis of variance (one-way ANOVA) was carried out on the chemical and microbiological analyses, whereas two-way

ANOVA was used on sensory analysis, considering *formulation* and *panelist* as independent variables. Significant differences among the values of all parameters were determined at $p \leq 0.05$. All data were processed by the XLStat software (Addinsoft SARL, New York, NY, USA).

3. RESULTS AND DISCUSSION

Table 1 shows the mean values and the results of a statistical analysis (one-way ANOVA) of the scores attributed to *odour* and *taste* during the consumer test performed on six different types of olive-based pâtés. The acceptability of the formulations was evaluated in order to identify the most appreciated, by considering only formulations scored more than 6 on a 0-10 scale.

Table 1. Means values and standard deviation of the results of consumer test performed for the six different ingredient formulations of olive-based pâtés.

Pâtés	Pleasant odour	Pleasant taste
F1	4.55±2.22 ^{d}	5.19±2.15 ^{c}
F2	5.16±2.49 ^{c}	6.42±2.74 ^{b}
F3	6.01±2.60 ^{b}	6.62±2.45 ^{b}
F4	6.50±2.12 ^{ab}	7.22±2.26 ^{a}
F5	7.00±2.03 ^{a}	6.64±2.00 ^{b}
F6	5.80±2.31 ^{bc}	6.37±2.21 ^{b}

a-d, different letters indicate a significant difference at $p \leq 0.05$.

The data showed significant differences among all the considered samples. Relatively to *odour* descriptor, scores higher 6, on linear scale 0-10 cm, were observed in F3, F4, and F5 pâtés. The F4 sample showed the highest *taste* score, even if all the other formulations (except F1) were scored more than 6, with no significant differences among them.
The most appreciated formulations in terms of *odour* were characterized by completely different ingredients among them, demonstrating that it is possible to set up several pleasant combinations, assuming that the components are quantitatively well-balanced. In particular, F3 and F4 were characterized by the presence of different vegetables that contributed to make a specific *odour* more intense which would have otherwise been flat if only table olives had been used, as evidenced for F1. Moreover, in the F1 sample probably the use of almonds flattened both the *odour* and the *taste*. F5 had a higher score than F6, although containing similar ingredients. This result could be attributed to a positive effect of additional ingredients, such as balsamic vinegar and arugula, or salted anchovies.
On the basis of the scores obtained for both *odour* and *taste*, three ingredient formulations were selected: F3, F4, and F5. They reached a mean *odour* score of 6.01, 6.50, and 7.00, respectively, and a mean *taste* score of 6.62, 7.22, and 6.64, respectively. These results evidenced a real possibility to market these products.
Table 2 shows the chemical composition of the three selected pâté formulations and the results of one-way ANOVA. The pH level ranged between 4.95 and 6.76, with significantly lower values in F4, due to the use of dried tomatoes and mushrooms (which needed to be blanched in water/vinegar, 1:1 v/v). Higher values of pH observed in the other formulations were due to the use of olives, which underwent the Spanish debittering

method, involving the use of NaOH. Moisture content was higher in F4, due to the use of a greater amount of vegetables, than in F3 and F5. Fat content, on the contrary, was higher in F3 and F5 than in F4 due to the higher amount of olive and oil used in their recipe.
Protein and ash content were significantly higher in F5, probably due to the presence of salted anchovies, whereas carbohydrate content was higher in F4.
Microbiological analyses (Table 3) performed on the selected pâté formulations shown as coliforms and *E. coli* cell density were below the limit indicated in the EC Regulation No 2073/2005 on microbiological criteria for foodstuffs. Significantly higher values were determined in F5, probably due to the use of salted anchovies (PATIR *et al*., 2006). Biochemical and serological tests on *Salmonella* spp. and *L. monocytogenes* allow to exclude the presence of pathogens, in agreement with the requirements of the above EC Regulation No 2073/2005.

Table 2. Mean values and standard deviation of percent composition of three selected olive-based pâtés.

Pâtès	pH	Moisture	Fat	Proteins	Carbohydrates	Ashes
F3	6.76±0.03^{a}	59.65±0.98^{b}	31.00±0.20^{a}	1.13±0.02^{c}	6.86±1.06^{b}	1.36±0.11^{c}
F4	4.95±0.04^{b}	67.72±0.84^{a}	21.27±0.44^{b}	1.63±0.02^{b}	7.37±0.78^{a}	2.01±0.15^{b}
F5	6.13±0.01^{a}	58.94±0.32^{b}	31.85±0.40^{a}	1.82±0.02^{a}	4.94±0.18^{c}	2.45±0.19^{a}

a-c, different letters indicate a significant difference at $p \leq 0.05$.

Table 3. Mean values (cfu/g) and standard deviation of the results of microbiological analyses of three selected olive-based pâtés.

Samples	*Coliforms*	*Salmonella* spp.	*Listeria monocytogenes*	*Escherichia coli*
F3	<1000 ^{b}	Not found in 25 g	Not found in 25 g	<100 (not encountered pathogenic strains)
F4	<1000 ^{b}	Not found in 25 g	Not found in 25 g	<100 (not encountered pathogenic strains)
F5	(2.0±0.2) × 10$^{3\ a}$	Not found in 25 g	Not found in 25 g	<100 (not encountered pathogenic strains)

a-b, different letters indicate a significant difference at $p \leq 0.05$.
cfu, colony-forming units.

Fig. 1 shows the GC-MS chromatograms related to headspace analysis of F3, F4, and F5 pâtés. It is possible evidence the good peaks separation and the more complex volatile compounds pattern in F4, in which the main volatile compounds were evidenced. In particular, a total of 77 volatile compounds were identified and grouped in relation to the chemical class they belonged to (Table 4). Generally, the volatile compounds of the examined pâtés were affected by the different ingredients used, but the exact contribution of each ingredient to the volatile fraction of pâtés was difficult to point out: in fact, the major volatile compounds identified were shared by different raw materials, as evidenced by their preliminary headspace analysis (data not showed). In particular, the most abundant volatile compound was acetic acid, significantly more represented in F4 than in F3 and F5. The analysis of raw materials evidenced that the abundance of this compound was mainly attributable to the presence of eggplants, capers, dried tomatoes, and mushrooms, the latter blanched in water/vinegar. The use of capers could justify also the preponderance of other carboxylic acids in F4, with respect to F3 and F5, such as hexanoic and heptanoic acids (ROMEO *et al*., 2007).

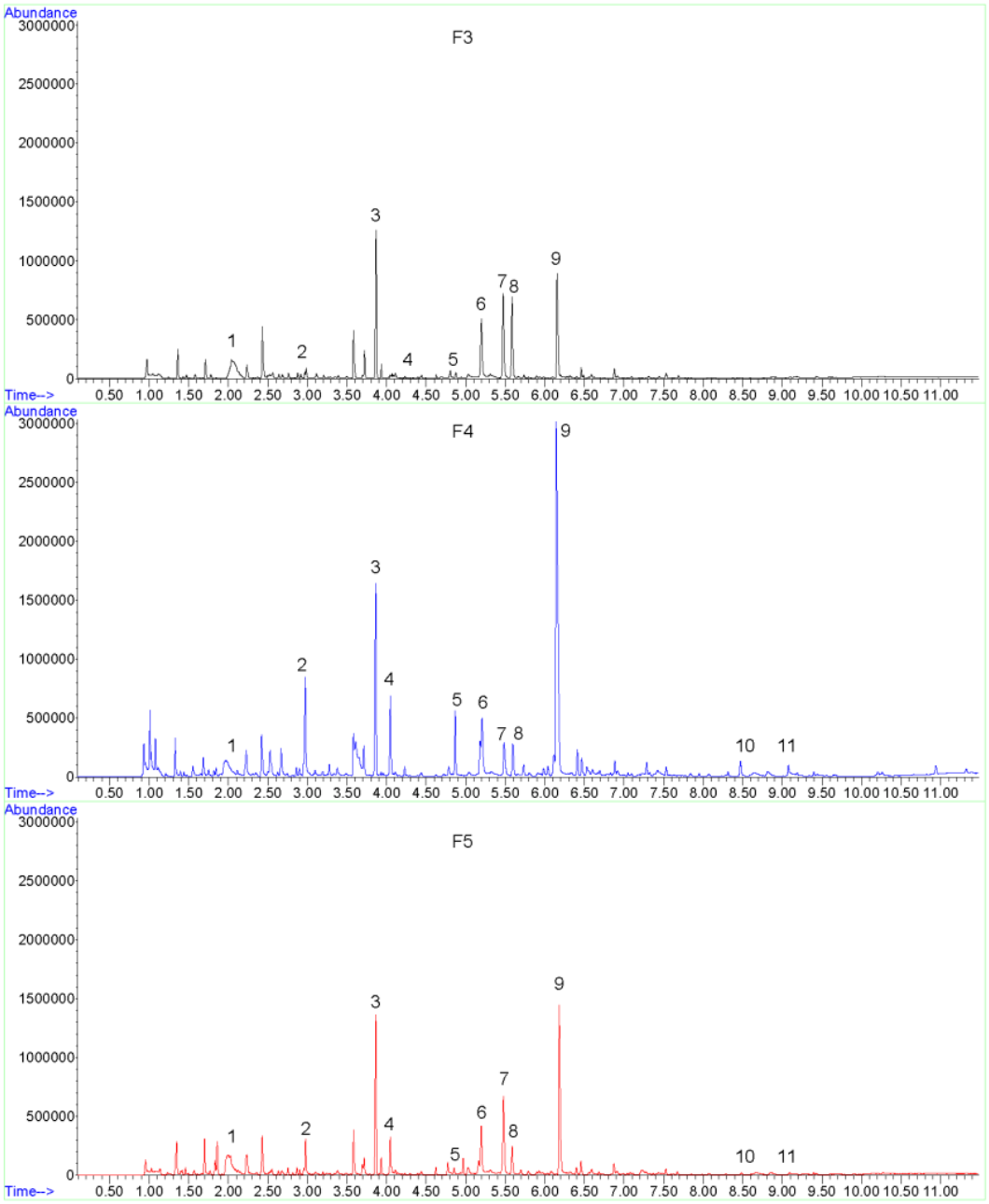


Figure 1. Chromatograms of the headspace of F3, F4, F5 formulations. Number peaks identify the main volatile compounds. 1 = ethanol; 2 = hexanal; 3 = eucalyptol; 4 = 2-hexenal; 5 = 6-methyl-5-hepten-2-one; 6 = 1-hexanol; 7 = (Z)-3-hexen-1-ol; 8 = 2-hexen-1-ol; 9 = acetic acid; 10 = hexanoic acid; 11 = heptanoic acid (Cosmai *et al*.).

The presence of vegetables (eggplants, red peppers, mushrooms, and dried tomatoes) made F4 richer in terpenic compounds more than F3 and F5, such as eucalyptol (contributed by olives and eggplants), D-limonene (from olives, mushrooms, and dried tomatoes), terpinen-4-ol (from olives, dried tomatoes, and eggplants) and linalool (from olives and dried tomatoes). The latter was very abundant also in F5, where it was contributed by olives and arugula. The F4 pâté was also characterized by higher levels of 6-methyl-5-hepten-2-one, related to pungent and green sensory notes. This volatile

derived from the dried tomatoes, being considered as a marker of the lycopene degradation (CREMER and EICHNER, 2000).

Table 4. Mean values of integrated total area of volatile compounds of three selected olive-based pâtés.

Volatile compounds	F3	F4	F5	*p*-value
Terpenes, phenols, lactones and thiols				
α-Pinene	nd	3.39^{a}	2.00^{b}	<0.001
β-Pinene	3.59^{a}	4.47^{a}	2.30^{b}	0.010
α-Phellandrene	2.56	nd	nd	<0.001
(+)-4-Carene	1.31	nd	nd	0.085
D-Limonene	17.91^{ab}	18.75^{a}	12.10^{b}	0.095
Eucalyptol	114.19^{b}	159.50^{a}	127.60^{b}	<0.001
2,4-Quinolinediol	nd	4.23^{ns}	5.07^{ns}	<0.001
Acetophenone	nd	6.94	nd	<0.001
Butyrolactone	3.03^{ns}	3.03^{ns}	nd	0.005
Benzothiazole	nd	4.67	nd	<0.001
α-Farnesene	2.75^{ns}	nd	3.20^{ns}	<0.001
2-Ethylphenol	3.30	nd	nd	<0.001
Linalool	8.12^{b}	14.34^{a}	12.86^{a}	<0.001
Terpinen-4-ol	4.53^{c}	14.33^{a}	10.73^{b}	<0.001
Esters				
Methyl acetate	2.34^{b}	3.09^{b}	4.59^{a}	0.006
Ethyl acetate	12.91^{b}	13.84^{b}	24.23^{a}	<0.001
Ethyl butanoate	3.10^{a}	1.14^{b}	3.04^{a}	<0.001
Ethyl 2-methylbutanoate	3.77^{b}	2.38^{c}	4.84^{a}	<0.001
Ethyl 3-methylbutanoate	3.78^{c}	6.75^{a}	5.11^{b}	<0.001
Hexyl acetate	nd	nd	1.04	<0.001
(Z)-3-Hexen-1-ol acetate	3.51^{c}	9.76^{a}	4.61^{b}	<0.001
Methyl benzoate	0.88^{b}	3.76^{a}	nd	<0.001
Alcohols				
Ethanol	83.65^{ns}	80.46^{ns}	91.49^{ns}	0.363
1-Butanol	nd	51.34^{ns}	36.14^{ns}	0.007
1-Penten-3-ol	nd	nd	8.80	<0.001
2-Methyl-1-butanol	2.68^{ns}	2.26^{ns}	2.49^{ns}	0.514
3-Methyl-1-butanol	4.59^{ns}	4.72^{ns}	5.11^{ns}	0.376
1-Pentanol	2.85^{b}	4.37^{a}	3.24^{b}	0.001
(Z)-2-Penten-1-ol	4.68^{c}	8.00^{b}	11.17^{a}	<0.001
1-Hexanol	68.98^{a}	71.78^{a}	54.50^{b}	0.001
(Z)-3-Hexen-1-ol	93.94^{a}	43.14^{b}	95.96^{a}	<0.001
(Z)-2-Hexen-1-ol	82.38^{a}	36.06^{b}	28.96^{c}	<0.001
1-Octen-3-ol	1.66^{b}	4.85^{a}	4.75^{a}	0.017
1-Heptanol	0.89^{b}	4.69^{a}	3.58^{a}	0.009
1-Octanol	4.76^{b}	7.51^{a}	7.65^{a}	0.082
Aldehydes				
Propanal	nd	nd	9.38	<0.001
2-Methylpropanal	nd	nd	2.68	<0.001
2,2-Dimethylpropanal-3-pentanone	12.41	nd	nd	<0.001

2-Methylbutanal	nd	5.57[b]	10.54[a]	<0.001
3-Methylbutanal	nd	9.41[b]	26.29[a]	<0.001
Pentanal	nd	24.62	nd	<0.001
Hexanal	6.20[c]	74.17[a]	26.94[b]	<0.001
2-Hexenal	2.47[c]	70.11[a]	28.47[b]	<0.001
(E)-2-Heptenal	3.44[b]	8.34[a]	10.96[a]	0.010
Nonanal	nd	4.83[b]	12.64[a]	<0.001
(E,E)-2,4-Heptadienal	nd	nd	5.36	<0.001
Furfural	nd	9.34[a]	2.45[b]	<0.001
Benzaldehyde	nd	21.74[a]	6.91[b]	<0.001
2-Decenal	nd	2.53[b]	3.13[a]	<0.001
3,5-Dimethylbenzaldehyde	1.36[b]	2.92[a]	nd	0.035
Ketones				
Acetone	1.34[b]	3.35[a]	2.73[a]	0.009
2-Butanone	2.79[b]	4.68[a]	2.90[b]	0.012
3-Hexanone	nd	nd	1.94	<0.001
6-Methyl-5-hepten-2-one	4.52[b]	47.14[a]	6.07[b]	<0.001
Sulfur compounds				
Dimethyl sulfide	1.11[ns]	2.16[ns]	2.05[ns]	0.112
Dipropyl disulfide	nd	nd	12.55	<0.001
Furans				
2-Pentylfuran	nd	2.05	nd	<0.001
Acids				
Acetic acid	43.21[c]	517.62[a]	218.83[b]	<0.001
Propanoic acid	nd	4.22	nd	<0.001
Pentanoic acid	nd	3.63	nd	0.001
Hexanoic acid	nd	21.94[a]	2.33[b]	<0.001
Heptanoic acid	nd	10.71[a]	2.94[b]	<0.001
2-Methyl-2-propenoic acid	nd	2.94	nd	<0.001
Other compounds				
Octane	19.54[ns]	20.45[ns]	26.40[ns]	0.132
1-Octene	0.73[ns]	nd	0.75[ns]	0.089
(Z)-2-Octene	nd	nd	0.79	<0.001
Decane	nd	2.94[ns]	2.53[ns]	0.003
3-Ethyl-1,5-octadiene	nd	nd	4.82	<0.001
4,8-Dimethyl-1,7-nonadiene	nd	nd	4.26	<0.001
Ethylbenzene	1.39[b]	2.98[a]	nd	0.037
p-Xylene	2.14[b]	2.80[b]	4.62[a]	<0.001
(E)-5-Octadecene	nd	2.56[b]	9.43[a]	<0.001
Styrene	1.43[ns]	2.74[ns]	1.36[ns]	0.216
3-Cyclohexene-1-methanol *α-α*, 4-Trimethyl	8.44[a]	8.50[a]	6.32[b]	0.039
α-α, Dimethyl benzyl alcohol	nd	2.49	nd	<0.001
1,2-Dimethoxy-4-(2-propenyl)benzene	2.59[b]	4.90[a]	2.18[b]	<0.001
Methoxy-phenyl-oxime	nd	8.20	nd	<0.001

a-c, different letters indicate a significant difference at $p \leq 0.05$. Least squares means expressed as total area counts x 10^{-5}.
nd, not detected. ns, not significant.

The headspace composition of the examined pâtés also was characterized by the presence of high amounts of alcohols, above all ethanol, 1-butanol, 1-hexanol, (E)-3-hexen-1-ol, and (Z)-2-hexen-1-ol, as well as by aldehydes, such as hexanal and 2-hexenal (significantly higher in F4 and attributed to the presence of mushrooms and dried tomatoes).
The majority of alcohols are by-products of some pathways involving the aldehydes. Once formed, the aldehydes undergo a series of enzymatic transformations mediated by isomerases and alcohol dehydrogenases that generate C6 alcohols (CAVALLI *et al*., 2004). C6 volatile alcohols are also important components of the flavour of fruits, vegetables, and leaves (SCHWAB *et al*., 2008).
Regarding the most abundant alcohols, no significant differences were observed for ethanol in the three selected formulations, and for 1-butanol between F4 and F5 (attributable to the use of capers and salted anchovies, respectively). High levels of 1-hexanol were observed for F3 and F4 pâtés, as well as of (Z)-3-hexen-1-ol for F3 and F5, and (Z)-2-hexen-1-ol for F3. The presence of these volatiles is principally linked to the use of table olives and extra-virgin olive oil; a further contribution of 1-hexanol could be attributed to mushrooms and, above all, to dried tomatoes where it is considered one of the most important volatile compounds (CHRISTENSEN *et al*., 2007). On the other hand, (Z)-3-hexen-1-ol could be related to arugula, whose headspace analysis showed this compound as the major contributor of aroma (data not shown). Significantly higher values of benzaldheyde were found in F4 more than in the other two formulations. This compound, present in all the examined raw materials, but at particularly high levels in roasted vegetables, is a powerful volatile having an aroma of bitter almond. It can be thermally generated from phenylalanine (CHU and YAYLAYAN, 2008) via the Strecker degradation.
The presence of salted anchovies in F5 could justify the significantly higher content of 2-methylbutanal, 3-methylbutanal, (E)-2-heptenal, (E,E)-2,4-heptadienal, and nonanal. The first two volatile compounds derived from catabolism of specific amino acids such as isoleucine and leucine, respectively, through the non-enzymatic Strecker reaction (ESTÉVEZ *et al*., 2011) while the other volatile compounds were oxidation-derived compounds, dueto the effect of salting that caused a marked increase of these compounds with respect to fresh sample (SOLIMAN *et al*., 1983). In particular, (E)-2-heptenal is associated to oxidized, tallow, pungent aroma; (E,E)-2,4-heptadienal to boiled-potato like odour (RUSTAD, 2009) and fatty, rancid aroma; nonanal to fatty, waxy, and pungent aroma.
The F5 pâté was also rich in esters, especially ethyl acetate, related to the presence of balsamic vinegar (DEL SIGNORE, 2001) and anchovies (HUMAID and JAMAL, 2014) among the ingredients. Moreover, the presence of dipropyl disulfide in F5 is due to the addition of onions to its formulation (CARSON, 1987).
Fig. 2 shows the results of quantitative descriptive sensory analysis and the results of two-way ANOVA, performed on the three selected formulations.
From the textural point of view, the three formulations did not show a phase separation, indicating a good homogenization and consequently an appropriate ratio among the ingredients. No significant differences were found among the tested formulations. The consistency (Fig. 3), instead, was significantly higher in F3 than F4 and F5, probably due to the different water content of the ingredients used. In terms of colour, all the examined samples showed generally low and high values for brightness and colour homogeneity, respectively.

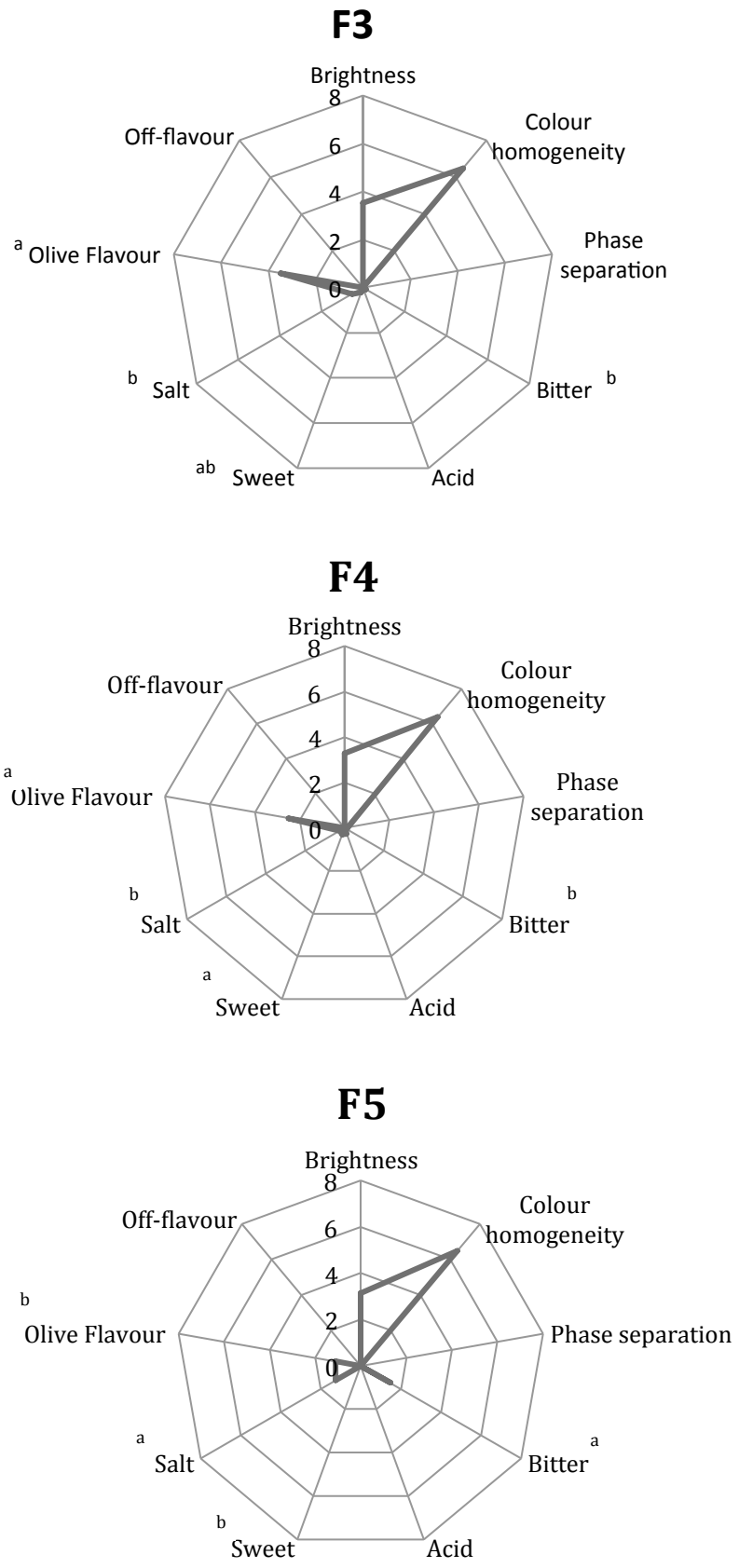


Figure 2. Sensory profile of the three selected olive-based pâtés. a-b, different letters indicate a significant difference at $P \leq 0.05$ (Cosmai *et al.*).

About taste descriptors, the intensity of their perception was low, except in F5 for bitter - where was attributable to the use of onion and arugula - and for salty sensations, due to the use of salted anchovies. Moreover, the presence of anchovies could explain the significant lower olive flavour assessed in F5. No off-flavour was perceveid in the tested formulations.

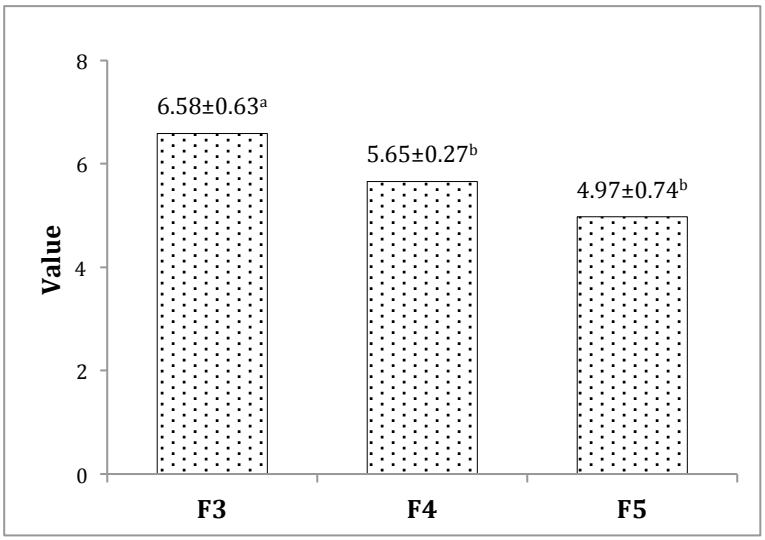


Figure 3. Consistency values of the three selected olive-based pâtés. a-b, different letters indicate a significant difference at $P \leq 0.05$ (Cosmai *et al.*).

4. CONCLUSIONS

The obtained results showed a good overall acceptability of the examined pâtés. The ingredients used in the recipe allowed their clear differentiation and identification. The microbiological analyses ensured the safeness of the product. Moreover, the obtained results evidenced that it is possible to produce innovative formulations of olive-based pâtés by adding new ingredients to their recipes, and representing also a viable alternative use to unsuitable table olives not to be marketed as such.

ACKNOWLEDGEMENTS

This work was funded by Ministero dell'Istruzione, dell'Università e della Ricerca, Ministero dello Sviluppo Economico and Fondo Europeo di Sviluppo Regionale (PON02_00186_3417037, project PROINNO_BIT).

REFERENCES

Abdul-Raouf U.M., Beuchat L.R. and Ammar M.S. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Appl. Environ. Microbiol. 59:1999.

Alvarenga N.B., Lidon F.J.C., Silva A., Martins G., Cruz T., Palma V. and Canada J. 2012. Production and characterization of green and black olive paste using cream of animal and vegetable origins. Emir. J. Food Agric. 24:12.

Anon. 1996. Rapporti ISTISAN ISSN 1123–3117, 96/35.

Aponte M., Ventorino V., Blaiotta G., Volpe G., Farina V., Avellone G., Lanza C.M. and Moschetti G. 2010. Study of green Sicilian table olive fermentations through microbiological, chemical and sensory analysis. Food Microbiol. 27:162.

Arroyo-López F.N., Querol A., Bautista-Gallego J. and Garrido-Fernández A. 2008. Role of yeasts in table olive production. Int. J. Food Microbiol. 128:189.

AOAC, 2000. "Official methods of analysis" of Association of Official Analytical Chemists International". 17th Ed. AOAC Press, Arlington, Method 982.27.

Bleve G., Tufariello M., Durante M., Grieco F., Ramires F.A., Mita G., Tasioula-Margari M. and Logrieco A.F. 2015. Physico-chemical characterization of natural fermentation process of Conservolea and Kalamata table olives and developement of a protocol for the pre-selection of fermentation starters. Food Microbiol. 46:368.

Campaniello D., Bevilacqua A., D'Amato D., Corbo M.R., Altieri C. and Sinigaglia M. 2005. Microbial characterization of table olives processed according to Spanish and natural styles. Food Technol. Biotechnol. 43:289.

Carson J.F. 1987. Chemistry and biological properties of onions and garlic. Food Rev. Int. 3:71.

Cavalli J.F., Fernandez X., Lizzani-Cuvelier L. and Loiseau A.M. 2004. Characterization of volatile compounds of French and Spanish virgin olive oils by HS-SPME:Identification of quality-freshness markers. Food Chem. 88:151.

Christensen L.P., Edelenbos M. and Kreutzmann S. 2007. Fruits and vegetables of moderate climate. Ch. 7. In: "Flavours and Fragrances Chemistry, Bioprocessing and Sustainability". R.G. Berger (Ed.), p. 135. Springer-Verlag, Berlin.

Chu F.L. and Yaylayan V.A. 2008. Model studies on the oxygen-induced formation of benzaldehyde from phenylacetaldehyde using pyrolysis GC-MS and FTIR. J. Agric. Food. Chem. 56:10697.

Commission Regulation (EC) No 2073/2005. 2005. Official Journal of the European Union L 338/1, on microbiological criteria for foodstuffs.

Cremer D.R. and Eichner K. 2000. Formation of volatile compounds during heating of spice paprika (*Capsicum annuum*) powder. J. Agric. Food. Chem. 48:2454.

Del Signore A. 2001. Chemometric analysis and volatile compounds of traditional balsamic vinegars from Modena. J. Food Eng. 50:77.

Estévez M., Ventanas S. and Heinonena M. 2011. Formation of Strecker aldehydes between protein carbonyls – α-aminoadipic and γ-glutamic semialdehydes – and leucine and isoleucine. Food Chem. 128:1051.

Humaid S.A. and Jamal M.T. 2014. Effect of storage temperature on histamine formation and bacterial growth in whole three fish species (*Rastrelliger kanagurta, Sardinella gibbosa and Lethrinus lentjan*). Life Sci. J. 11:927.

Hurtado A., Reguant C., Esteve-Zarzoso B., Bordons A. and Rozès N. 2008. Microbial population dynamics during the processing of *Arbequina* table olives. Food Res. Int. 41:738.

International Olive Council (IOC). 2014. Key Figures On The Work Marked For Table Olives. Available at http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive.

International Olive Council (IOC). 2004. Trade Standard Applying to Table Olives. Resolution No. RES-2/91-IV/04.

ISO 8587. 1988. Sensory analysis. Methodology. Ranking. International Organization for Standardization.

IUPAC. 1979. International Union of Pure and Applied Chemistry Standard methods for the analysis of the oils, fats and derivatives. 6th ed. Pergamon Press, Oxford.

Lanza B. 2012. Nutritional and Sensory Quality of Table Olives. Ch. 16. In "Olive Germplasm – The Olive Cultivation, Table Olive and Olive Oil Industry in Italy". I. Muzzalupo (Ed.), p. 343. InTech. Available from:http://www.intechopen.com/books/olive-germplasm-the-olive-cultivation-tableolive-and-olive-oil-industry-in-italy/nutritional-and sensory quality of-table olives. Rijeka, Croatia.

Malheiro R., Sousa A., Casal S., Bento A. and Pereira J.A. 2011. Cultivar effect on the phenolic composition and antioxidant potential of stoned table olives. Food Chem. Toxicol. 49:450.

Montaño A., Sánchez A.H., Casado F.J., De Castro A. and Rejano L. 2003. Chemical profile of industrially fermented green olives of different varieties. Food Chem. 82:297.

Panagoua E.Z., Schillingerb U., Franzb Charles M.A.P. and Nychasa George-John E. 2008. Microbiological and biochemical profile of cv. *Conservolea* naturally black olives during controlled fermentation with selected strains of lactic acid bacteria. Food Microbiol. 25:348.

Pasqualone A., Nasti R., Montemurro C. and Gomes T. 2014. Effect of natural-style processing on the oxidative and hydrolytic degradation of the lipid fraction of table olives. Food Control. 37:99.

Patir B., Gurel Inanli A., Oksuztepe G. and Ilhak O.I. 2006. Microbiological and chemical qualities of salted grey mullet (*Chalcalburnus tarichii* PALLAS, 1811). Int. J. Sci. Technol. 1:91.

Pereira A.P., Pereira J.A., Bento A. and Estevinho M.L. 2008. Microbiological characterization of table olives commercialized in Portugal in respect to safety aspects. Food Chem. Toxicol. 46:2895.

Pérez R.A., Navarro T. and De Lorenzo C. 2007. HS–SPME analysis of the volatile compounds from spices as a source of flavour in 'Campo Real' table olive preparations. Flavour Frag J. 22:265.

Romeo F.V., Piscopo A. and Poiana M. 2010. Effect of acidification and salt concentration on two black brined olives from Sicily. Grasas y Aceites. 61:251.

Romeo V., Ziino M., Giuffrida D., Condurso C. and Verzera A. 2007. Flavour profile of capers (*Capparis spinosa* L.) from the Eolian Archipelago by HS-SPME/GC–MS. Food Chem. 101:1272.

Rustad T. 2009. Lipid oxidation. Ch. 7. In "Handbook of Seafood and Seafood Products Analysis". L.M.L. Nollet and F. Toldrá (Eds.), p. 87. CRC Press.

Sabatini N. and Marsilio V. 2008. Volatile compounds in table olives (*Olea Europaea* L., *Nocellara del Belice* cultivar). Food Chem. 107:1522.

Sabatini N., Perri E. and Marsilio V. 2009. An investigation on molecular partition of aroma compounds in fruit matrix and brine medium of fermented table olives. Innov. Food Sci. Emerg. 10:621.
Schwab W., Davidovich-Rikanati R. and Lewinsohn E. 2008. Biosynthesis of plant-derived flavor compounds. Plant J. 54:712.

Soliman M.M., Fahmy A.A., El Sawy A.A. and Osman F. 1983. Effect of salting treatment on the flavour of oyster, donax and sea urchin. Agr. Biol Chem. 47:1655.

Tassou C.C., Panagou E.Z. and Katsaboxakis K.Z. 2002. Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines. Food Microbiol. 19:605.

Ünlüsayin M., Bilgin S., İzci L. and Günlü A. 2007. Chemical and sensory assessment of hot-smoked fish pâté. J. FisheriesSciences.com. 1:20.

Paper Received March 3, 2016 Accepted December 17, 2016

PAPER

EFFECT OF PULSED LIGHT ON SELECTED PROPERTIES OF CUT APPLE

L. MANZOCCO, P. COMUZZO, M. SCAMPICCHIO[a] and M.C. NICOLI
Dipartimento di Scienze Agro-Alimentari, Ambientali e Animali, Università degli Studi di Udine,Via Sondrio 2/A, 33100, Udine, Italy
[a]Faculty of Science and Technology, Libera Università di Bolzano, Piazza Università 1, 39100, Bolzano, Italy
*Corresponding author. lara.manzocco@uniud.it

ABSTRACT

The effect of pulsed light (0, 8.8 and 17.5 J cm^{-2}) on selected properties of cut apple (thermal power, oxygen consumption, volatile compounds, colour and firmness) was investigated during storage at 30 °C for up to 6 days. Samples exposed to pulsed light showed lower heat production due to a decrease in tissue respiration. Pulsed light treated samples also showed different evolution of volatile compounds (ethanol, acetaldehyde and ethyl acetate), browning and tissue softening. Modification of tissue metabolisms by pulsed light could be exploited in the processing of fresh-cut fruit and vegetables leading to advantages well beyond its germicidal activity.

Keywords: fresh-cut, isothermal microcalorimetry, light, respiration, volatile

1. INTRODUCTION

Pulsed light processing consists in exposing food to consecutive intense flashes of a radiation with a spectrum similar to that of the sun, including not only ultraviolet light but also visible and infrared radiation (170-2600 nm) (MORARU and UESUGI, 2009; FALGUERA *et al*., 2011). Because of its germicidal effect, pulsed light has been investigated as an interesting technological approach to decontaminate the surface of fresh-cut fruits and extend their shelf life (GÒMEZ-LÒPEZ *et al*., 2007; OMS-OLIU *et al*., 2010; RAMOS-VILLARROEL *et al*., 2011; GÒMEZ *et al*., 2012). The antimicrobial efficacy of pulsed light has been attributed to localized photothermal and photophysical effects but especially to the capability of its UV light component to modify the structure of biomolecules (WEKHOF, 2000; TAKESHITA *et al*., 2003; WANG *et al*., 2005; KRISHNAMURTHY *et al*., 2008). Pulsed light has actually the potential of modifying protein conformation and configuration, leading to significant modification in their biologic activity (MANZOCCO, 2015). These changes promote the death of microorganisms but also the inhibition of oxidative enzymes (GÒMEZ *et al*., 2012; MANZOCCO *et al*., 2013a; 2013b; IGNAT *et al*., 2014). It is thus likely that pulsed light could modulate the metabolic activity of vegetable tissues following adaptation mechanisms to the light stressor (LUCKEY, 1980). A circumstantial evidence supporting this hypothesis is the well-known physiological response of plant tissue to UV-C light, which is part of the pulsed light spectra. Common response to UV-C light in plants actually involves: (i) enhancement of biosynthesis of phenols toxic to pathogens; (ii) reduced ethylene production, and (iii) development of antisenescent putrescine exerting opposite physiological effects to ethylene (STEVENS *et al*., 1998; MAHARAJ *et al*., 1999). Physical effects could also be expected since UV-C light actually promotes moisture redistribution in the plant tissue due to local thermal effects deriving from electron excitation of food components (MANZOCCO *et al*., 2011a). It has been hypothesised that modification of water availability could further modify tissue metabolic activity (MANZOCCO *et al*., 2011b). Nevertheless, limited information is available about effects of pulsed light other than the germicidal one.
The aim of the present work was to evaluate the effect of pulsed light on some metabolism-related properties of cut apple. In particular, Golden Delicious apple tissues were submitted to pulsed light in a fluence range typically applied to inactivate microorganisms and inhibit enzymatic browning (up to 17.5 J cm^{-2}). Samples were then analysed for heat production, oxygen consumption, carbon dioxide formation, evolution of volatile compounds, colour and firmness during storage at 30 °C.

2. MATERIALS AND METHODS

2.1. Sample preparation

Golden delicious apples were purchased at the local market. Fruits were washed with water and rinsed. Apples were cored using a perforated cylinder having 4 mm internal diameter and 50 mm length. The extremities of the cored apple tissue were discarded to get a 35 mm long cylinder of apple mesocarp. A cylindrical shape was chosen to guarantee homogeneous exposure of sample surface to pulsed light and allow sample introduction in the microcalorimetry vials.

2.2 Pulsed light treatments

Pulsed light treatments were carried out at room temperature by using a pulsed light mobile decontamination unit (Claranor, Avignon, France) equipped with 4 xenon lamps with maximum emission in the range 200-1000 nm (200-400 nm: 41%; 400-700 nm: 51%; 700-1000 nm: 8%). Lamps were positioned at each side of a quartz plaque held in the centre of the cube shaped chamber. Two lamps were symmetrically positioned above and below the apple cylinder at a distance of 1 cm. Two lamps were symmetrically positioned at the lateral side of the apple cylinder at 1 cm distance from the quartz plaque, which corresponded to 3 cm from the sample. Apple cylinders were thus individually exposed to increasing light fluence up to 17.50 J cm^{-2}, by means of increasing number of light pulses. According to the manufacturer's instructions, each pulse delivered to the sample a light fluence of 1.75 J cm^{-2}. Pulse duration was 0.50 μs and repetition rate was 0.50 Hz.
Treated apple cylinders were individually introduced in 1.2 mL capacity vials (Lab Logistic Group GmbH, Meckenheim, Germany), hermetically sealed in the presence of air with butyl septa and metallic caps (Lab Logistic Group GmbH, Meckenheim, Germany), and stored for increasing time up to 6 days at 30 °C (Climacell 222, MMM Group, Gräfelfing, Germany). This temperature was chosen to emphasise metabolic activity of apple tissue.
Analogous sample not exposed to pulsed light were prepared as control.

2.3. Temperature

Temperature was measured by a Testo 805 pyrometer (Testo, Settimo Milanese, Italy). Sample temperature was measured immediately after the pulsed light treatment. Interval time between the end of the pulsed light treatment and sample temperature measurement was less than 10 s.

2.4. Isothermal microcalorimetry

Isothermal calorimetry was performed by a multichannel microcalorimeter (TAM III Air isothermal calorimetry, TA instruments, New Castle, Delaware, USA) equipped with an oil bath thermostat (accuracy 0.0001 °C) operating through a Peltier element. The instrument has a sensitivity of ± 100 nW and allows accurate maintenance of temperature in the 25 ± 5 °C temperature range. Vials containing the apple cylinders were placed in the calorimeter and isothermal traces recorded at 30 °C for 5 days. The thermal profile was expressed as W g^{-1} by normalising the heat flux (W) of each sample based on the sample weight.

2.5 Firmness

Firmness was measured by Warner-Blatzler shear test using an Instron 4301 (Instron LTD. High Wycombe, United Kingdom). The instrumental settings and operations were accomplished using the software Automated Materials Testing System (version 5, Series IX, Instron LTD, High Wycombe, United Kingdom). The blade was lowered into the apple sample perpendicularly to the cylinders at a speed of 5 cm/min. Force was measured over time and sample firmness was taken as the force (kN) required to shear apple cylinders.

2.6 Image analysis

Images of apple cylinders were acquired by using an image acquisition cabinet (Immagini & Computer, Bareggio, Italy) equipped with a digital camera (EOS 550D, Canon, Milan, Italy). In particular, the digital camera was placed on an adjustable stand positioned 60 cm above a black cardboard base where the apple sample was placed. Light was provided by 4 100 W frosted photographic floodlights, in a position allowing minimum shadow and glare. Other camera settings were: shutter time 1/125 s, F-Number F/6.0, focal length 60 mm. Images were saved in *jpeg* format. Image-Pro® Plus (ver. 6.3, Media Cybernetics, Inc., Bethesda, MD, USA) was used to analyzed apple browning. Brown pixels in the apple images presented $103 < R < 194$, $76 < G < 188$, $5 < B < 100$. Browning was defined as the percentage ratio between brown pixels and pixels corresponding to the apple area.

2.7 Gas chromatographic analyses

A Fisons 8000 Series gas chromatogram, equipped with a thermal conductivity detector Fisons HWD (both from Fisons Instruments, Milan, Italy), was used for analysis of oxygen and carbon dioxide in the headspace of vials containing apple cylinders. Compounds were separated on two glass columns (2 m x 2 mm i.d.), packed with Porapaqs (80/100 mesh), in isothermal conditions (column temperature 70 °C). Carrier gas was nitrogen, at a flow rate of 27 ml min^{-1}; injector and detector temperatures were 180 and 120 °C respectively. The temperature of the filament was 170 °C. Samples were equilibrated at 25 °C before injection; then, 200 μL of headspace was sampled by a 500 μL gastight manual syringe (Dynatech, Batonrouge, Lousiana, USA) and immediately injected in the GC system.
Capillary gas chromatography (GC) and solid-phase microextraction (SPME) were used for the analysis of low-boiling volatile compounds in the headspace of the vials containing apple cylinders. The fiber used was a 1 cm 85 μm Carboxen/PDMS (Supelco, Bellefonte, PA, USA). SPME was run for 2 min at 25 °C; vials were preliminary equilibrated at the operating temperature for 15 min before microextraction. GC injection was carried out in split mode (split ratio 1:10) and the fiber remained in the injector for 1 min. The GC system was a HRGC 8560 Mega Series 2 gas chromatograph (Carlo Erba, Milan, Italy), equipped with a flame ionisation detector (FID); carrier gas was helium, at a linear flow rate of 35 cm s^{-1}. Compounds were separated on a J&W DB-Wax capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness), purchased from Agilent Technologies Inc. (Santa Clara, CA, USA). The column temperature was programmed as follows: 35 °C for 2 min, then at 4 °C min^{-1} up to 44 °C; temperature was then further increased at 20 °C min^{-1} up to 240 °C, held for 15 min. Injector and detector temperature were set at 250 and 240 °C respectively. The concentration of the volatile compounds was expressed in absolute area units. The identity of the peaks was confirmed by comparing their retention time with those of standard compounds; all the standards were from Sigma-Aldrich (St. Louis, MO, USA).

2.8 Statistical analysis

Analyses were performed on at least duplicated samples. Colour and firmness analyses were performed on at least 8 duplicated samples. Results are reported as mean value ± SD. Pearson correlation analysis was performed by using Statistica for Windows (ver. 5.1, Statsoft Inc., Tulsa, USA, 1997). A p-value >0.05 was set as a statistical threshold for significance.

3. RESULTS AND DISCUSSION

Apple cylinders were submitted to pulse light treatments with increasing fluence at environmental temperature. After the treatment, the temperature of the apple cylinder surface never exceeded 30 °C. The effect of pulsed light was initially monitored by isothermal microcalorimetry. This technique was chosen since the evaluation of heat production provides a direct indication of the metabolic responses of raw materials, such as respiration and reaction to wounding stress (CRIDDLE *et al*., 1991; GÒMEZ GALINDO *et al*., 2005; WADSÖ and GÒMEZ GALINDO, 2009; ROCCULI *et al*., 2012). Apple tissue was thus exposed to increasing fluence of pulsed light and evaluated for heat production under isothermal conditions at 30 °C for up to 6 days (Fig. 1).

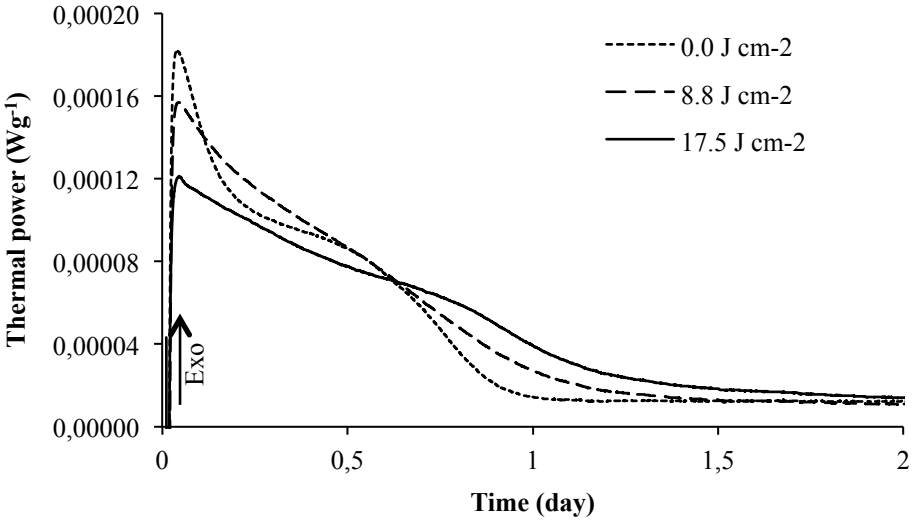


Figure 1. Calorimetric traces at 30 °C of apple tissue exposed to pulsed light with increasing fluence.

Independently on the fluence of the pulsed light treatment, calorimetric traces of all samples showed a main exothermal peak within 1-2 days of observation. Prolonging observation time, no further changes in calorimetric traces were detected (data not shown).

According to the literature, the exothermal peak (Fig. 1) should be related to the thermal effects of microbial growth and/or metabolic activity of the apple tissue (RIVA *et al*., 2001; GÓMEZ GALINDO *et al*., 2005). However, calorimetric peaks due to microbial growth are generally preceded by a plateau signal corresponding to the lag phase of micro-organisms. As clearly shown in Figure 1, no initial plateau signal was detected, suggesting the peak to be mainly attributable to heat generated by the respiration process of wounded apple tissue. It can be inferred that the hypoxic conditions that are quickly generated inside the vial upon apple tissue respiration may inhibit microbial growth making its contribution negligible as compared to that of tissue metabolism.

The calorimetric trace of the control untreated apple showed the occurrence of a sharp and narrow peak (Fig. 1). The peak shoulder could be explained considering the occurrence of physiologic activities with different metabolic rate depending on the storage period. After 1 day of storage at 30 °C, the calorimetric signal reached a plateau value. The latter was above the starting base line and indicated that beyond this storage time, there was still a slight but constant residual metabolic activity. When apple samples were exposed to

pulsed light with increasing fluence, the exothermal peak showed a progressively lower intensity and appeared broader. In addition, the plateau was reached in longer times and its level was lower than in the control sample. These results indicate that pulsed light treatment modifies tissue metabolism. To this regard, the UV-C light component of pulsed light was reported to reduce ethylene production in fresh tomato, favouring the accumulation of putrescine, an anti-senescence agent with an opposite physiological effect with respect to ethylene and delayed the appearance of the climacteric peak (MAHARAJ *et al*., 1999). A similar effect of UV-C radiation has been also reported for other climacteric fruits, such as apples and peaches (LU *et al*., 1991).
To verify the effect of pulsed light on apple tissue respiration, samples were stored for increasing time under the same temperature adopted during the calorimetric analysis (30 °C) and evaluated for oxygen consumption and carbon dioxide formation (Fig. 2).

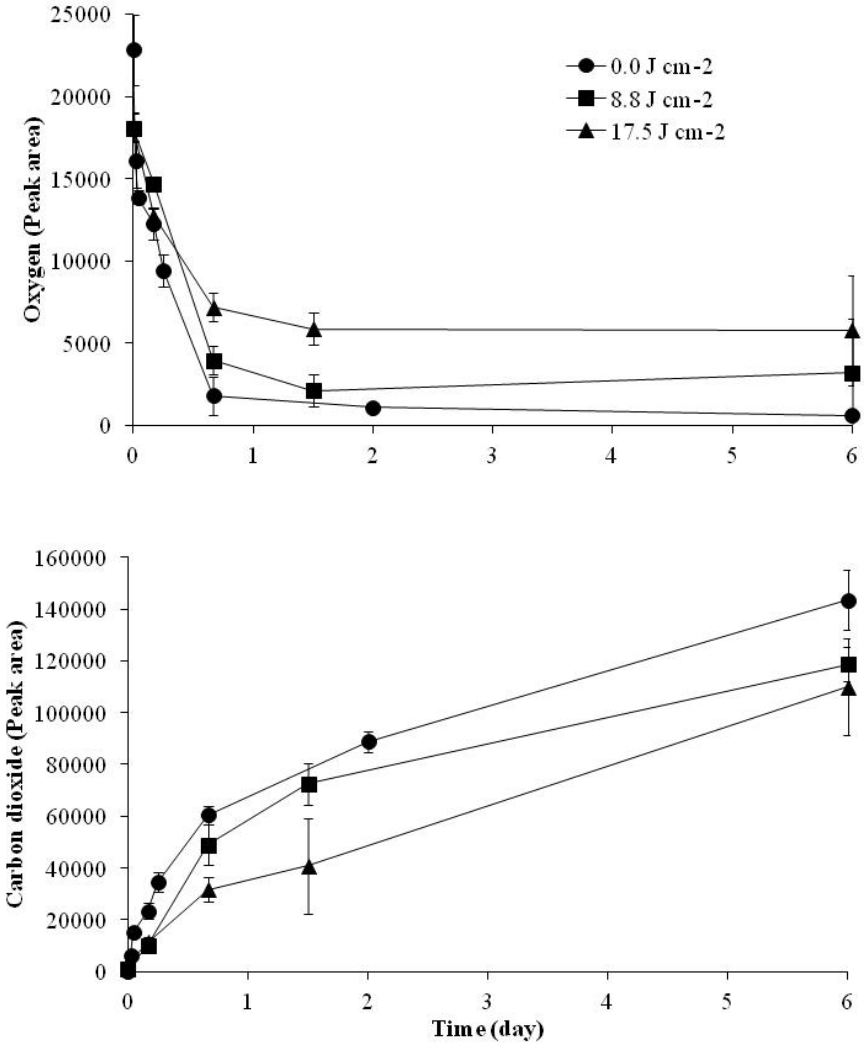


Figure 2. Headspace oxygen and carbon dioxide during storage at 30 °C of apple tissue exposed to increasing fluence of pulsed light.

Headspace oxygen was rapidly consumed during the first days of cut apple storage while carbon dioxide concomitantly accumulated following the typical gas evolution of plant

tissues performing a respiration metabolism. The respiration-driven oxygen consumption and formation of carbon dioxide resulted progressively less intense as the fluence of the pulsed light treatment was increased. This result apparently contradicts with the negligible effect of pulsed light on respiration of endive salad and mung bean sprouts (KRAMER *et al*., 2015). Contradictory results were also reported by RAMOS-VILLARROEL *et al*. (2011) with reference to fresh-cut avocado. These authors showed that pulsed light treatment reduced oxygen consumption but increased respiration products such as carbon dioxide and ethanol. Additional information can be obtained considering the plant respiration effects of UV-C light, which is an important component of the pulsed light radiation. UV-C light was reported to reduce respiration rates of fresh cut cantaloupe melon and intact Fuji apples (LAMIKANRA *et al*., 2005; YUJUAN *et al*., 2015). It can be hypothesised that the different effects of pulsed light on respiration rate may be strongly dependent not only on the nature of the plant matrix and its peculiar respiration metabolism but also on the spectral composition of the light radiation.

The progressive decrease of oxygen partial pressure in the sample headspace (Fig. 2) is well known to be associated to hypoxic conditions, leading to accumulation of ethanol and acetaldehyde involved in post harvest maturation and flavour development (DIXON and HEWETT, 2000; PESIS, 2005). Samples were thus analysed for ethanol, acetaldehyde and ethyl acetate (Fig. 3).

The latter was taken as an example of volatile compound that is expected to play a role in the flavour of fresh-cut apple derivatives (SONG and BANGERTH, 1996; DIXON and HEWETT, 2000). Gas chromatographic analyses were only performed on samples stored up to 2 days of storage due to the minimum metabolic activity on further storage (Figure 1 and 2). Pulsed light treated apple tissues showed significantly lower amounts of ethanol and higher values of acetaldehyde than the control sample (Fig. 3). In addition, when pulsed light was applied at the highest fluence (17.5 J cm^{-2}), it also modified the evolution of ethyl acetate (Fig. 3). As reported in the literature, piruvate is converted to acetaldehyde and CO_2 by the enzyme pyruvate decarboxilase, and acetaldehyde is reduced to ethanol by the enzyme alcohol dehydrogenase (MATHEWS and VAN HOLDE, 1996). Esterification of alcohols, although not fully understood in its biosynthetic pathway, is then responsible for the formation of esters contributing to apple flavour during maturation (DIXON and HEWETT, 2000). Data shown in Figure 3 suggest that exposure to pulsed light radiation could modulate the activity of the enzymes of the anaerobic biosynthetic pathway for the formation of acetaldehyde, ethanol and esters. To this regard, light radiation is known to be absorbed by enzyme proteins due to the presence of endogenous chromophores within their structure (DAVIES and TRUSCOTT, 2001). As a consequence, light would modify the structure of enzymes leading both to their activation or inactivation (MANZOCCO *et al*., 2009). Results acquired in this experimentation indicate that pulsed light is able to modify the metabolic response of apple tissue to the wounding stress, modulating the kinetics of respiration and volatile formation (Figs. 2 and 3). These phenomena would be probably the result of a combination of different biological activities that are concomitantly monitored when measuring the thermal power of the sample (Fig. 1). In order to verify the capability of isothermal calorimetry to study the metabolic consequences of pulsed light treatment, correlation analysis was performed. Table 1 shows the correlation coefficients between thermal power and analytical parameters used to monitor respiration and volatile formation, during storage at 30 °C of cut apple exposed to increasing fluence of pulsed light.

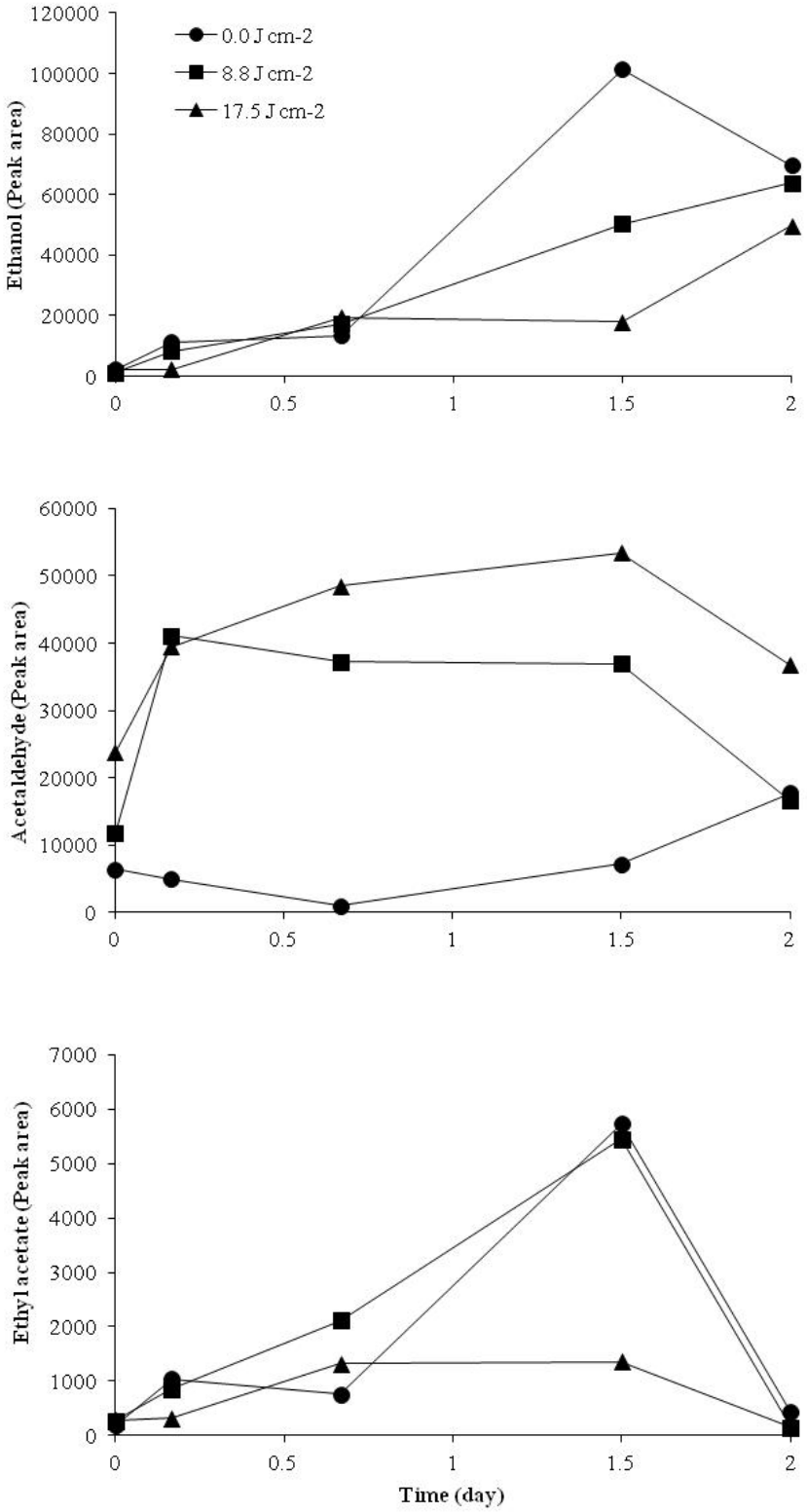


Figure 3. Headspace ethanol, acetaldehyde and ethyl acetate during storage at 30 °C of apple tissue exposed to increasing fluence of pulsed light.

Table 1. Correlation coefficients between thermal power and indices of respiration and volatile formation of cut apple exposed to increasing fluence of pulsed light and stored for increasing time at 30 °C.

	Thermal power
Oxygen	0.77[a]
Carbon dioxide	-0.73 [a]
Ethanol	-0.77 [a]
Acetaldehyde	0.05
Ethyl acetate	-0.32

[a] significant at $p < 0.05$

A very low correlation of thermal power with acetaldehyde and ethyl acetate formation was observed. This can be attributed to the fact that the evolution of these parameters is quite complex, being affected by a number of different concomitant and consequent reaction pathways. By contrast, a high correlation between changes in thermal power and oxygen, carbon dioxide and ethanol was detected. This result definitely indicates that the evolution of the calorimetric signal (Fig. 1) mainly accounts for the metabolic phenomena associated with the respiration process of apple tissue (GÓMEZ GALINDO *et al*., 2005).
The effects of pulsed light on respiration rate could be associated to modification of the metabolism pathways leading to tissue browning and softening. To verify this hypothesis, apple samples exposed to pulsed light were evaluated for the development of browning and the change in firmness. The effect of increasing pulsed light fluence on the percentage of brown colour on the surface of cut apple during storage is shown in Fig. 4.

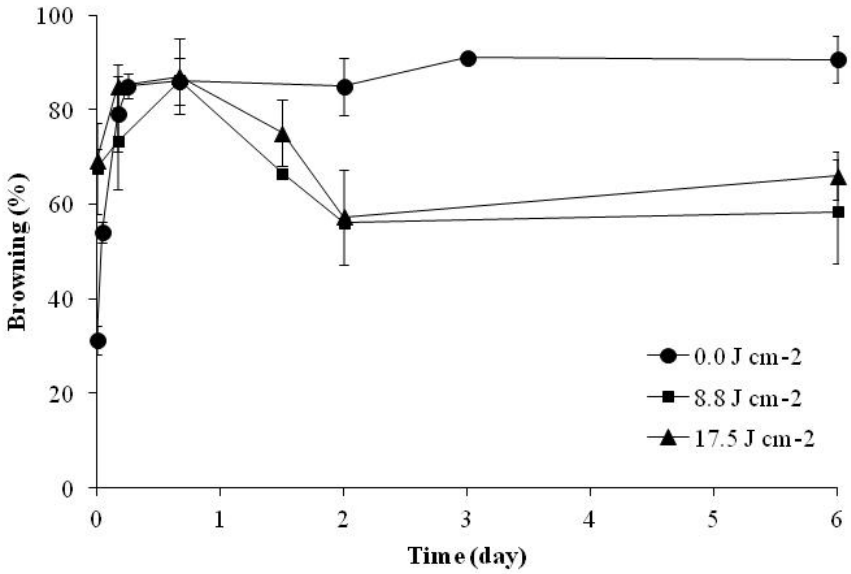


Figure 4. Browning of cut apple exposed to increasing fluence of pulsed light and stored at 30 °C.

The control sample showed a quick development of enzymatic browning during the first hours after cutting (SAPERS and DOUGLAS, 1987). No significant further colour changes were detected when the sample was maintained at 30 °C for up to 6 days. Apple samples exposed to pulsed light appeared browner just after their preparation (0 days). This is in

agreement with the enhancement of phenol biosynthesis as a common response of plant tissue to UV-C light (STEVENS *et al*., 1998; MAHARAJ *et al*., 1999). After 16 hours of storage, pulsed light treated samples showed a browning level analogous to that of the control sample. A significant decrease in browning was observed when pulsed light samples were stored beyond one day. This sample whitening could be attributed to the modification of the phenolic metabolism controlling the formation of brown polyphenols in apple tissue. However, it is not excluded that brown polyphenols could be further degraded to uncoloured compounds. A similar effect was reported as a consequence of pulsed light treatment of protein rich ingredients, such as egg white (MANZOCCO *et al*., 2013a). In that case, sample bleaching was attributed to melanoidin electronic transitions following light radiation absorption. Colour changes could also be accounted for by changes in physical structure of apple tissue. Water distribution could actually play a role in determining the overall sample colour as well as changes in apple tissue firmness. To this regard, Fig. 5 shows the evolution of firmness during storage at 30 °C of apple samples exposed to increasing fluence of pulsed light.

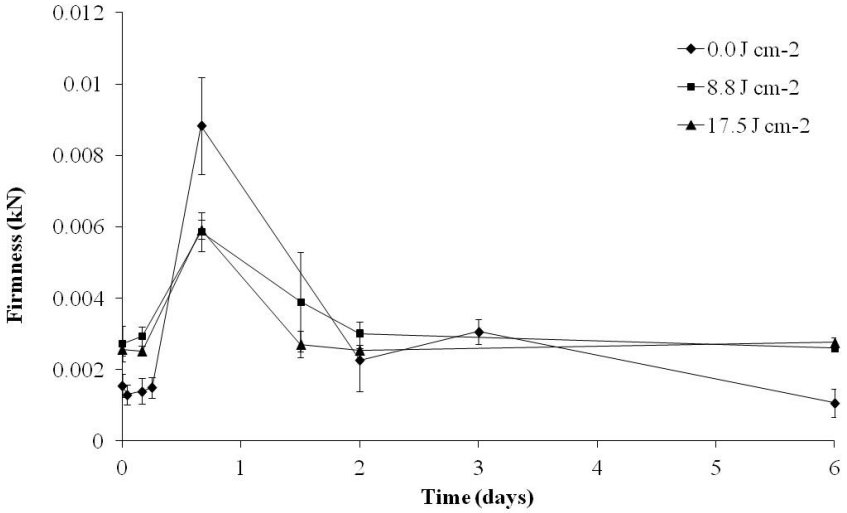


Figure 5. Firmness of cut apple exposed to increasing fluence of pulsed light and stored at 30 °C.

All samples showed an initial increase in firmness, reaching a maximum value after 16 hours of storage. Beyond this storage time, firmness decreased so that a maximum value was identified, followed by a plateau. The increase in firmness could be attributed to sample dehydration while the following decrease could account for progressive pectolityc activity (OMS-OLIU *et al*., 2010). The maximum value of firmness resulted lower in the case of the samples exposed to pulsed light. These result is in agreement with the fact that radiation can promote dehydration of a thin surface layer of the wounded plant tissue, begetting a protective film that hinders moisture migration from the inside to the environment (MANZOCCO and NICOLI, 2015).

4. CONCLUSIONS

Pulsed light has been investigated as an interesting technological approach to decontaminate the surface of fresh-cut fruits and extend their shelf life. Results reported in this work demonstrate that pulsed light exerts additional complex effects of different

metabolism-related properties of fresh-cut vegetables. In the case of apple, exposure to pulsed light modified the kinetics of respiration, volatile formation, browning and tissue softening. The possibility of pulsed light implementation in fresh-cut processing will be strictly dependent on the availability of detailed information about its consequences on tissue metabolisms, especially in relation to different temperature and atmosphere conditions during storage. These data should be merged with those relevant to the antimicrobial activity of pulsed light in order to select optimal fluence to be adopted during fruit treatment. In this context, isothermal calorimetry could represent a very useful methodological tool since potentially allowing to concomitantly quantify both metabolic and microbial activity.

ACKNOWLEDGEMENTS

This research was supported by Ministero dell'Istruzione, dell'Università e della Ricerca (Prot. 957/ric, 28/12/2012), through the Project 2012ZN3KJL "Long Life, High Sustainability".

REFERENCES

Criddle R.S., Breindenbach, R.W. and Hansen, L.D. 1991. Plant calorimetry:how to quantitatively compare apples and oranges. Thermochim. Acta 193:67.

Davies M.J. and Truscott R.J. 2001. Photo-oxidation of proteins and its role in cataractogenesis. J. Photochem. Photobiol. B 63:114.

Dixon J. and Hewett E.W. 2000. Factors affecting apple aroma/flavour volatile concentration:a review. New Zealand J. Crop Horticultural Sci. 28:155.

Falguera V., Pagán J., Garza S., Garvín A. and Ibarz A. 2011. Ultraviolet processing of liquid food:A review. Food Res. Int. 44:1580.

Gòmez Galindo F., Rocculi P., Wadsö L. and Sjöholm I. 2005. The potential role of isothermal calorimetry in monitoring and predicting quality changes during processing and storage of minimally processed fruits and vegetables. Trends Food Sci. Technol. 16:325.

Gòmez P.L., Salvatori D.M., Garcìa-Loredo A. and Alzamora S. M. 2012. Pulsed light treatment of cut apple: dose effect on color, structure and microbiological stability. Food Bioprocess Technol. 5:2311.

Gòmez-Lòpez V.M., Devlieghere F., Bonduelle V. and Debevere J. 2005. Intense light pulses decontamination of minimally processed vegetables and their shelf-life. Int. J. Food Microbiol. 103:79.

Gòmez-Lòpez V.M., Ragaert P., Debevere J. and Devlieghere F. 2007. Pulsed light for food decontamination:a review. Trends Food Sci. Technol. 18:464.

Ignat A., Manzocco L., Maifreni M., Bartolomeoli I. and Nicoli M.C. 2014. Surface decontamination of fresh-cut apple by pulsed light:effects on structure, colour and sensory properties. Postharvest Biol. Technol. 91:122.

Kramer B., Wunderlich J. and Muranyi P. 2015. Pulsed light decontamination of endive salad and mung bean sprouts and impact on color and respiration activity. J. Food Protection 78:340.

Krishnamurthy K., Tewari J.C., Irudayaraj J. and Demirci A. 2008. Microscopic and Spectroscopic evaluation of inactivation of Staphylococcus aureus by pulsed UV light and infrared heating. Food Bioprocess Technol. 3:93.

Lamikanra O., Kueneman D., Ukuku D. and Bett-Garber K.L. 2005. Effect of processing under ultraviolet light on the shelf life of fresh-cut cantaloupe melon. J. Food Sci. 70:C534.

Lu Y.J., Stevens C., Khan V.A. and Kabwe M. 1991. The effect of ultraviolet irradiation on shelf-life and ripening of peaches and apples. Food Quality 14:299.

Luckey T.D. (Ed.). 1980. "Hormesis with ionizing radiation" p. 222. CRC Press, Boca Raton, FL.

Manzocco L. 2015. Photo-induced modification of food protein structure and functionality. Food Eng. Rev. 7:346.

Manzocco L. and Nicoli M.C. 2015. Surface processing:existing and potential applications of ultraviolet light. Crit. Rev. Food Sci. Nutr. 55:469.

Manzocco L., Panozzo A. and Nicoli M.C. 2013a. Effect of pulsed light on selected properties of egg white. Innov. Food Sci. Emerg. Technol. 18:183.

Manzocco L., Panozzo A. and Nicoli M.C. 2013b. Inactivation of polyphenoloxidase by pulsed light. J. Food Sci. 78:E1183.

Manzocco L., Da Pieve S. and Maifreni M. 2011a. Impact of UV-C light on safety and quality of fresh-cut melon. Innov. Food Sci. Emerg. Technol. 12:13.

Manzocco L., Da Pieve S., Bertolini A., Bartolomeoli I., Maifreni M., Vianello A. and Nicoli M.C. 2011b. Surface decontamination of fresh-cut apple by UV-C light exposure:Effects on structure, colour and sensory properties. Postharv. Biol. Technol. 61:165.

Manzocco L., Quarta B. and Dri A. 2009. Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. Innov. Food Sci. Emerg. Technol. 10:506.
Maharaj R., Arul J. and Nadeau P. 1999. Effect of photochemical treatment in the preservation of fresh tomato (Lycopersicon esculentum Mill cv. Capello) by delaying senescence. Postharv. Biol. Technol. 15:13.

Mathews C.K. and van Holde K.E. (Eds) 1996. "Biochemistry". 2nd edition (p. 1159). The Benjamin/Cummings Publishing Company, Inc., Redwood City, CA.

Moraru C. and Uesugi A. 2009. Pulsed-light treatments:principles and applications. In T. Koutchma, L. J. Forney, and C. I. Moraru (Eds.) 2011. "Ultraviolet Light in Food Technology. Principles and Applications" (pp. 235–265). CRC Press, Boca Raton, FL.

Oms-Oliu G., Aguilò-Aguayo I., Martin-Belloso O. and Soliva-Fortuny R. 2010. Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (Agaricus bisporus). Postharv. Biol. Technol. 56:216.

Pesis E. 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharv. Biol. Technol. 37:1.

Ramos-Villarroel A.Y., Martín-Belloso O. and Soliva-Fortuny R. 2011. Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. Eur. Food Res. Technol. 233:395.

Riva M., Fessas D. and Schiraldi A. 2001. Isothermal calorimetry approach to evaluate shelf life of foods. Thermochimica Acta, 370:73.

Rocculi P., Panarese V., Tylewicz U., Santagapita P., Cocci E., Gòmez Galindo F., Romani S. and Dalla Rosa M. 2012. The potential role of isothermal calorimetry in studies of the stability of fresh-cut fruits. Food Sci. Technol. 49:320.

Sapers G.M. and Douglas F.W. 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruit. J. Food Sci. 52:1258.

Song J. and Bangerth F. 1996. The effect of harvest date on aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production. Postharv. Biol. Technol. 8:259.

Stevens C., Liu J., Khan V.A., Lu J.Y., Wilson C.L., Igwegbe E.C.K., Kabwe M.K., Chalutz E. and Droby S. 1998. Application of hormetic UV-C for delaying ripening and reduction of Rhizopus soft rot in tomatoes:the effect of tomatine on storage rot development. J Phytopathol. 146:211.

Takeshita K., Shibato J., Sameshima T., Fukunaga S., Isobe S., Arihara K. and Itoh M. 2003. Damage of yeast cells induced by pulsed light irradiation. Int. J. Food Microbiol. 85:151.

Wadsö L. and Gòmez Galindo F. 2009. Isothermal calorimetry for biological applications in food science and technology. Food Control 20:956.

Wang T., Macgregor S.J., Anderson J.G. and Woolsey G.A. 2005. Pulsed ultra-violet inactivation spectrum of Escherichia coli. Water Res. 39:2921.

Wekhof A. 2000. Disinfection with flash lamps. PDA J. Pharm. Sci. Technol. 54:264.

Yujuan L., Ming'an Y. and Xiaolin R. 2015. Effects of UV-C radiation on storability and quality in harvested apple. Food Sci. China 36:244.

Paper Received January 12, 2016 Accepted January 25, 2017

PAPER

APPLICATION OF ROSEMARY FOR THE PROLONGATION OF MICROBIAL AND OXIDATIVE STABILITY IN MECHANICALLY DEBONED POULTRY MEAT FROM CHICKENS

E. HAĆ-SZYMAŃCZUK*[1], A. CEGIEŁKA[2], E. LIPIŃSKA[1] and K. PIWOWAREK[1]

[1]Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences SGGW (WULS SGGW), 159c Nowoursynowska Street, 02-787 Warsaw, Poland

[2]Department of Food Technology, Faculty of Food Sciences, Warsaw University of Life Sciences SGGW (WULS SGGW), 159c Nowoursynowska Street, 02-787 Warsaw, Poland

*Corresponding author. elzbieta_hac_szymanczuk@sggw.pl

ABSTRACT

In this study, we aimed to determine the effect of rosemary (*Rosmarinus officinalis* L.) preparations on microbial quality and oxidative stability in vacuum-packed mechanically deboned poultry meat (MDPM) from chickens stored at -18 °C for 4 months. We used MDPM originating from four production batches in which rosemary was added to in the form of dried spice (2.0%), extracts (2.0%) such as aqueous and ethanol (40 and 70% (v/v)), and essential oil (0.2%). MDPM control sample did not contain added rosemary. According to the results, the microbial quality of MDPM depended on the type of rosemary preparation used. Compared to the control sample, total bacterial count was considerably lower in samples with added essential oil and ethanol extract (70%, v/v). Essential oil was found to be the most effective in inhibiting psychrotrophic bacteria growth in vacuum-packed MDPM during storage. During the entire storage period, the use of rosemary preparations did not have a significant effect on the count of *Enterobacteriaceae*, but it significantly limited the growth of the coliform bacteria. Based on the index value of thiobarbituric acid reactive substances, rosemary preparations also showed, except for aqueous extract, a decrease in lipid oxidation in vacuum-packed MDPM from chickens stored at -18 °C for 4 months.

Keywords: antimicrobial effect, antioxidant effect, mechanically deboned poultry meat, rosemary, storage

1. INTRODUCTION

Mechanically deboned poultry meat (MDPM) obtained from chickens constitutes raw material commonly used in the meat industry, particularly in the production of homogenized products. The use of MDPM is justified for economic reasons as well as for the pursuit of rational usage of the elements of carcasses, which would be difficult to use otherwise (PIETRZAK *et al.*, 2011). The basic raw material to obtain MDPM from chickens is bones that remains from the deboning of the largest muscles (breast and thigh) and carcasses of chicken of lower quality (STANGIERSKI *et al.*, 2011).
The MDPM production, storage, and processing conditions have been established in Regulation (2004). Despite the continuous improvements in methods and machines used to obtain MDPM, Polish and European poultry industries most commonly use high-pressure methods for the production of MDPM, which is destructive for the bone structure (NAGY *et al.*, 2007; BOTKA-PETRAK *et al.*, 2011; BEŁKOT *et al.*, 2013). This leads to the lower stability of the raw material upon storage than hand-trimmed or machine-trimmed chicken meat. This poor stability is primarily due to the high level of fragmentation and aeration during production, which contributes to a higher susceptibility toward oxidation processes and an increase in the growth of microflora (GRABOWSKI and KIJOWSKI, 2004; MICHALSKI and POMYKAŁA, 2008).
In a situation of inability for immediate use of MDPM in processing, the raw material is stored in a frozen state (GRABOWSKI and KIJOWSKI, 2004). Addition of natural substances from plant origin, exhibiting antimicrobial and antioxidant effect, constitutes an additional factor in prolonging the stability of meat and meat products during storage. However, the latest literature search (SHAH *et al.*, 2014) reveals that the majority of the studies of the effectiveness of plant preparations on the stability of meat products during storage involves mammal meat and its products. Study results demonstrate that inter alia rosemary preparations may be used to minimize the oxidative changes in meat and meat products such as aged beef (COLLE *et al.*, 2016), raw pork batters (HERNÁNDEZ-HERNÁNDEZ *et al.*, 2009), fresh (GEORGANTELIS *et. al.*, 2007) and thermally processed pork sausage (SEBRANEK *et al.*, 2005), wiener (CORONADO *et al.*, 2002) and bologna sausages (VIUDA-MARTOS *et al.*, 2010), frankfurters (ESTÉVEZ and CAVA, 2006), and reduced nitrite liver pâtés (DOOLAEGE *et al.*, 2012). Due to the application of rosemary preparations, microbial quality of different meat products can be improved, inter alia in modified atmosphere-packaged fresh pork and vacuum-packed ham slices (ZHANG *et al.*, 2009), and in the African fresh sausage (MATHENJWA *et al.*, 2012). However, there is little information on the application possibilities of plant preparations for the prolongation of storage stability of MDPM (HASSAN and LAM SWET FAN, 2005; HAĆ-SZYMAŃCZUK *et al.*, 2014) and its products (MOHAMED and MANSOUR, 2012; JIRIDI *et al.*, 2015).
Rosemary (*Rosmarinus officinalis* L.), from the *Lamiaceae* family, is a plant with both strong antioxidant and antimicrobial activities. It is used in food in the form of fresh or dried leaves, essential oil, and aqueous and alcoholic extracts from leaves (ERKAN *et al.*, 2008; HAĆ-SZYMAŃCZUK *et al.*, 2010). Various studies have demonstrated that the complex biologically active substances of rosemary have an inhibitory effect on a wide spectrum of bacteria, including *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Klebsiella pneumoniae* (DIMITRIJEVIC *et al.*, 2007; ZHANG *et al.*, 2009; HAĆ-SZYMAŃCZUK *et al.*, 2010). As an alternative to synthetic antioxidants, rosemary preparations have been used in food processing (BALENTINE *et al.*, 2006; VELASCO and WILLIAMS, 2011).
In this study, we aimed to determine the effect of rosemary (*R. officinalis* L.) on lipid oxidation and microbial quality of MDPM from chickens stored at -18 °C for 4 months.

The results of this study may contribute to the understanding of the innovative methods of MDPM preservation.

2. MATERIALS AND METHODS

We used MDPM that was obtained from a production plant in northeast Poland. MDPM was prepared using high-pressure separation method on breast muscle scraps of broilers. The chilled MDPM (6 kg) was collected from the wholesalers in Warsaw and transported to the Division of Food Biotechnology and Microbiology of the Faculty of Food Sciences under refrigerated conditions. Rosemary (*R. officinalis* L.) was added to the MDPM in dried form ("Kamis," McCormic, Stefanowo, Poland) and as an aqueous extract, ethanol extracts, and essential oil (own production) under laboratory conditions. In the following parts of the paper, they will be called rosemary preparations.

Each of the batches of MDPM from chickens was analyzed for fat, protein, and water content. The determination of these chemical components in MDPM was performed in accordance with the requirements of the Association of Official Analytical Chemists (AOAC, 2007). We used a FoodScan™Lab near-infrared spectrometer (Foss Analytical A/S, Hillerød, Denmark) working in the spectral range of 850-1050 nm and using a calibration based on the artificial neural network model.

The aqueous extract and ethanol extracts from dried rosemary ("Kamis," McCormic, Stefanowo, Poland) were obtained via continuous extraction in a Soxhlet apparatus (a universal extraction system B-811, Büchi Labortechnik AG, Flawil, Switzerland). The extraction process parameters were established in the preliminary study (results unpublished). For the preparation of each extract, 40 g of dried rosemary was distributed onto 8 extraction thimbles (5 g per thimble). Distilled water and ethyl alcohol with 40 and 70% (v/v) concentration were, respectively, used as solvents. The raw material in each thimble was extracted with 150 mL of the appropriate solvent for 15 cycles, maintaining the boiling point of a solvent. The portions obtained from each extract were combined, resulting in approximately 550 mL of raw extracts. The raw extracts were filtered using 180-μm thick filter paper (Whatman GE, LaboPlus Sp. z o.o., Warsaw, Poland). Subsequently, each extract was concentrated in a rotary evaporator (Rotovaporator R-205; Büchi Labortechnik AG) until approximately 40 g of the extract was left, corresponding to the weight of dried rosemary used to obtain the extract.

To obtain essential oil from rosemary, the method of BIAŁECKA-FLORIAŃCZYK and WŁOSTOWSKA (2007) was followed. Around 30 g of fresh rosemary leaves were crumbled and covered with 400 mL of water. This was subjected to distillation in a Deryng apparatus by Simax until essential oils were obtained. The chilled distillate was four times extracted using dichloromethane in a separatory funnel. Then, water was removed by adding anhydrous magnesium sulfate. The obtained extract was concentrated in a rotary evaporator (Rotovaporator R-205). The solvent was evaporated at a temperature of 30 °C and at a pressure of 540-560 hPa.

The chemical composition of the aqueous extract, ethanol extracts, and essential oil from rosemary was analyzed for the identification and determination of chemical compounds.

The determination of volatile compounds in essential oil was performed by gas chromatography (GC) equipped with flame ionization detector (FID) (Perkin Elmer, Autosystem XL) based on the literature (BURT, 2004; DJEDDI *et al.*, 2007). The following parameters were used for separation: HP-5 column (30 m × 0.32 mm × 0.25 μm), helium as a carrier gas (3 cm^3/min), split mode (1:100) for sample injection, injection temperature at 270 °C, and FID temperature at 300 °C. The following program of oven temperature was used: initial temperature 35 °C/5 min, 30 °C/min temperature increase up to 60 °C

followed by 6 °C/min to 200 °C and 30 °C/min until a temperature of 280 °C was achieved.
The identification and determination of the amount of selected chemical compounds in rosemary extracts was performed based on the literature (LONGARAY DELAMARE *et al.*, 2007, TAWAHA *et al.*, 2007). In this study, we performed high performance liquid chromatography (HPLC) using Agilent 1200 liquid chromatography coupled with diode array detector (DAD). Zorba Eclipse XDB C18 (4.6 × 150 mm) column was used with the following parameters: 5 μl injection volume, 0.8 cm^3/min flow rate, and UV detection at a wavelength of 210 and 325 nm. Separation was performed in gradient elution with two eluents: A-acetonitrile and B-0.05% trifluoroacetic acid. Data were analyzed using EZ Elite Chrome program.
In each experimental series, six samples of MDPM were prepared (each weighing 1 kg), differing in the type of rosemary preparation added: Control-sample without addition of rosemary, D-2.0% addition of dried rosemary, WE-2.0% addition of aqueous extract from rosemary, E40-2.0% addition of 40% (v/v) ethanol extract from rosemary, E70-2.0% addition of 70% (v/v) ethanol extract from rosemary, and EOS-0.2% addition of essential oil from rosemary.
The amount of rosemary preparations added to MDPM was established based on the recommendations of the producer or based on the literature (GEORGANTELIS *et al.*, 2007). After MDPM samples were thoroughly mixed with rosemary preparations, each sample was divided into four portions (250 g each) and vacuum-packed in plastic bags (PE/PA, thickness 75 μm) using a vacuum machine C200 (Multivac Sepp Haggenmüller GmbH & Co. K.G., Wolfertschwenden, Germany). These samples were stored at a temperature of -18° C for 4 months. After each month, microbial analyzes were performed and TBARS index values were determined for all MDPM samples including the control sample. Before the analyzes, each sample was defrosted (+4 °C, 4 h) without opening the packaging. Moreover, directly after the delivery of the raw material to the laboratory, the same determinations were carried out only on the MDPM control sample.
The microbial analyzes were conducted following Polish Standard (PN-EN ISO, 2005). They include the determination of the total bacteria count (TBC) (PN-EN ISO, 2013), number of psychrotrophic bacteria (PN-ISO, 2004), *Enterobacteriaceae* (PN-ISO, 2005), coliform bacteria (PN-ISO, 2007), and *Salmonella* spp. (PN-EN ISO, 2003). The number of bacteria was expressed as $\log_{10}$ colony forming units per gram (log CFU/g). TBARS was determined using the extraction method of PIKUL et al. (1989). TBARS index value was expressed in milligram of malondialdehyde per kilogram of sample (mg MAD/kg).
The experiment was repeated four times, preparing MDPM samples from different production batches. The statistical analysis of the results was performed using Statistica version 10.0 program (2011). The significance was tested using one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test at a significance level of α=0.05.

3. RESULTS

In this study, the analysis of chemical composition of rosemary preparations revealed that they differed in the chemical profile. Each of them consisted of a complex mixture of different substances. The results of an earlier work (HAĆ-SZYMAŃCZUK *et al.*, 2015) demonstrated that the dominating compounds of the aqueous extract of rosemary were rosmarinic, ferulic, and chlorogenic acids. In the ethanol extracts of rosemary, the major compounds present were rosmarinic acid, carnosol, and ferulic acid (Table 1), whereas in

the essential oil, the major compounds present were camphor, borneol, and R(+)limonene (Table 2).

Table 1. Chemical composition of extracts from rosemary.

Chemical compound	Retention time (min)	Ethanol extract (40%, v/v)	Ethanol extract (70%, v/v)
		Concentration (mg/cm^3)	
Chlorogenic acid	2.83	0.068	0.108
Epicatechin	3.80	nd	0.002
Caffeic acid	4.33	0.012	0.026
Rutoside	5.83	0.060	0.104
p-coumaric acid	7.04	0.006	0.020
Ferrulic acid	8.08	0.112	0.166
Benzoic acid	11.87	0.012	0.018
Rosemarinic acid	12.70	4.006	5.756
Myricetin	12.80	nd	0.002
Resveratrol	15.51	nd	0.002
Quercetyn	18.69	0.006	0.012
Carnosol	25.73	0.468	0.178
Curcumin	26.03	nd	0.026

nd – not detected

Table 2. Chemical composition of essential oil from rosemary.

Chemical compound	Retention time (min)	Concentration (mg/cm^3)
α-pinene	7.88	0.20
β-pinene	8.79	0.10
Myrcene	9.21	0.22
1,4-cineole	9.72	0.10
p-cymene	9.92	0.53
R(+) limonene	10.13	22.47
γ-terpinene	10.71	0.75
Linalol	11.70	1.33
Camphor	12.26	51.87
Borneol	13.15	27.90
Carvone	14.93	0.44
Bergamol	15.23	0.18
Thymol	15.99	0.11
Carwacrol	16.20	6.70
Eugenol	17.42	0.92
β-caryophyllene	18.73	0.62

Based on the results of microbial analysis of MDPM during storage (Figs. 1-4), it was found that the tested rosemary preparations showed different antimicrobial activity. During the storage period, TBC was found to be highest in the control sample (Fig. 1). In the EOS, E70, and E40 samples, a reduction in TBC was observed from 2 months of

storage. After 4 months of storage, the EOS and E70 samples were characterized by significantly ($p \leq 0.05$) lower TBC than control sample. Of all the tested rosemary preparations, essential oil was found to be the most efficient in inhibiting the growth of psychrotrophic microorganisms (Fig. 2). EOS significantly lowered the number of microorganisms compared to control, D, and WE after 4 months of storage. In each of the examined MDPM samples, a significantly ($p \leq 0.05$) higher *Enterobacteriaceae* bacterial count was found after 2 months of storage (Fig. 3). The use of rosemary preparations did not significantly ($p > 0.05$) influence the count of *Enterobacteriaceae* in the MDPM samples during the entire storage period. In each of the examined MDPM samples, coliform bacteria was also detected (Fig. 4). However, in comparison to the control sample, addition of rosemary preparations to MDPM significantly ($p \leq 0.05$) restricted the growth of coliform bacteria during the entire storage period. *Salmonella* spp. was not determined in any of the examined MDPM samples.

The fat, protein, and water content in MDPM from chickens was on average 15.93, 15.72, and 66.31%, respectively.

Based on TBARS index values (Fig. 5.), the addition of rosemary preparations to MDPM had an influence on the course of oxidative changes in lipids. Among the tested preparations, the weakest antioxidant activity was exhibited by the aqueous rosemary extract. However, other rosemary preparations significantly ($p \leq 0.05$) slowed down the processes of lipid oxidation in the MDPM samples during the storage period. Our results also demonstrated that for EOS and E70 samples, the TBARS index value after 4 months of storage was significantly ($p \leq 0.05$) lower than the TBARS index value after 1 month of storage.

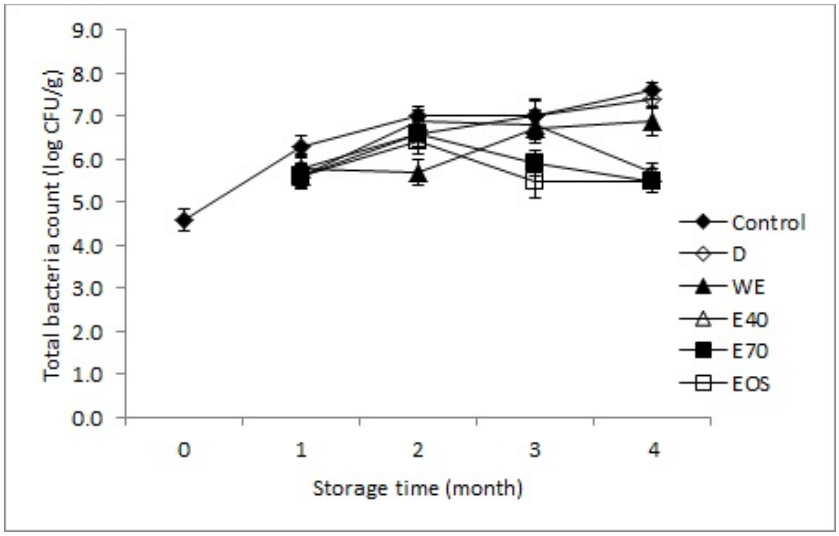


Figure 1. Effect of addition of rosemary preparations on the total bacteria count in mechanically deboned poultry meat (MDPM) from chickens stored at -18 °C. Notes: Control-control sample, without addition of rosemary; D-2.0% addition of dried rosemary; WE-2.0% addition of aqueous extract from rosemary; E40-2.0% addition of 40% (v/v) ethanol extract from rosemary; E70-2.0% addition of 70% (v/v) ethanol extract from rosemary; EOS-0.2% addition of essential oil from rosemary.

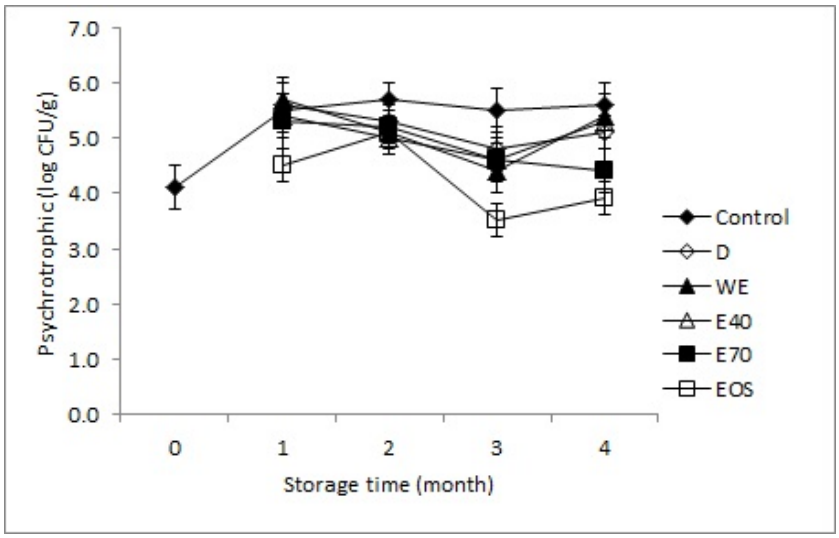


Figure 2. Effect of addition of rosemary preparations on the number of psychrotrophic bacteria in mechanically deboned poultry meat (MDPM) from chickens stored at -18 °C. Notes: Control-control sample, without addition of rosemary; D-2.0% addition of dried rosemary; WE-2.0% addition of aqueous extract from rosemary; E40-2.0% addition of 40% (v/v) ethanol extract from rosemary; E70-2.0% addition of 70% (v/v) ethanol extract from rosemary; EOS-0.2% addition of essential oil from rosemary.

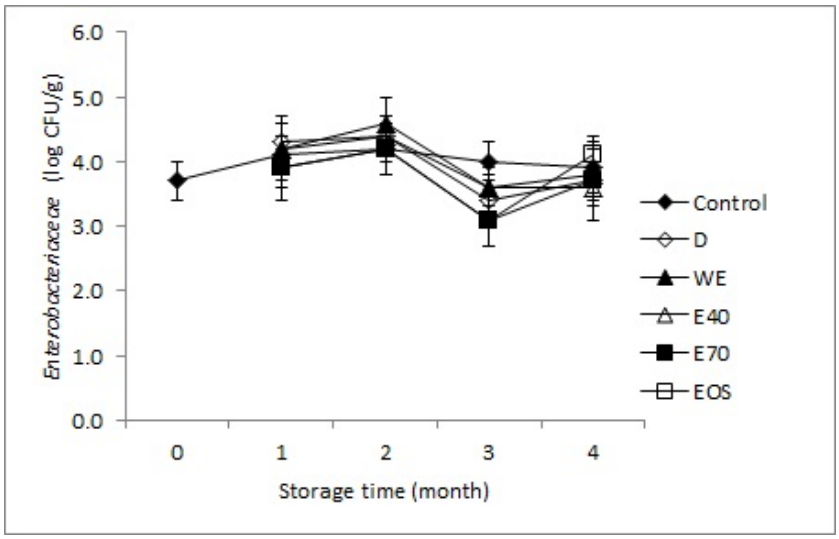


Figure 3. Effect of addition of rosemary preparations on the number of *Enterobacteriaceae* bacteria in mechanically deboned poultry meat (MDPM) from chickens stored at -18 °C. Notes: Control-control sample, without addition of rosemary; D-2.0% addition of dried rosemary; WE-2.0% addition of aqueous extract from rosemary; E40-2.0% addition of 40% (v/v) ethanol extract from rosemary; E70-2.0% addition of 70% (v/v) ethanol extract from rosemary; EOS-0.2% addition of essential oil from rosemary.

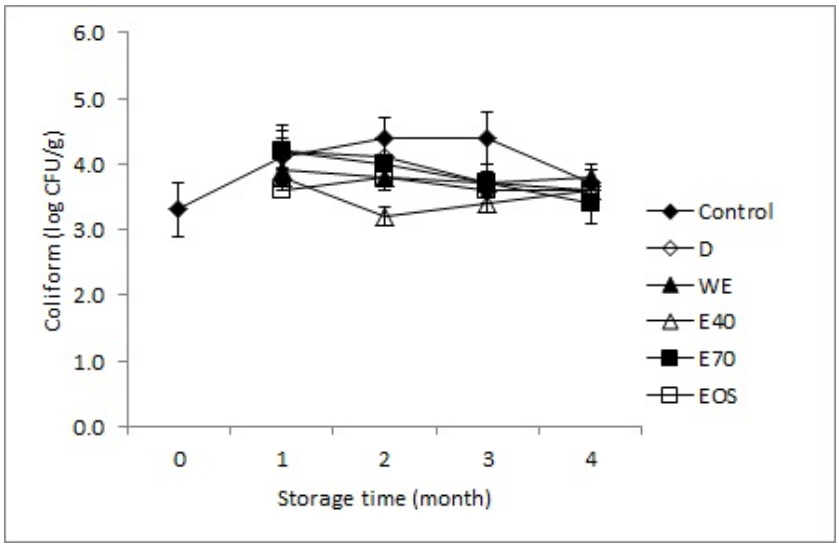


Figure 4. Effect of addition of rosemary preparations on the number of coliform bacteria in mechanically deboned poultry meat (MDPM) from chickens stored at -18 °C. Notes: Control-control sample, without addition of rosemary; D-2.0% addition of dried rosemary; WE-2.0% addition of aqueous extract from rosemary; E40-2.0% addition of 40% (v/v) ethanol extract from rosemary; E70-2.0% addition of 70% (v/v) ethanol extract from rosemary; EOS-0.2% addition of essential oil from rosemary.

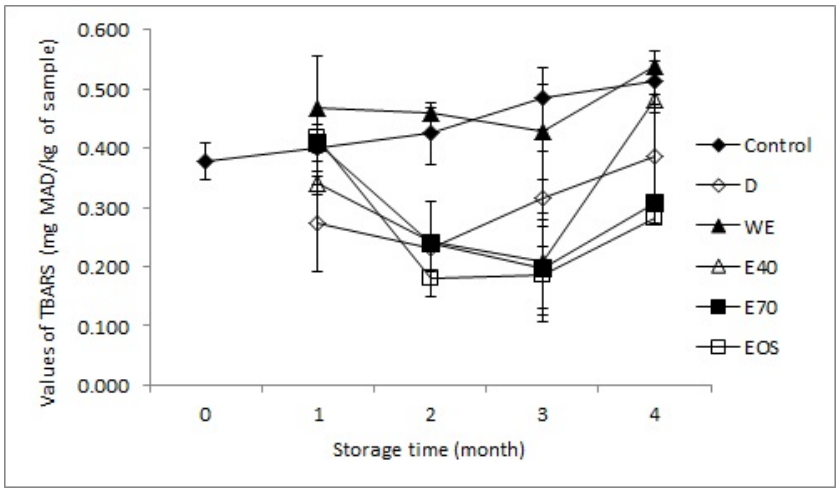


Figure 5. Effect of addition of rosemary preparations on thiobarbituric acid reactive substances (TBARS) index value in mechanically deboned poultry meat (MDPM) from chickens stored at -18 °C. Notes: Control-control sample, without addition of rosemary; D-2.0% addition of dried rosemary; WE-2.0% addition of aqueous extract from rosemary; E40-2.0% addition of 40% (v/v) ethanol extract from rosemary; E70-2.0% addition of 70% (v/v) ethanol extract from rosemary; EOS-0.2% addition of essential oil from rosemary.

4. DISCUSSION

The results of this study showed that MDPM from chickens contained a high amount of fat (15.93%). However, it was in compliance with the requirements of non-compulsory Polish Standard (PN, 1992), which states that MDPM from burring poultry should not contain fat more than 20%, protein less than 12%, and water more than 75%. The fat present in MDPM is susceptible to oxidation, which might be due to the presence of the unsaturated fatty acids and phospholipids along with the catalytic effects of heme iron

(GRABOWSKI and KIJOWSKI, 2004; PIETRZAK *et al*., 2011; BEŁKOT *et al*., 2013). Since lipid oxidation is the major cause of quality loss in MDPM, in our opinion the application of rosemary preparations as sources of natural antioxidants seems to be interesting option for preserving the shelf life of this raw material.

The raw material used in this study met the food safety criteria with respect to *Salmonella* and aerobic bacteria and *E. coli* count as specified in Commission Regulation (2005). More discussion in this area is difficult because the available literature lacks the information on antimicrobial effect of rosemary extracts in MDPM.

HAĆ-SZYMAŃCZUK *et al.* (2009) and OKOH *et al.* (2010) have found that the antimicrobial efficiency of rosemary extracts varies, which could be attributed to the type and method of its preparation (e.g., distillation and extraction). This could be due to the different chemical profiles of these preparations. In their study, OKOH *et al.* (2010) found that rosemary oil obtained by solvent free microwave extraction exhibited stronger inhibitory effect on the examined bacteria (*Staphylococcus aureu*s, *E. coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae*) in comparison to the oil obtained through hydro-distillation. However, HAĆ-SZYMAŃCZUK *et al.* (2009) found that rosemary oil as well as aqueous extract did not inhibit the growth of *S. aureus* and *K. pneumoniae* on Mueller-Hinton Agar medium.

ROMANO *et al.* (2009) found that the addition of rosemary leaf extract limited the growth of *E. coli.* According to the authors the minimal inhibitory concentration (MIC) of this extract was 105 μg/mL. They found a stronger antimicrobial activity than benzoic acid and butylated hydroxytoluene (BHT), whose concentration was 250 μg/mL. However, based on the comparative study of antimicrobial properties of essential oils from *Lamiaceae* plants, ŽIŽOVIC *et al.* (2009) found that the minimum inhibitory concentration (MIC) for *Escherichia* and *Salmonella* bacteria was above 1250 μg/mL.

Similar studies on the use of rosemary preparations, solely or mixed with other components of plant origin, have demonstrated efficiency in inhibiting the growth of microflora in meat and meat products. According to ABDEL-HAMIED *et al.* (2009), a significant inhibition of psychrotrophic microorganisms in minced meat stored at 4 °C and -18 °C was obtained by using a mixed addition of rosemary and salvia extracts. According to them, the addition of 0.05% of extracts to the meat stored at 4 °C for 10 days reduced the number of psychrotrophic microbes from 31.64 log CFU/g in the control to 14.12 log CFU/g in the extract sample. For meat stored at -18 °C for 100 days, the bacterial count was 7.16 and 20.31 log CFU/g, respectively, for the extracts and control samples.

ZHANG *et al.* (2009) studied antimicrobial activity of 14 different extracts toward pathogenic bacteria causing pork meat spoilage such as *Listeria monocytogenes*, *E. coli*, *Pseudomonas fluorescens*, and *Lactobacillus sake*. According to their results, the modified-atmosphere-packed meat stored at 4 °C for 28 days demonstrated the effectiveness of combination of rosemary and liquorice extracts as a natural preservative, which significantly inhibited the growth of the studied microorganisms.

According to PHAM *et al.* (2013), a mixture of rosemary extract (addition level: 2000 ppm) and green tea extract (100–300 ppm) can be used to limit the growth of psychrotrophic bacteria in raw pork sausage stored at -20 °C for 6 months. According to MATHENJWA *et al.* (2012), the use of plant extracts and chitosan in the production of traditional South African pork and beef sausage can lower or eliminate the addition of sulfur dioxide (SO_2) as a preservative. They found that the combination of rosemary extract (addition level: 260 mg/kg), chitosan (10 mg/kg), and SO_2 (100 mg/kg) or rosemary extract with chitosan had an equally efficient antimicrobial effect in sausages as SO_2 (250 mg/kg).

The results of microbiological quality evaluation of MDPM obtained in this study confirm the bacteriostatic properties of rosemary formulations. Literature data indicate, however,

that some of the active substances present in these preparations may exhibit bactericidal effect. Thymol and carvacrol are chemical compounds whose mechanism of action on bacterial cells has been most comprehensively evaluated so far. The presence of these compounds has been confirmed in rosemary oil used in this study. Their mechanism of action on Gram-negative bacteria is based on the disintegration of the cell membrane, by releasing lipopolysaccharides (LPS) and increasing the permeability of the plasma membrane for adenosine triphosphate (ATP), the loss of which ultimately leads to cell death (HELANDER *et al*., 1998).

In case of Gram-positive bacteria, carvacrol interacts with the cell membrane, changing its permeability toward H+ and K+ cations. Change in the gradient of these cations causes disruption of the basic processes in cell and ultimately leads to cell death. In Gram-positive bacteria, increase in membrane permeability toward ATP is not observed as for Gram-negative bacteria (ULTEE *et al*., 2002).

The present literature does not provide information on the antioxidant activity of rosemary extracts in MDPM, and the majority of studies concern the storage stability of slaughtered mammal meat and its products (HERNÁNDEZ-HERNÁNDEZ *et al*., 2009; WÓJCIAK *et al*., 2011; PHAM *et al*., 2013; ARMENTEROS *et al*., 2016).

WÓJCIAK *et al*. (2011) compared the antioxidant activity of aqueous extracts from different plants added to pork meat and found that after 30 days of storage under refrigeration conditions, the highest efficiency was observed in rosemary extract. However, HERNÁNDEZ-HERNÁNDEZ *et al*. (2009) recommend the addition of alcoholic extract of rosemary based on the study performed on model pork batters to slow down the lipid oxidation processes. According to them, the strong antioxidant property of the extract might be due to high concentration of carnosic acid and carnosol and the presence of numerous other active components. The antioxidant activity of rosemary extracts was also confirmed by PHAM *et al*. (2013) on raw pork sausage and by MATHENJWA *et al*. (2012) on pork and beef sausage, which were stored in a frozen state for 180 and 100 days, respectively.

According to MIELNIK *et al*. (2003), the use of commercial rosemary preparations may also constitute an alternative method for the improvement of oxidative stability and prolongation of MDPM from turkey upon storage. To obtain a satisfactory quality of vacuum-packed raw material stored at -25 °C for 7 months, an individual selection of the type and amount of rosemary preparation is necessary, which complies with our results.

In contrast to the above-cited literature, COLLE *et al*. (2016) reported that the use of rosemary extract together with ascorbic acid did not significantly limit the processes of lipid oxidation in beef steaks in comparison to the control product. SZCZEPANIK (2007) conducted a comparative study of antioxidant activity of extracts from dill, coltsfoot, rosemary, horsetail, salvia, and thyme in the breast muscle of chickens and turkeys during a 6-month frozen storage (-25 °C). The author also found that none of the used extracts significantly slowed down the oxidation process of lipids contained in the chicken muscles.

Although we did not evaluate it in this work, the use of natural preservatives of plant origin could be helpful in controlling the oxidation of other ingredients that exhibit nutritional value in meat products. NIETO *et al*. (2013) explored the mechanisms behind the protection of protein against oxidation by natural plant antioxidants. The oxidative stability of the meat proteins in pork patties was evaluated as loss of thiols and as formation of myosin cross-links. Essential oil of rosemary was found to have an antioxidative effect on protein thiol loss. Furthermore, protein disulfide cross-link formation was inhibited in pork patties with added essential oil of rosemary. These and other properties of rosemary preparations are due to a large range of chemical compounds.

Both the results of the analysis of the chemical composition of rosemary preparations obtained by the authors of this study and those presented by other researchers (BURT, 2004; DJEDDI *et al*., 2007) demonstrate that these preparations are mixtures of many different compounds. According to DJEDDI *et al*. (2007) the chemical profile of preparations from rosemary depends not only on the methods of obtaining them but also on the habitat of plants. When reviewing the literature, the authors concluded that the climatic differences between south Europe and North Africa Mediterranean areas might have a significant impact on the content of ingredients such as 1,8-cineole, α-pinene, and camphor in rosemary essential oil. KASPARAVIČIENE *et al*. (2013) reported that ethanol extracts of rosemary contain primarily three groups of compounds: phenolic diterpenes, flavonoids, and phenolic acids. ABRAMOVIČ *et al*. (2012), among the dominant phenolic diterpenes of these extracts, mentioned-after COUVELIER *et al*. (1996) carnosol, carnosic acid, methyl carnosate, and phenolic acids from caffeinic and rosmarinic acid.

5. CONCLUSIONS

Our results indicate that the addition of rosemary preparations constitute an auxiliary factor in the preservation of MDPM from chickens stored in a frozen state for 4 months. The tested preparations differed in their chemical composition and antimicrobial and antioxidant activities. The addition of 0.2% essential oil and 2.0% of 70% (v/v) ethanol extract was the most efficient in restricting the growth of microflora and inhibiting lipid oxidation in MDPM from chickens.

ACKNOWLEDGEMENTS

This work is a part of the project titled "Studies on the antimicrobial and antioxidant effects of extracts, essential oils and dried spices in poultry meat," which was financially supported by the Polish Ministry of Science and Higher Education in 2011–2015 (grant No. N N312 257040).

REFERENCES

Abdel-Hamied A.A, Nassar A.G. and El-Badry N. 2009. Investigations on antioxidant and antibacterial activities of some natural extracts. World J. Dairy Food Sci. 4:1-7.

Abramovič H., Terpinc P., Generalia I., Skroza D., Klančnik A., Katalinic V. and Možina S.S. 2012. Antioxidant and antimicrobial activity of extracts obtained from rosemary (*Rosmarinus officinalis*) and vine (*Vitis vinifera*) leaves. Croat. J. Food Sci. Technol. 4(1):1-8.

AOAC. 2007. "Official Methods of Analysis" 18th ed. Association of Official Analytical Chemists, Gaithesburg, MD, USA.

Armenteros M., Morcuende D., Ventanas J. and Estévez M. 2016. The application of natural antioxidants via brine injection protects Iberian cooked hams against lipid and protein oxidation. Meat Sci. 116:253-259.

Balentine C., Crandall P., O'Bryan C., Duong D. and Pohlman F. 2006. The pre- and post-grinding application of rosemary and its effects on lipid oxidation and color during storage of ground beef. Meat Sci. 73:413-421.

Bełkot Z., Ziomek M. and Gondek M. 2013. Nutritional value of mechanically recovered goose and chicken meat. Med. Weter. (PL). 69:499-504.

Białecka-Florjańczyk E. and Włostowska J. (Ed.). 2007 "Laboratory experiments in organic chemistry", (PL), 2nd ed. Wyd. SGGW, Warsaw.

Botka-Petrak K., Hraste A., Lucić H., Gottstein Ž., Gomerčić M.D., Jakšić S. and Petrak T. 2011. Histological and chemical characteristics of mechanically deboned meat of broiler chickens. Vet. Arhiv. 81:273-283.

Burt S. 2004. Essential oils, their antibacterial properties and potential applications in foods – a review. Int. J. Food Microbiol. 94:223-252.

Colle M.J., Richard R.P., Colle M.C., Loucks W.I. and Doumit M.E. 2016. Effects of ascorbic acid and rosemary extract on quality characteristics and sensory perception of extended aged beef. Meat Sci. 112:141.

Commission Regulation 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L338. 1-26.

Coronado S.A., Trout G.R., Dunshea F.R. and Shah N.P. 2002. Antioxidant effects of rosemary extract and whey powder on the oxidative stability of wiener sausages during 10 months frozen storage. Meat Sci. 62:217-224.

Couvelier M.E., Richard H., Berset C. 1996. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. J. Am. Oil Chem. Soc. 74:645-652.

Dimitrijevic S.I., Mihajlovski K.R., Antonovic D.G., Milanovic-Stevanovic M.R. and Mijin D.Z. 2007. A study of the synergistic antilisterial effects of a sublethal dose of lactic acid and essential oils from *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum vulgare* L. Food Chem. 104:774-782.

Djeddi S., Bouchenah N., Settar I. and Skaltsa H.D. 2007. Composition and antimicrobial activity of the essential oil of Rosmarinus officinalis from Algeria. Chem. Nat. Comp. 43:487-490.

Doolaege E.H.A., Vossen E., Raes K., De Meulenaer B., Verhé R., Paelinck H and De Smet S. 2012. Effect of rosemary extract dose on lipid oxidation, colour stability and antioxidant concentrations in reduced nitrite liver pâtés. Meat Sci. 90:925-931.

Erkan N., Ayranci G. and Ayranci E. 2008. Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesame oil. Food Chem. 110:76-82.

Estévez M. and Cava R. 2006. Effectiveness of rosemary essential oil as inhibitor of lipid and protein oxidation: Contradictory effects in different types of frankfurters. Meat Sci. 72:348-355.

Georgantelis D., Ambrosiadis I., Katikou P., Blekas G. and Georgakis S.A. 2007. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. Meat Sci. 76:172-181.

Grabowski T. and Kijowski J. 2004. Technologia przetworów drobiowych. In:„Mięso i przetwory drobiowe. Technologia, Higiena, Jakość". T. Grabowski and J. Kijowski (Ed.), (PL), pp. 265-269. WNT, Warsaw.

Hać-Szymańczuk E., Cegiełka A., Lipińska E. and Ilczuk P. 2014. Effect of sage on the microbial quality and TBARS value of mechanically deboned poultry meat. Med. Weter. (PL). 70:704-708.

Hać-Szymańczuk E., Cegiełka A., Lipińska E. and Czapska S. 2015. Evaluation of chemical composition and antibacterial activity of water extracts from selected spices. Zesz. Prob. Post. Nauk Rol. (PL). 582:3-11.

Hać-Szymańczuk E., Roman J. and Bednarczyk K. 2009. A study of the antibacterial activity of rosemary (*Rosmarinus officinalis* L.). Nauka Przyr. Technol. (PL). 3:1-9.

Hać-Szymańczuk E., Roman J. and Bednarczyk K. 2010. Estimation of the antibacterial activity of the rosemary (*Rosmarinus officinalis* L.) essential oil, water extract and commercial preparation. Bromat. Chem. Toksykol. (PL). 42:979-984.

Hassan O. and Lam Swet Fan 2005. The anti-oxidation potential of polyphenol extract from cocoa leaves on mechanically deboned chicken meat (MDCM). LWT – Food Sci. Technol. 38:315-321.

Helander I.M., Alakomi H.L., Latva-Kala K., Mattila-Sandholm T., Pol I., Smid E.J., Gorris L.G.M. and Von Wright A. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. J. Agr. Food Chem. 46:3590–3595.

Hernández-Hernández E., Ponce-Alquicira E., Jaramillo-Flores M.E. and Guerrero Legarreta I. 2009. Antioxidant effect rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters. Meat Sci. 81:410-417.

Jridi M., Siala R., Fakhfakh N., Ayadi M.A., Elhatmi M., Taktak M.A., Nasri M. And Zouari N. 2015. Effect of rosemary leaves and essential oil on turkey sausage quality. Acta Alimentaria 44:534-541.

Kasparavičiene G., Ramanauskiene K., Savickas A., Velžiene S., Kalveniene Z., Kazlauskiene D., Ragažinskiene O. and Ivanauskas K. 2013. Evaluation of total phenolic content and antioxisdant activity of different *Rosmarinus officinalis* L. ethanolic extracts. Biologija 59(1):39-44.

Longaray Delamare A.P., Moschen-Pistorello I. T., Artico L., Atti-Serafini L. and Echeverrigaray S. 2007. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem. 100:603-608.

Mathenjwa S.A., Hugo C.J., Botha C. and Hugo A. 2012. Effect of alternative preservatives on the microbial quality, lipid stability and sensory evaluation of boerewors. Meat Sci. 91:165-172.

Michalski M. and Pomykała R. 2008. Microbiological quality of mechanically deboned poultry meat. Acta Sci. Pol., Medicina Veterinaria (PL). 7:43-49.

Mielnik M., Aaby K. and Skrede G. 2003. Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. Meat Sci. 65:1147-1155.

Mohamed H.M.H. and Mansour H.A. 2012. Incorporating essential oils of marjoram and rosemary in the formulation of beef patties manufactured with mechanically deboned poultry meat to improve the lipid stability and sensory attributes. LWT – Food Sci. Technol. 45:79-87.

Nagy J., Lenhardyty L., Korimova L., Dicakova Z., Pipova M. and Tomkova I. 2007. Comparison of the quality of mechanically deboned poultry meat after different method of separation. Meso 9:92-95.

Nieto G., Jongberg S., Andersen M.L. and Skibsted L.H. 2013. Thiol oxidation and protein cross-link formation during chill storage of pork patties added essential oil of oregano, rosemary, or garlic. Meat Sci. 95:177-184.

Okoh O.O., Sadimenko A.P. and Afolayan A.J. 2010. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem. 120:308-312.

Pham A.J., Williams J.B., Perez S.M. and Schilling M.W. 2013. Changes in the volatile composition of fresh pork sausage with rosemary (*Rosmarinus officinalis* L.) and green tea (*Camella sinensis* L.) extracts during long-term frozen storage followed by retail display. Meat Sci. 96:446.

Pietrzak D., Słowiński M. and Mroczek J. 2011. Mechanically deboned poultry meat. Przem. Spoż. (PL). 65:68-71.

Pikul J., Leszczyński D.E. and Kummerow F.A. 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J. Agric. Food Chem. 37:1309-1313.

PN 1992. Mechanically deboned poultry meat. Polish Standard PN-A-86522:1992. Polish Committee for Standardization, Warsaw, Poland.

PN-EN ISO 2003. Horizontal method for the detection of *Salmonella* spp. PN-EN ISO Standard 6579:2003. Microbiology of food and animal feeding stuffs. Polish Committee for Standardization, Warsaw, Poland.

PN-ISO 2004. Horizontal method for the detection of psychrotrophic microorganisms. PN-ISO Standard 17410:2004. Microbiology of food and animal feeding stuffs. Polish Committee for Standardization, Warsaw, Poland.

PN-EN ISO 2005. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2:Specific rules for the preparation of meat and meat products. PN-EN ISO Standard 6887-2:2005. Microbiology of food and animal feeding stuffs. Polish Committee for Standardization, Warsaw, Poland.

PN-ISO 2005. Horizontal method for the detection and enumeration of *Enterobacteriaceae*. Part 2:Colony-count method. PN-ISO Standard 21528-2:2005. Microbiology of food and animal feedings stuffs. Polish Committee for Standardization, Warsaw, Poland.

PN-ISO 2007. Horizontal method for the enumeration of coliforms. Colony-count technique. PN-ISO Standard 4832:2007. Microbiology of food and animal feeding stuffs. Polish Committee for Standardization, Warsaw, Poland.

PN-EN ISO 2013. Horizontal method for the enumeration of microorganisms. Colony count at 30 degrees C by the pour plate technique. PN-EN ISO Standards 4833-1:2013-12. Microbiology of the food chain. Polish Committee for Standardization, Warsaw, Poland.

Regulation 2004. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union L139. 55-205.

Romano C.S., Abadi K. and Repetto V. 1989. Synergistic antioxidant and antimicrobial activity of rosemary plus butylated derivatives. Food Chem. 115:456-461.

Sebranek J.G., Sewalt V.J.H., Robbins K.L and Houser T.A. 2005. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Sci. 69:289-296.

Shah M.A., Bosco S.J.D. and Mir S.A. 2014. Plant extracts as natural antioxidants in meat and meat products. Meat Sci. 98:21-33.

Stangierski J., Kijowski J. and Konieczny P. 2011. The quality and use of mechanically separated poultry meat. Zesz. Nauk. Uniw. Ekon. Pozn. (205):202-211.

StatSoft, Inc. 2011. STATISTICA (data analysis software system), version 10.0 Tulsa, OK, USA. www.statsoft.com

Szczepanik G. 2007. The influence of extracts of fennel, coltsfoot, rosemary, horsetail, sage and thyme on oxidation inhibition of lipids extracted from breast tissue of chickens and turkeys. Zywn-Nauk. Technol. Ja. (PL). 4:89-98.

Tawaha K., Alali F.Q. and Gharaibeh M. 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem. 104:1372-1378.

Ultee A., Bennink M.H.J. and Moezelaar R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microb. 68:1561-1568.

Velasco V. and Williams P. 2011. Improving meat quality through natural antioxidants. Chilean J. Agric. Res. 71:313-321.

Viuda-Martos M., Ruiz-Navajas Y., Fernández-López J. and Pérez-Álvarez J.A. 2010. Effect of orange dietary fibre, oregano essential oil and packaging conditions on shelf-life of bologna sausages. Food Cont. 21:436-443.

Wójciak K.M., Dolatowski Z.J. and Okoń A. 2011. The effect of water plant extracts addition on the oxidative stability of meat products. Acta Sci. Pol., Technologia Alimentaria 10:175-188.

Zhang H., Kong B., Xiong Y.L. and Sun X. 2009. Antimicrobial activities of spices extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4 °C. Meat Sci. 81:686-692.

Žižovic I., Mišic D., Ašanin R. and Ivanović J. 2009. Antibacterial activity of essential oils of some *Lamiaceae* family species isolated by different methods. Zbornik radova Tehnološkog fakulteta u Leskovcu 19:20-26.

Paper Received October 23, 2016 Accepted January 15, 2017

PAPER

THE PHYSICAL, OPTICAL AND RECONSTITUTION PROPERTIES OF APPLES SUBJECTED TO ULTRASOUND BEFORE DRYING

A. FIJALKOWSKA, M. NOWACKA* and D. WITROWA-RAJCHERT
Warsaw University of Life Sciences, Faculty of Food Sciences, Department of Food Engineering and Process Management, Nowoursynowska159c, 02-776 Warsaw, Poland
*Corresponding author. Tel.: +48 225937579; fax: +48 225937576
E-mail address: malgorzata_nowacka@sggw.pl

ABSTRACT

The purpose of the study was to present the influence of ultrasound pre-treatment on the physical, optical and reconstitution properties before convective drying of apple slices. The apples were subjected to ultrasonic treatment (35 kHz) for 10, 20 and 30 minutes and dried at a temperature of 70 °C and at an air velocity of 2 m/s. The ultrasonic waves reduced the drying time by 5-13% and affected the properties of dried apples. The longest pre-treatment resulted in colour changes and increased the porosity of dried apples. It also decreased the density, hardness and hygroscopic properties of dried apples.

Keywords: ultrasound, colour, texture, structure, reconstitution properties, drying

1. INTRODUCTION

Apples are one of the most important horticultural crops in the world and they constitute the greater part of the fruit production. Their consumption is increasing in the world, mostly in the form of fresh fruit, juice and dried apples (RODRÍGUEZ *et al.*, 2014). Research conducted by HERTOG *et al.* (1993) showed that the consumption of 110 g of apples a day reduces the risk of heart attack by 49% when compared with the daily consumption of only 18 g of apples.

Dehydration by hot-air is probably the most common and effective preservation method, used to imbue a food product with long shelf-life. Drying adds new values to food by limiting the spoilage and reducing the mass of the product (MULET *et al.*, 2003; CHONG *et al.*, 2008; GAMBOA-SANTOS *et al.*, 2013; KEK *et al.*, 2013, WITROWA-RAJCHERT *et al.*, 2014). However, the convective drying conditions such as temperature and air velocity may negatively affect the quality of a final product, causing changes in the microstructure, physical properties and nutritional value of food products (GAMBOA-SANTOS *et al.*, 2013; CHONG *et al.*, 2014). The texture modification, the degradation of vitamin, the loss of essential amino acids and changes in colour and flavour occur during hot-air drying (AZOUBEL *et al.*, 2010; OZUNA *et al.*, 2014). In order to determine the changes that appear during treatment and after drying food is tested on its reconstitution properties such as rehydration and hygroscopic properties (RZĄCA and WITROWA-RAJCHERT, 2007). Although convective drying is widely used, the method is related to high energy consumption, which results in the high cost of this technique (GAMBOA-SANTOS *et al.*, 2014).

Because of the growing need for the production of higher quality dried food products at lower processing cost, the traditional convective drying is combined with non-thermal techniques of pre-treatment such as high hydrostatic pressure, pulsed electric field and power ultrasound (WITROWA-RAJCHERT *et al.*, 2014).

Due to the low heating effect, the ultrasound treatment is a very promising method in the food industry (OZUNA *et al.*, 2014). Ultrasound waves indicate compression and expansion cycles in the material, which leads to micro-channels formation (FERNANDES *et al.*, 2008a; NOWACKA *et al.*, 2014; NOWACKA and WEDZIK, 2016). This phenomenon improves the rate of mass transfer and accelerates diffusion during dehydration (GAMBOA-SANTOS *et al.*, 2013; RODRÍGUEZ *et al.*, 2014). Moreover, the sonication generates cavitation, which can cause the removal of strongly attached moisture (MULET *et al.*, 2003; GAMBOA-SANTOS *et al.*, 2014). For example, the ultrasound pre-treatment reduced the drying time by 5-40% in the case of dried banana (AZOUBEL *et al.*, 2010), by 31-40% in the case of dried apples (NOWACKA *et al.*, 2012) and in the case of pineapple by over 30% (FERNANDES *et al.*, 2008c).

The ultrasound application reduces the drying time, and the process can be carried out at a lower temperature (NOWACKA *et al.*, 2012), which is relevant for food containing thermo-labile compounds. The ultrasonic effects during dehydration depend on the kind of the product and the ultrasound power. The visual appearance such as colour and texture are important sensory criteria for both the manufacturer and the customer (CYBULSKA *et al.*, 2011; PINGRET *et al.*, 2013). The drying process linked to the ultrasound pre-treatment can improve the quality of dried fruits and modify food properties (OZUNA *et al.*, 2014).

The purpose of the research was to investigate the effects of ultrasound pre-treatment at different treatment times on the quality factors of dried products, which affect the consumers' choice such as colour, mechanical, rehydration and hygroscopic properties. Moreover, the influence of sonication on the kinetics of apple drying was analysed.

2. MATERIALS AND METHODS

2.1. Sample preparation

Apples (var. Idared) from the Experimental Fields (Orchards) located in the district of Wilanow in Warsaw served as the experimental material. The production of apples is carried out by employees of the Faculty Horticulture, Biotechnology and Landscape Architecture (Warsaw University of Life Sciences WULS-SGGW). Apples were harvested and transported to the Faculty of Food Sciences in WULS-SGGW. While the experiments were performed, the apples were stored at 90% air humidity and a temperature of 4-8 °C for 1 month.
The material was cut into slices of a thickness of 0.005±0.001 m and a diameter of 0.030±0.001 m, excluding peel and core. In order to prevent enzymatic browning reactions, apple slices were immersed in the 0.1% citric acid solution, then blotted with filter paper and pre-treated with ultrasound.

2.2. Ultrasound pre-treatment

The material was submitted to ultrasound treatment for 10, 20 and 30 minutes in an ultrasonic bath with a frequency of 35 kHz (InterSonic, Olsztyn, Poland, IS-3 model, internal dimensions: 240×135×100 mm), while the ultrasound intensity equalled 4 W/cm^2. Pre-treatment was carried out in distilled water at room temperature (22±1 °C) and, in order to prevent flowing out of the samples, the slices were covered with a metal net. The ratio of raw material to water was 1:4, as recommended by NOWACKA *et al.* (2014). Afterwards, the samples were blotted with filter paper and placed in a dryer.
The material mass, the content of dry matter and a temperature of medium were measured before and after pre-treatment. The experiments were performed in duplicate.

2.3. Convective drying

The apple slices were dried in a laboratory convective dryer (Warsaw, Poland) at a temperature of 70 °C with parallel air-flow at an air velocity of 2 m/s. The scheme of the dryer is presented in Fig. 1. The main part of the dryer is the chamber within the shelf, where the dried product is placed. The shelf is connected with the balance. The product is dried in the hot air flow, which is heated by the heater system placed on the air inlet. The temperature of the air flow is measured by the thermocouples. The thermal sensors and the balance are connected to the computer, which records the data during the drying process. The heaters and thermal sensors are connected to the control panel, which is used to set up temperature and air velocity. Firstly, the dryer was preheated to a set-point temperature and then loaded with 0.25 kg (1,92 kg/m^2) of apple slices spread over the two nets in a single layer. Drying was carried out until constant weight was achieved. The experiments were conducted in duplicate.
The relative moisture ratio and the effective water diffusivities (D_{eff}) were calculated using the equations (1) and (2), respectively (SLEDZ *et al.*, 2013):

$$MR = \frac{u}{u_0} \qquad (1)$$

where: u – water content during drying (kg moisture/kg d.m.), u_o – initial water content (kg moisture/kg d.m.)

$$MR = \frac{8}{\pi^2} \cdot \exp\left(\frac{-\pi^2 \cdot D_{eff} \cdot \tau}{4 \cdot L^2} \right) \quad (2)$$

where: D_{eff} – diffusion coefficient (m^2/s), τ - drying time (s), L – half-thickness of the sample (m).

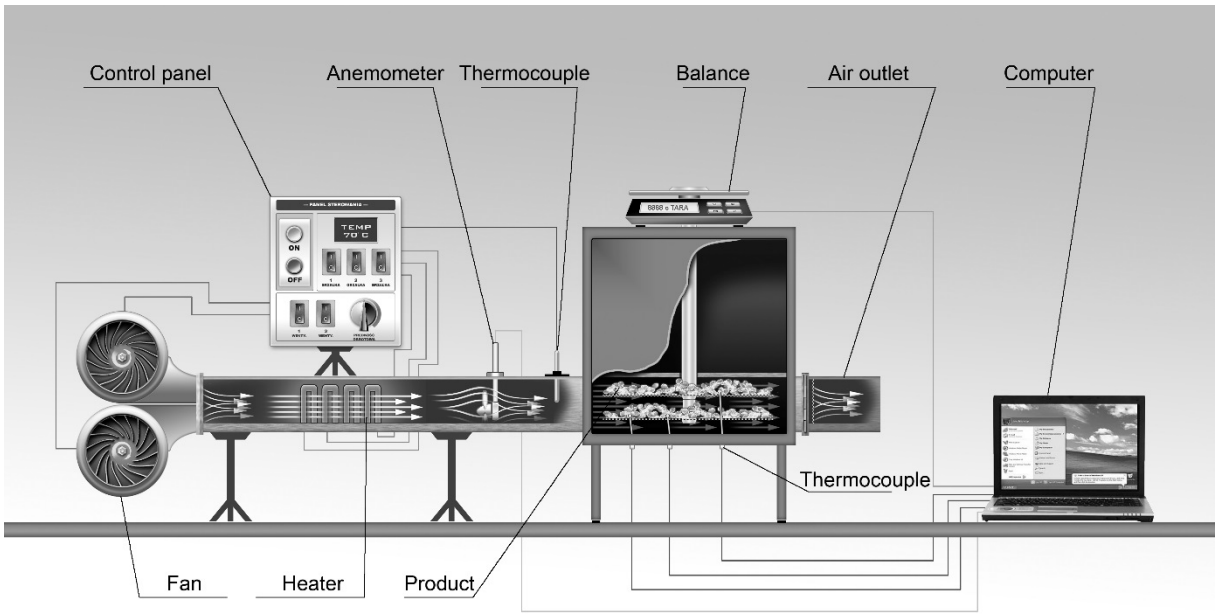


Figure 1. The scheme of a laboratory convective dryer (figure made by www.ct.waw.pl)

2.4. Physical properties

The dry matter in the raw, pre-treated, dried and rehydrated material was measured according to the Polish Standard PN-90/A-75101/03 by drying at a temperature of 105 °C to the constant weight.
The water activity of the untreated, ultrasound treated and dried samples were examined using hygrometer Aqua Lab CX-2 (Dekagon Device Inc., USA) with the accuracy of ±0.001. The equipment was switched on around 30 minutes before measuring and checked with the water. The raw apple slices were placed in a plastic vessel, covering the bottom of the vessel. After ultrasound pre-treatment the samples were blotted with filter paper and placed in the vessel. The apple slices after drying were stored in a sealed bag for three days to compensate the humidity of the sample after drying. The measurements were made thrice for each sample at a temperature of 25 °C.
Based on the measurement of the mass and volume of the dried sample, density and porosity were calculated (ANDRÉS *et al.*, 2004).

2.5. Colour measurement

A hand-held Minolta CR-300 chromameter (Minolta, Japan) was used to measure the colour of the dried apple slices (diffuse illumination, 0º viewing geometry). Colour was recorded with the CIE L*a*b* system. In this colour space, the L^* parameter defines

lightness and ranges from 0 to 100, the a^* parameter denotes chromaticity on a green (-) to red (+) axis and the b* parameter represents chromaticity on a blue (-) to yellow (+) axis. The L^*, a^* and b^* coefficient were used to calculate the chroma (C^*) which indicates the purity and saturation of colour and the value of the colour difference (ΔE) (GONÇALVES *et al.*, 2007; FIJALKOWSKA *et al.*, 2016) according to equations (3) and (4), respectively.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

where: ΔL^*, Δa^*, Δb^* - the change of lightness, a^* and b^* parameter value between pre-treated and untreated dried apple.
The experiments were performed in 15 repetitions.

2.6. Structure

The internal structure of dried tissue was examined using a scanning electron microscope HITACHI TM 3000 Tabletop Microscope (Tokio, Japan). The internal part of the slices was cut in half with a scalpel and put into the vacuum chamber of the microscope. The images were examined under the magnification of 100.

2.7. Mechanical properties

Mechanical properties were analysed using the cutting test in a texture analyser (TA-TX2i model, Stable Micro Systems, Godalming, England). In order to conduct this test, a knife of 0.062 m in length, 0.024 m in width and 0,0005 m in thickness was slid inside a metal table with a slot. The load cell was calibrated to 250 N and the cutting was applied at a velocity of 0.001 m/s. The cutting began when the sample resisted and carried out to complete intersection. Both the maximum cutting force and the cutting energy were recorded using program "Texture" and then calculated. The mean value for ten replicates of apple slice was averaged for each ultrasound treatment.

2.8. Rehydration properties

In order to analyse rehydration kinetics, two slices of dried apples were weighed with an accuracy of $\pm 0.1 \cdot 10^{-6}$ kg and placed in a glass with 10^{-4} m^3 of distilled water. The process was carried out at a temperature of 20 °C and the samples were immersed for 30, 60 and 180 minutes. After a given rehydration time, the slices were drained on a sieve and then on a blotting paper. The experiments were repeated twice for each kind of the dried material.
The rehydration properties were calculated according to the following equation:

$$X = \frac{X_\tau}{X_0} \quad (5)$$

where: X_τ – moisture of a rehydrated sample at time τ (kg water/ kg d.m.), X_0 – initial moisture of a fresh sample (kg water/kg d.m.).

The soluble solid loss during rehydration was calculated using the following equation:

$$SSL = \frac{m_\tau \cdot dm_\tau}{m_0 \cdot dm_0} \quad (6)$$

where: m_τ – sample weight after rehydration at time τ (kg), m_0 – sample weight before rehydration (kg), dm_τ – dry matter content of a sample after rehydration at time τ (%), dm_0 – dry matter content of a sample before rehydration (%).

2.9. Hygroscopic properties

In order to determine the hygroscopic properties of the apple tissue after drying the samples were weighed with an accuracy of $\pm 0.1 \cdot 10^{-6}$ kg and placed in a desiccator over NaCl solution of a water activity $a_w = 0.75$. The kinetics of adsorption was determined for 72 hours at 25 °C. After a specific time of 0.5, 1, 3, 5, 8, 10, 24, 48 and 72 h the samples were weighed again. The experiments were performed in triplicate for each kind of the dried material

2.10. Statistical analysis

The significance of the ultrasound treatment and the drying process were examined by the analysis of variance (ANOVA) using Statgraphics Plus 5.0 programme. The homogeneity of variance was verified using Levene's test. Duncan's multiple range tests with a probability of 0.05 were used to determine homogeneous groups.

3. RESULTS

3.1. Ultrasound treatment

During sonication, the distilled water temperature increased by 4.3, 7.3 and 10.8 °C for 10, 20 and 30 minutes of treatment, respectively (Table 1). Due to a series of rapid compressions and expansions of plant tissue generated by ultrasound waves, water included in the raw material could flow out to the surroundings. After 10 and 20 minutes of pre-treatment, the apples lost 0.89±0.02 and 0.59±0.03% of weight, respectively (Table 1), whereas 30 minutes of ultrasound application increased the weight of the samples (1.47±0.10%). The phenomenon that occurred during a longer time of immersion, can be caused by water penetration into the tissue as a result of osmotic concentration differences. Statistical analysis showed significant differences between mass changes for all pre-treatment time. The similar relation was observed in NOWACKA *et al.* (2012) research where the smallest weight loss of apple tissue (0.8±0.4%) was obtained for samples pre-treated for 30 minutes with an ultrasound frequency of 35 kHz. For 10 and 20 minutes of sonication, the weight loss was equal to 2.3±0.1 and 3.0±0.2%, respectively. The opposite tendency was observed by FERNANDES *et al.* (2008b), who subjected papaya tissue to ultrasound waves with a frequency of 25 kHz. The enhancement of water loss was obtained with increasing time of treatment in the range of 10 to 30 minutes.
Raw apple tissue contains 15.1±0.8% of dry matter. The samples submitted to ultrasonic treatment lost from 26.5 to 28.4% of soluble solids because of their flowing out to a liquid medium. The decrease of this parameter was significant in the case of each time of pre-treatment in comparison with the untreated apple slices (Table 1). The length of

ultrasound application did not have statistically meaningful influence on the dry matter content of the material.

Table 1. Changes of mass, dry matter content and medium temperature after ultrasound pre-treatment.

Type of treatment	Weight gain (+)/loss(-) [%]	Dry matter [%]	Medium temperature increase [°C]
Untreated	-	15.1±0.8^{a}	-
US 10 min	-0.89±0.02^{a}	10.8±1.4^{b}	4.3±0.8^{a}
US 20 min	-0.59±0.03^{b}	11.0±0.5^{b}	7.3±0.3^{b}
US 30 min	1.47±0.10^{c}	11.1±0.5^{b}	10.8±0.8^{c}

a, b, c: the same letters indicate homogeneous groups.

3.2. Drying characteristics

The application of ultrasound resulted in a reduction of the convective drying time by 5-13% in relation to the untreated material. The drying time decreased significantly with the increasing time of ultrasound pre-treatment (Table 2). Similarly, the shorter time of drying process after ultrasound treatment was noticed in the literature where a time reduction of 4.5% was observed for banana (AZOUBEL *et al.*, 2010), 3-22% for papaya (FERNANDES *et al.*, 2008b), 7-39% for pineapple (FERNANDES *et al.*, 2008c), 27% for apple cubes, 18-23% for red bell pepper (SCHÖSSLER *et al.*, 2012) and 50-75% for eggplant (PUIG *et al.*, 2012). These examples confirm the assumptions of FEUNTE-BLANCO *et al.* (2006) who noticed in the influence of ultrasound on the fruit tissue facilitating water diffusion during air-drying. This phenomenon can be caused by micro-channels formation during sonication, which can enable easier water diffusion from interior material to the surface (FERNANDES *et al.*, 2008a).

Table 2. Drying time of the apple slices to obtain 0.09 kg moisture/kg d.m., the value of the effective moisture diffusion coefficient (D_{eff}), dry matter content and water activity of the dried apples.

Type of treatment	Drying time [min]	Deff·10^{9} [m^{2}/s]	Dry matter [%]	Density [kg/m^{3}]	Porosity [%]	Water activity [%]
Untreated	133±3^{c}	1.037	92.3±0.9^{a}	524± 100^{bc}	65.6±5.7 ^{a}	0.264±0.012^{b}
US 10 min	126±1^{b}	1.058	92.1±0.2^{a}	464±30^{ab}	69.5±1.8^{ab}	0.286±0.010^{c}
US 20 min	123±1^{b}	1.064	93.2±0.4^{a}	545±60^{bc}	64.2±3.5 ^{a}	0.246±0.011^{a}
US 30 min	116±2^{a}	1.102	93.1±1.0^{a}	388±40^{a}	74.5±2.2 ^{b}	0.290±0.009^{c}

a, b, c: the same letters indicate homogeneous groups.

Furthermore, the values of the effective water diffusivity (D_{eff}) for the ultrasound treated samples were growing with the increase of treatment time, and the highest value of this parameter was noted for the apples subjected to 30 minutes of sonication. In this case the effective moisture diffusion coefficient increased by 6% in comparison with the untreated material. The values of this coefficient were related to drying time (NOWACKA *et al.*, 2012), which was proved by the shortest time of drying in the case of the apples treated by ultrasound for 30 minutes (Table 2).
The apple slices were dried to obtain 0.09 kg moisture/kg d.m., where the dry matter content of untreated tissue equalled 92.3±0.9% and there was no significant influence of

ultrasound treatment on this parameter value (Table 2). However, statistical analysis confirmed that sonication caused significant changes in the density and porosity of the dried tissue (Table 2). The density of the material is closely related to its porosity. A low density product must possess high porosity. The tissue exposed to ultrasound waves for 30 minutes after drying exhibited the lowest density of 388±40 kg/m^3 and the highest porosity (74.5±2.2%). The difference between the dried treated samples and the untreated ones was statistically significant. Similar results were reported by NOWACKA *et al.* (2012) for dried cubes of apples. Moreover, the statistical changes were found in the water activity of the ultrasound treated samples. The samples that were subjected to ultrasound for 10 and 30 minutes obtained higher values of water activity in comparison to the untreated material, whereas the tissue treated for 20 minutes was characterized by a lower value of this parameter. However, all dried samples revealed low water activity, which proves the microbiological safety of food.

3.3. Optical properties

Colour is one of the most important factors of raw and dried fruits' quality because the external appearance influences the consumer acceptability (SINGH and REDDY, 2006; NUNCIO-JÁUREGUI *et al.*, 2014).
The colour parameters L^*, a^*, b^*, C^* and ΔE of the untreated and pre-treated dried apple slices are presented in Table 3. In the case on lightness, the L^* value of dried apples significantly increased after 30 minutes of sonication in comparison with the untreated sample. However, a shorter time of ultrasound treatment did not have any significant impact on this colour parameter.
The higher value of a^* parameter denotes the increase of red colour saturation, which can be associated with the enzymatic browning reaction occurring during pre-treatment and the drying process (VADIVAMBAL and JAYAS, 2007). This trend can be observed for the samples treated with ultrasound waves for 20 minutes, where parameter a^* equalled 1.45±0.75, but statistical analysis did not show any significant differences in relation to the untreated dried apple tissue (Table 3). However, the lowest values of this parameter were obtained by the samples subjected to ultrasound for 10 and 30 minutes. As compared to the untreated material the statistically significant effect occurred only after 30 minutes of pre-treatment, where a^* value was equal to -0.53±0.83.

Table 3. L*, a*, b*, chroma C* values of the dried apple slices and total colour differences (ΔE) in comparison to the dried untreated apples.

Type of treatment	L*	a*	b*	C*	ΔE
Untreated	82.07±1.87^{a}	0.91±0,98^{bc}	21.42±3.23^{a}	21.45±2.93^{a}	-
US 10 min	84.27±1.33^{ab}	0.02±0.75^{ab}	19.81±0.92^{a}	19.82±0.81^{a}	2.93±1.42
US 20 min	82.01±1.22^{a}	1.45±0.75^{c}	22.01±1.27^{a}	22.07±1.15^{a}	1.36±1.24
US 30 min	84.57±0.73^{b}	-0.53±0.83^{a}	20.77±2.21^{a}	20.79±1.82^{a}	3.57±0.33

a, b, c: the same letters indicate homogeneous groups.

In the case of b^* parameter, the lowest value was observed after 10 minutes of ultrasound application (19.81±0.92) (Table 3), whereas the highest level of this parameter was noted for the samples subjected to ultrasound for 20 minutes (22.01±1.27). The statistical analysis did not show any significant differences between the untreated and ultrasound pre-treated

samples. The same tendency was noticed for chromaticity C^* (Table 4) which determines the purity or saturation of the colour (GONÇALVES *et al.*, 2007).
The L^*,a^* *and* b^* colour parameters were used to calculate the total colour differences (ΔE). CHOI *et al.* (2002) revealed that ΔE value higher than 2 confirms the visible difference. After 10 and 30 minutes of pre-treatment, the obtained results showed higher ΔE value than 2, which means that the colour was changed noticeably in these cases (Table 3). However, the invisible colour changes were observed for the apple tissue treated with ultrasound for 20 minutes, hence ΔE value was lower than 2.

3.4. Structure of the ultrasound treated apple tissue

Fig. 2 shows a photo of the dried apple tissue with and without ultrasound treatment. Dried untreated apple tissue is characterized by high density and small pores with elongated cell shape. In the case of the dried tissue subjected to ultrasound treatment, the changes in the structure were observed (Fig. 1). The results confirm that the ultrasound treated material exhibited a lower density and more porous form (Table 2). However, it was impossible to clearly determine the effect of ultrasound treatment time on microstructure changes, as it was done by other researchers (FERNANDES *et al.*, 2008a; FENANDES and RODRIGUES, 2009; NOWACKA *et al.*, 2012; NOWACKA and WEDZIK, 2016). Moreover, it was not noticed that ultrasound treatment before drying caused the formation of a microscopic channel in dried tissue as reported by NOWACKA and WEDZIK (2016) in carrot or FERNANDES *et al.* (2008a) in pineapple.

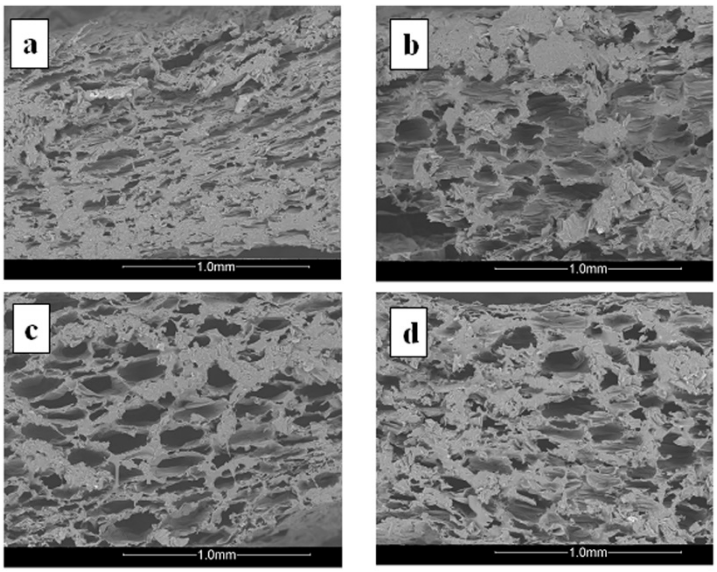


Figure 2. Photos of dried apples taken by using scanning electronic microscopy at a magnification of 100: untreated samples (a), 10 min (b), 20 min (c), 30 min (d) ultrasound treated samples.

3.5. Mechanical properties

Texture evaluation of the product is an important feature to determine the quality of dried fruits (CHONG *et al.*, 2008; KEK *et al.*, 2013). Fig. 3 shows texture changes of the ultrasound treated dried apple slices, where results of the maximum cutting force and the

cutting energy of dried samples were presented. The ultrasound treatment caused alteration of apple tissue, which resulted in changes of textural properties of the dried material. Moreover, it was observed that the maximum cutting force and cutting energy decreased with increasing ultrasound treatment time. Taking into account the maximum cutting force and cutting energy, the apple slices subjected to ultrasound for 10 minutes were the most similar to the untreated dried material. The dried material was characterized by the highest hardness; however, the differences were not statistically significant.
The lowest hardness of dried apple slices was observed for the material treated with ultrasound for 30 minutes. These samples had significantly lower values of maximum cutting force (196.0±37.5 N) and cutting energy (233.4±47.2 mJ) in comparison with the untreated dried tissue, which was probably related to lower density and porosity.

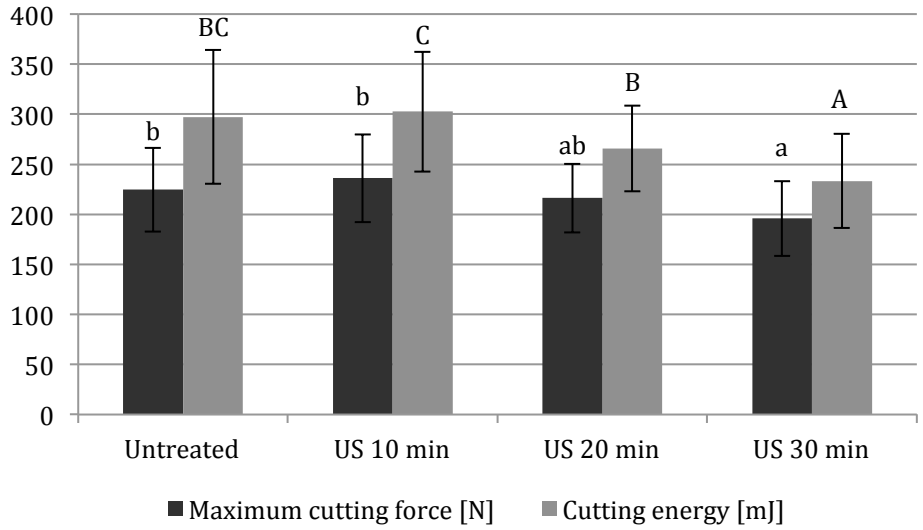


Figure 3. The maximum cutting force and the cutting energy of dried apple; a, b: the same letters indicate homogeneous groups for maximum cutting force; A, B, C: the same letters indicate homogeneous groups for cutting energy.

3.6. Rehydration properties

A rehydration is a significant quality criterion of dried food. High temperature during drying causes irreversible structure changes of plant tissue. In order to determine these changes, the reconstitution characteristics are investigated (CIURZYNSKA *et al.*, 2011). The gain of weight and volume occur during the rehydration process due to water uptake, while the water soluble compounds are removed from the interior of tissue to the surrounding. The rate of the rehydration process is determined by the level of tissue destruction (NOWACKA *et al.*, 2012).
The changes of rehydration properties are presented in Table 4. The obtained results showed that the prolongation of rehydration time resulted in the increase of moisture uptake. The same tendency was observed during rehydration of apple cylinders var. Fuji (Deng and Zhao, 2008) and apple cubes var. Idared (NOWACKA *et al.*, 2012). However, the use of different ultrasound frequencies (FIJALKOWSKA *et al.*, 2016) and different treatment times (NOWACKA *et al.*, 2012) did not significantly influence rehydration

properties. The amount of water content in the untreated apple slices after 3 hours of rehydration was equal to 12.0±2.2 kg moisture/kg d.m., while the ultrasound pre-treated samples were characterized by higher levels of moisture content, but the statistical analysis showed that these differences were not significant. The results calculated to the initial moisture of the fresh sample confirmed that after 30 minutes of rehydration the dried apples did not obtain the initial water content. After 60 minutes of rehydration the water content of the samples subjected to ultrasound for 10 and 20 minutes were similar to the fresh apple, while the untreated samples and the samples treated with ultrasound for 30 minutes and after 180 minutes of rehydration were characterized by a higher moisture content than the fresh raw material (Table 4).
The rehydration process is also related to the loss of water soluble components of dry matter - the loss is growing with the increasing time of rehydration. After 30 minutes of dried apple rehydration, the amount of their dry matter content was higher than for the fresh sample. The rehydration for 60 minutes of the samples treated with ultrasound for 10 and 20 minutes resulted in the amount of dry matter content similar to the intact sample. However, the untreated apples and the ones subjected to ultrasound for 30 minutes and rehydrated for 60 minutes and all the samples rehydrated for longer time (180 min) were characterized by lower dry matter content in comparison to the raw material, which was associated with high loss of water soluble solids from the dried tissue (Table 4).

Table 4. Water content and loss of water soluble solids after different time of rehydration expressed in kg moisture/kg d.m. and calculated percentage of the initial moisture recovery compared to the fresh fruit.

Type of treatment	Rehydration time [min]					
	30	60	180	30	60	180
	Water content [kg moisture/kg d.m.]			Loss of water soluble solids [kg d.m./kg d.m.]		
Untreated	4.0±0.6^{a}	6.3±1.0^{a}	12.0±2.4^{a}	0.71±0.03^{ab}	0.58±0.06^{a}	0.39±0.06^{a}
US 10 min	4.5±0.4^{a}	5.5±0.6^{a}	13.3±1.7^{a}	0.67±0.02 ^{b}	0.65±0.06^{a}	0.37±0.02^{a}
US 20 min	4.2±0.4^{a}	5.5±0.6^{a}	15.3±1.7^{a}	0.73±0.03 ^{a}	0.64±0.03^{a}	0.32±0.03^{a}
US 30 min	4.7±0.7^{a}	6.5±0.6^{a}	13.7±2.8^{a}	0.67±0.05^{ab}	0.56±0.04^{a}	0.36±0.05^{a}
	Water content [%]			Loss of water soluble solids [%]		
Untreated	-29±11^{a}	12±18^{a}	113±43^{a}	34±16^{ab}	-8±13^{a}	-48±9^{a}
US 10 min	-20±8^{a}	-3±11^{a}	136±30^{a}	22±10 ^{b}	3± 9^{a}	-53±6^{a}
US 20 min	-26±8^{a}	-2±11^{a}	172±30^{a}	29±11 ^{a}	2±10^{a}	-59±5^{a}
US 30 min	-17±12^{a}	16±11^{a}	144±49^{a}	19±16^{ab}	-11±8^{a}	-54±8^{a}

a,b: the same letters indicate homogeneous groups in column.

3.7. Hygroscopic properties

Hygroscopic properties of plant materials can specify the changes induced by the drying process and other treatment. The course of adsorption kinetics is mainly influenced by the method and drying parameters, as well as by the structure, shrinkage and porosity of a dried product. The smaller shrinkage and the higher porosity result in faster absorption of water vapour (RZĄCA and WITROWA-RAJCHERT, 2007; ACEVEDO *et al.*, 2008). According to the creation of microchannels by ultrasonic waves (FERNANDES *et al.*, 2008a; NOWACKA *et al.*, 2014; NOWACKA and WEDZIK, 2016) the hygroscopic properties may provide information about changes in the structure of the tissue.

After 72 hours of water vapour absorption from the NaCl solution with a water activity of 0.75, the dried apple rings absorbed from 0.36 to 0.38 g H_2O/g d.m. (Fig. 4). Analyzing the water sorption curves, it was observed that the process was most intense in the initial phase for 24 hours. At a later stage, there was a decrease in the rate of moisture adsorption. The untreated samples displayed the greatest ability to absorb water. In most cases, the amount of absorbed water did not depend on the type of pre-treatment. However, the apple tissue treated with ultrasound waves for 30 minutes before drying demonstrated lower hygroscopic properties. This dried tissue was characterized by a higher porosity and a lower density (Table 2), which may affect the water binding capacity reduction. At the same time, the lower ability of water absorption could be related to the fact that longer ultrasound treatment resulted in greater damage to the structure of the raw material (Fig. 1), which has been proved by other researchers (FERNANDES *et al.*, 2008a; NOWACKA *et al.*, 2014; NOWACKA and WEDZIK, 2016).

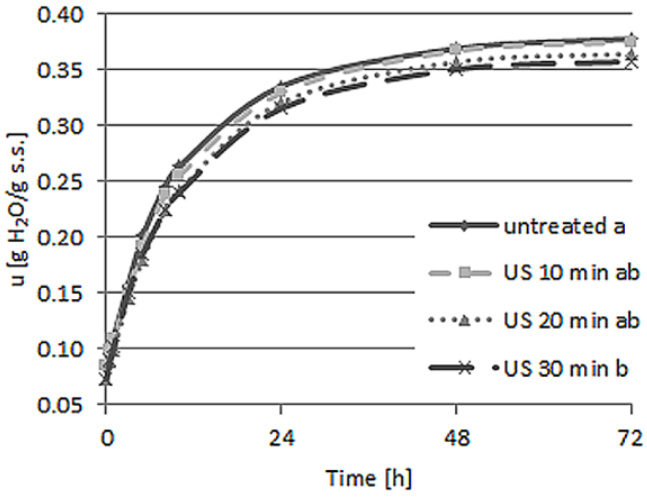


Figure 4. The hygroscopic properties of the ultrasound treated dried apples a, b: the same letters indicate homogeneous groups after 72 h of water vapour absorption.

4. CONCLUSIONS

Ultrasound waves used as a method of pre-treatment enhanced the drying of apple slices. The application of ultrasound induced a reduction of the convective drying time by 5-13% in relation to the untreated material. Moreover, the effective water diffusivity was the highest for the apples treated with ultrasound for 30 minutes and it increased by 6% in comparison with the untreated material.

Furthermore, ultrasound treatment impacted on physical, optical and reconstitution properties of the dried apple tissue; however, the effect of the overall quality of the product was not obviously stated. The ultrasound treatment resulted in the decrease of dry matter content, which is not a very favourable effect due to the loss of the tissue water-soluble components. Moreover, after 30 minutes of treatment the significant decrease of density and increase of porosity was observed, which was confirmed by micro-structure assessment. The changes in the structure had an impact on the acceleration of the drying process.

The conducted research indicated that all dried samples demonstrated low water activity, which provides microbiological safety of food. Additionally, the changes of total colour

differences (ΔE) were unnoticeable for the samples sonicated for 20 minutes, or slightly changed for the tissues ultrasound treated for 10 and 30 minutes.
On the other hand, longer time of ultrasound pre-treatment resulted in a significant decrease of dried slices hardness, which could be considered as a negative effect of ultrasonic treatment because a product that should be crispy like apple chips is softened. Furthermore, it was observed that with increasing ultrasound treatment time the maximum cutting force and the cutting energy of the dried samples decreased. Furthermore, the apples subjected to ultrasonic treatment for 30 minutes exhibited lower hygroscopic properties. However, sonication did not influence rehydration properties in comparison to the untreated sample.

REFERENCES

Acevedo N.C., Briones V., Buera P. and Aguilera J.M. 2008. Microstructure affects the rate of chemical, physical and color changes during storage of dried apple discs. J. Food Eng. 85(2):222-231.

Andrés A., Bilbao C. and Fito P. 2004. Drying kinetics of apple cylinders under combined hot air–microwave dehydration. J. Food Eng. 63 (1):71-78.

Azoubel P.M., Baima M.A.M., Amorim M.R. and Oliveira S.S.B. 2010. Effect of ultrasound on banana cv Pacovan drying kinetics. J. Food Eng. 97(2):194-198.

Choi M.H., Kim G.H. and Lee H.S. 2002. Effects of ascorbic acid retention on juice color and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. Food Res. Int. 35(8):753-759.

Chong C.H., Law C.L., Cloke M., Luqman C.A. and Wan Daud W.R. 2008. Drying kinetics and product quality of dried Chempedak. J. Food Eng. 88(4):522-527.

Ciurzynska A., Janowicz M., Piotrowski P., Pomarańska-Łazuka W., Sitkiewicz I. and Lenart A. 2011. Reconstitutional properties of vacuum-dried strawberries. Zeszyty Problemowe Postępów Nauk Rolniczych (Advances of Agricultural Sciences Problem Issues) 569:35-50 (in Polish).

Cybulska J., Zdunek A. and Konstankiewicz K. 2011. Calcium effect on mechanical properties of model cell walls and apple tissue. J. Food Eng. 102:217-223.

Deng Y. and Zhao Y. 2008. Effect of pulsed vacuum and ultrasound osmopretreatments on glass transition temperature, texture, microstructure and calcium penetration of dried apples (Fuji). LWT – Food Sci. Technol. 41(9):1575-1585.

Fenandes F.A.N. and Rodrigues S. 2009. Effect of osmosis and ultrasound on pineapple cell tissue structure during dehydration. J. Food Eng. 90:186-190.

Fenandes F.A.N., Gallao M.I. and Rodrigues S. 2008a. Effect of osmotic dehydration and ultrasound pre-treatment on cell structure: melon dehydration. LWT-Food Sci. Technol. 41(4):604-610.

Fernandes F.A.N., Oliveira F.I.P. and Rodrigues S. 2008b. Use of ultrasound for dehydration of papayas. Food Bioprocess Technol. 1(4):339-345.

Fernandes F.A.N., Linhares Jr. F.E., Rodrigues S. 2008c. Ultrasound as pre-treatment for drying of pineapple. Ultrason. Sonochem. 15(6):1049-1054.

Feunte-Blanco S., Sarabia E.R.F., Acosta-Aparicio V.M., Blanco-Blanco A. and Gallego-Juárez J.A. 2006. Food drying process by power ultrasound. Ultrasonics 44:523-527.

Fijalkowska A., Nowacka M., Wiktor A., Sledz M. and Witrowa-Rajchert D. 2016. Ultrasound as a pretreatment method to improve drying kinetics and sensory properties of dried apple. J. Food Process Eng. 9(3):256-265.

Gamboa-Santos J., Montilla A., Cárcel J.A., Villamiel M. and Garcia-Perez J.V. 2014. Air-borne ultrasound application in the convective drying of strawberry. J. Food Eng. 128:132-139.

Gamboa-Santos J., Soria A.C., Pérez-Mateos M., Carrasco J.A., Montilla A. and Villamiel M. 2013. Vitamin C content and sensorial properties of dehydrated carrots blanched conventionally or by ultrasound. Food Chem. 136(2):782-788.

Gonçalves B., Silva A.P., Moutinho-Pereira J., Bacelar E., Rosa E. and Meyer A.S. 2007. Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus Avium* L.). Food Chem. 103(3):976-984.

Hertog M.G.L., Feskens E.J.M., Hollman P.C.H., Katan M.B. and Kromhout D. 1993. Dietary antioxidants flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet 342:1007-1011.

Kek S.P., Chin N.L. and Yusof Y.A. 2013. Direct and indirect power ultrasound assisted pre-osmotic treatments in convective drying of guava slices. Food and Bioprod. Process. 91(4):495-506.

Mulet A., Cárcel J.A., Sanjuán N. and Bon J. 2003. New Food Drying Technologies - Use of Ultrasound. Food Sci. Technol. Int. 9(3):215-221.

Nowacka M., Tylewicz U., Laghi L., Dalla Rosa M. and Witrowa-Rajchert D. 2014. Effect of ultrasound treatment on the water state in kiwifruit during osmotic dehydration. Food Chem. 144:18-25.

Nowacka M. and Wedzik M. 2016. Effect of ultrasound treatment on microstructure, colour and carotenoid content in carrot tissue. Appl. Acoust. 103:163-171.

Nowacka M., Wiktor A., Sledz M., Jurek N. and Witrowa-Rajchert D. 2012. Drying of ultrasound pretreated apple and its selected physical properties. J. Food Eng. 113(3):427-433.

Nuncio-Jáuregui N., Calín-Sánchez A., Carbonell-Barrachina A. and Hernández F. 2014. Changes in quality parameters, proline, antioxidant activity and color of pomegranate (*Punica granatum* L.) as affected by fruit position within tree, cultivar and ripening stage. Scientia Horticulturae 165: 181-189.

Ozuna C., Álvarez-Arenas T.G., Riera E., Cárcel J.A. and Garcia-Perez J.V. 2014. Influence of material structure on air-borne ultrasonic application in drying. Ultrason. Sonochem. 21:1235-1243.

Pingret D., Fabiano-Tixier A-S. and Chemat F. 2013. Degradation during application of ultrasound in food processing: A review. Food Control 31(2):593-606.

Polish Standard PN-90/A-75101/03. Fruit and vegetable products. Determination of dry matter content by gravimetric method (in Polish).

Puig A., Perez-Munuera I., Carcel J.A., Hernando I. and Garcia-Perez J.V. 2012. Moisture loss kinetics and microstructural changes in eggplant (*Solanum melongena* L.) during conventional and ultrasonically assisted convective drying. Food and Bioprod. Process. 90(4):624-632.

Rodríguez Ó., Santacatalina J.V., Simal S., Garcia-Perez J.V., Fermenia A. and Rosselló C. 2014. Influence of power ultrasound application on drying kinetics of apple and its antioxidant and microstructural properties. J. Food Eng. 129:21-29.

Rząca M. and Witrowa-Rajchert D. 2007. The influence of drying technique and storage conditions on dried apples reconstitution and hygroscopic properties. Acta Agrophysica 9(2):471-480 (in Polish).

Schössler K., Jäger H. and Knorr D. 2012. Effect of continuous and intermittent ultrasound on drying time and effective diffusivity during convective drying of apple and red bell pepper. J. Food Eng. 108(1):103-110.

Singh K.K. and Reddy B.S. 2006. Post-harvest physic-mechanical properties of orange peel and fruit. J. Food Eng. 73:112-120.

Sledz M., Nowacka M., Wiktor A. and Witrowa-Rajchert D. 2013. Selected chemical and physico-chemical properties of microwave-convective dried herbs. Food and Bioprod. Process. 91(4):421-428.

Vadivambal R. and Jayas D.S. 2007. Changes in quality of microwave-treated agricultural products-a review. Biosystems Eng. 98(1):1-16.

Witrowa-Rajchert D., Wiktor A., Sledz M. and Nowacka M. 2014. Selected emerging technologies to enhance the drying process. A review. Dry. Technol. 32(11):1386-1396.

Paper Received October 18, 2016 Accepted January 18, 2017

PAPER

RAPID DETERMINATION OF CRUCIAL PARAMETERS FOR THE OPTIMIZATION OF MILLING PROCESS BY USING VISIBLE/NEAR INFRARED SPECTROSCOPY ON INTACT OLIVES AND OLIVE PASTE

V. GIOVENZANA[a], R. BEGHI*[a], R. CIVELLI[a], S. TRAPANI[b], M. MIGLIORINI[c], E. CINI[d], B. ZANONI[b] and R. GUIDETTI[a]

[a]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

[b]Department of Agricultural Biotechnology, Food Technology Section, University of Florence, Via Donizetti 6, 50144 Firenze, Italy

[c]Promofirenze, Special Agency of the Florence Chamber of Commerce, Laboratorio Chimico Merceologico Unit, Via Orcagna 70, 50121 Firenze, Italy

[d]Department of Economics, Engineering, Science and Technology Agriculture and Forestry, University of Florence, Via San Bonaventura 13, 50145 Firenze, Italy

*Corresponding author. Tel.: +39 0250316843; fax: +39 0250316845

E-mail address: roberto.beghi@unimi.it

ABSTRACT

The aim of this work is the application of vis/NIR spectroscopy in order to correlate spectral data acquired on intact olives just before or in pastes during the milling process, to the crucial parameters for the optimization of the process. Physical measurements (i.e. yield point force and total deformation energy) were performed on the olive samples; after the fruit were crushed for olive paste production, chemical analysis (moisture, oil and sugars content) and maturity index (MI) were measured and correlated to the spectral data. The obtained results were encouraging for chemical, texture and MI parameters, demonstrating the feasibility of real-time prediction of important indices for the milling plant settings.

Keywords: chemometrics, milling plant, olive pastes, optical analysis, ripening, texture

1. INTRODUCTION

In olive fruits, physical parameters such as weight, color, pulp-to-stone ratio, and texture, and chemical parameters such as oil content, composition of fatty acids, levels of polyphenols, tocopherols, and sterols, change during the ripening process (BELTRÁN *et al*., 2004; YOUSFI and GARCÍA, 2005). These features are of high commercial importance as they influence the olive oil shelf life. In fact, olive oils derived from ripe fruit results in a less stable shelf life due to an increase in polyunsaturated fatty acids and a decrease in total polyphenol content (CAPONIO *et al*., 2001; MARSILIO *et al*., 2001; MORELLO *et al*., 2004; BELTRÁN *et al*., 2005). Phenolic compound content is considered an important parameter in the evaluation of virgin olive oil quality because of phenols contribute to oil flavour and aroma. Phenols also, protect oil from autoxidation. In addition, olives processed at an over ripened stage may result in unstable oil during shelf-life owing to the low phenolic compound content (CHERUBINI *et al*. 2009). Early-harvested fruits produce oil with high polyphenol concentrations and a degradation may occur during the processing and shelf-life. Degradation may result in variations in nutritional quality of the product since antioxidant content decreases and free radical content increases. This can lead to sensory modification and to an appreciation reduction of the product, since aroma, colour, taste and flavour attributes change and some unpleasant sensory attributes may occur (ZANONI *et al*., 2005; DIRAMAN and DIBEKLIOGLU, 2009).

For these reasons, the influence of harvesting time on the quality stability and sensory characteristics of olive oils is of crucial interest for the growers. An investigation of the olives' characteristics before the milling process could allow the quality of the oil output to be controlled. Better monitoring of the oil production process also depends on controlling the paste, the intermediate product between the olives inlet in the process and the oil outlet from the mill, to establish correlations among olives, paste and oil. Nowadays, established methods for quality assessment are generally based on tedious and time-consuming techniques that are impractical for processing a large number of samples. Therefore, there is a lack of real-time information during the milling process in order to monitor the operating parameters continuously. Hence, there is a strong need in the modern oil industry for a simple, rapid, and easy-to-use method for (i) objectively evaluating the level of olive ripening and the characteristics of the paste, (ii) early detection of possible failure, (iii) permanent monitoring of the production process, and (iv) assessment of oil process at any desired time in order to control the oil quality deriving from the process. The rapid analysis of olives during consignment and paste during the process would allow preliminary separation of homogeneous classes and a more efficient decision-making process about the destination of lots. Therefore, the sector could be helped by optical non-destructive and rapid applications for olive oil chain optimization.

Despite there being some works regarding the application of NIR spectroscopy for olive oil analysis (ARMENTA *et al*., 2010), few works about the characterization of intact olives and olive pastes using spectroscopy can be found. FERNÁNDEZ-ESPINOSA (2016) combined chemometric analysis with NIR spectroscopy to monitor quality parameters in intact olives to determine the optimal harvesting time; SALGUERO-CHAPARRO *et al*. (2013) used NIR spectroscopy for the online determination of the oil content, moisture and free acidity parameters in intact olive fruits; and BELLINCONTRO *et al*. (2012) studied the application of a portable NIR for on-field prediction of phenolic compounds during olive ripening. CAYUELA *et al*. (2009) determined the effectiveness of a portable NIR spectrometer for the prediction of oil free acidity, oil yield, oil content in fresh fruit, oil content in fruit dry matter and fruit moisture content, analyzing intact fruits.

For olive oil fruits, textural properties could be used as indices of ripeness to meet requirements for the technological processes and oil characterization. Texture-measuring

instruments are time-consuming, and there are high costs for the devices. The parameter setting of the process operations (i.e. crushing, malaxation, and extraction using decanter) is highly influenced by ripeness degree and olive texture. Therefore, there is nowadays a lack of available information that could allow feedback-based real-time control of the plant, for better oil quality and the reduction of process wastes. BEGHI *et al*. (2013) and GIOVENZANA *et al*. (2015) conducted preliminary studies on the laboratory scale of the capability of portable visible/near infrared (vis/NIR) and NIR spectrophotometers to investigate different textural indices for the characterization of olive fruits entering the milling process.
On olive paste, GARCÍA SÁNCHEZ *et al*. (2005) tested the suitability of NIR and NMR spectroscopy for the determination of moisture and fat contents, while GALLARDO *et al*. (2005) used near-infrared spectroscopy for the real-time determination of moisture and fat content in olive pastes and solid-liquid wastes. HERMOSO *et al*. (1999) examined the applicability of NIR spectroscopy for the measurement of oil content and humidity in olive pomace.
The challenge of producing high-quality olive oil is of great concern, and the selection of olive fruit with defined properties that ensure positive attributes in olive oil is foreseeable using vis/NIR and NIR spectroscopy in olive oil production (ARMENTA *et al*., 2010). The olive oil sector is increasingly becoming more interested in the implementation of quality control systems in a mill industry context. However, limited work has been undertaken about the implementation of vis/NIR and NIR spectroscopy directly in the mill. Research regarding the application at the factory level is desirable, mainly concerning the definition of parameters related to the on-line spectrum acquisition (SALGUERO-CHAPARRO *et al*., 2012) and the testing of compact and low-cost devices also usable for the SME of the sector. Hence, in this study, the applicability of a vis/NIR low-cost and compact system was tested on intact olives, acquired just before the milling process, and on olive pastes, in order to correlate spectral data to the crucial parameters (yield point force, total deformation energy, moisture, oil and sugars content, and maturity index) for the optimization of the milling process. The predictive models calculated here could be applied in future on-line for the rapid monitoring of crucial parameters for the enhancement of extraction oil yield and the control of semi-finished products of the process.

2. MATERIALS AND METHODS

Two olive varieties were considered: Frantoio and Moraiolo (~50% of each). These varieties are typical of the Tuscan hills, in the province of Florence, Italy, and are cultivated in several European olive growing areas.

2.1. Sampling

Sampling was performed in 2013, from September to December, to obtain a wide sample variability. The sampling was conducted by hand once a week at 08:00 a.m. on a selected number of plants (about 10), belonging to the two different cultivars selected for the experiment. The olives were picked along plant circumference at approx. 1.7 m from the soil. A total of 54 olive samples (400-500 g), which presented no infection or physical damage, were quickly transported to the laboratory to be analysed and for each sample a homogeneous batch of olives (i.e. approx. 300 g) was selected. For each sample, 30 olive fruit vis/NIR spectra were acquired, for a total of 1620 optical measurements. Using a portable spectrophotometer, two spectral measurements were taken in reflectance mode

on individual fruit along their equatorial region and averaged. All the olives composing a sample were crushed using a laboratory crusher (Zeutec, Rendsburg, Germany), obtaining olive paste. Five acquisitions were performed for each paste sample through disposable laboratory cuvettes (12.5 mm x 12.5 mm x 45.0 mm).

2.2. Texture analysis

The firmness was assessed using a laboratory dynamometer (MTS Criterion® Systems, Eden Prairie, MN, USA) providing time-series data of product compression, allowing the calculation of textural attributes from load-extension traces. Table 1 shows the settings for the compression test. The measurements were carried out using a 5 cm diameter plate coupled with a load cell of 100 N. The sample was placed under the plate, without holder, with the major axis perpendicular to the direction of the compression test.

Table 1. Settings for the compression test.

Settings	Units	
Gage adjustment speed	mm s^{-1}	0.2
Gage adjustment load	N	0.6
Experimental speed	mm s^{-1}	1.0
Date acquisition rate	Hz	400.0
Break Threshold	N	5.0
Break sensibility	%	50.0
Strain end point	%	80.0

The textural parameters obtained from the elaboration of the load-extension curve were as follows:
- Yield point force (N): the maximum force recorded during the elastic deformation phase;
-Total deformation energy (mJ): the work required for complete compression of the olive pulp.
In Figure 1 is shown an example of load-extension curve obtained from the compression test for the identification of yield point force (N) and total deformation energy (mJ).

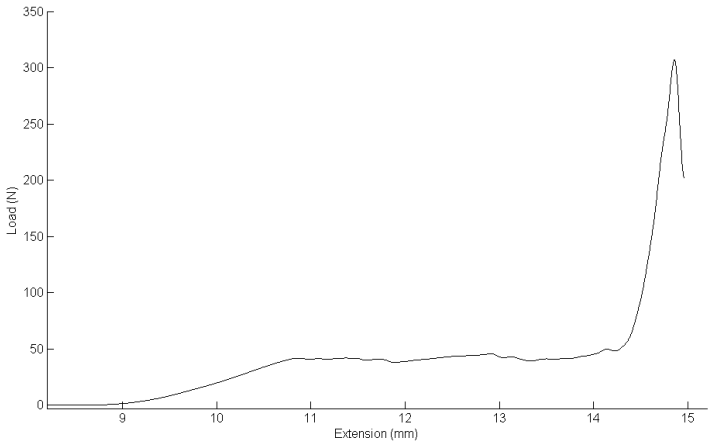


Figure 1. Example of load-extension curve obtained from the compression test.

2.3. Chemical analyses

The olive paste was used for the following chemical analyses.

2.3.1 Water content

The water content was measured on olive paste by heating 60 g of sample in an oven at 105°C until reaching constant weight (CECCHI *et al*. 2013). The results were expressed as moisture content (%).

2.3.2 Oil content

The total oil content was determined on 5 g of dried olive paste (see the oven method above). Samples were extracted with hexane in an automatic extractor (Randall mod. 148, VELP Scientifica, Milan, Italy), following the method of CHERUBINI *et al*. (2009). The results were expressed as oil content on dry matter basis (g kg^{-1}).

2.3.3 Sugar content

8 g of olive paste was cold extracted (6 ± 2°C) with distilled water in a 200 mL flask for 2 hours. The content of the flask was filtered on paper, and 10 mL of the obtained solution was diluted with water in a 20 mL flask. The measurements were performed by analyzing the obtained solution with an enzymatic method using an automatic ChemWell analyzer (Awareness Technology, ChemWell 9210, Palm City, FL). Three enzymatic kits were used to measure, respectively, (i) the sum of two monosaccharides contents, namely glucose and fructose; (ii) the sum of disaccharide sucrose content with glucose and fructose contents; (iii) the mannitol content. All kits were purchased from R-Biopharm (Darmstadt, Germany). The measurements were performed by means of external calibration standards: fructose and glucose (purity > 99%, Sigma Aldrich SrL, Milano, Italy), and mannitol (purity > 98%, Sigma Aldrich Srl, Milan, Italy). The results provided by the instrument were expressed in g L^{-1}; they were also converted in sugar content on dry matter basis (g kg^{-1}) as the average of two readings, carried out for each sample. The sucrose contents were determined by multiplying by 0.95 the difference between the sum of glucose, fructose, sucrose contents and the sum of glucose and fructose contents.

2.4. Maturity Index

MI was based on visual assessment according to UCEDA and FRIAS (1975). A sample of 100 drupes was classified into eight different classes according to pulp and skin colors. The values ranged from 0 (skin color deep green) to 7 (skin color black with all the flesh purple to the stone).

2.5. Visible/Near Infrared device

Spectral acquisitions were performed on samples (olives and pastes) using an optical portable system (JAZ vis/NIR spectrophotometer, OceanOptics, Inc., Dunedin, FL, USA) operating in the 400-1000 nm wavelength range. The system is composed of five components: 1) vis/NIR lighting system; 2) fiber-optic probe for reflection measurement; 3) spectrophotometer; 4) hardware for data acquisition and instrument control; 5) power battery.

Spectra were acquired in reflectance mode: light radiation was guided to the sample through a Y-shaped, bidirectional fiber optic probe (OceanOptics, Inc., Dunedin, FL, USA). A Y-shaped fiber allowed light from a halogen lamp to be guided to illuminate the sample while simultaneously collecting the radiation coming from the berry and guiding it back to the spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure contact with sample skin during measurements, minimizing environmental light interference. White background and black background were acquired before each acquisition session. The integrated spectrophotometer was equipped with a diffractive grating for spectral measurements, optimized in the range 400-1000 nm, and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm. Each spectrum corresponds to the average of five spectral acquisitions.

2.6. Data analysis

The data acquired were processed using chemometric techniques to extract maximum data information. Chemometric analysis was performed using The Unscrambler software package (version 9.8, CAMO ASA, Oslo, Norway). Different pre-treatments were applied to the vis/NIR spectra in order to maximize the model accuracy. Moving-averaged smoothed spectra (15 point-wide window corresponding to a window of 4.5) and multiplicative scatter correction (MSC) were applied before building the calibration models. These pre-treatments were applied to improve the signal-to-noise ratio in order to reduce the effects caused by the physiological variability of olive and paste samples.
The olive samples available were used for the calculation of a chemometric regression model for reference parameters by using partial least squares (PLS) regression analysis. The vis/NIR spectra acquired on the single olives were correlated to the textural parameters (one-to-one correlation) using the PLS regression algorithm, while the 30 olive spectra representing each experimental sample were averaged, and the resulting mean spectrum was correlated to the chemical indices and MI. Similarly, the olive paste samples were also correlated to chemical reference data and to MI to create PLS models.
To evaluate model accuracy, the statistics used were the coefficient of determination in calibration (R^2_{cal}), coefficient of determination in cross-validation (R^2_{cv}), root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV). Calibration models were evaluated using a cross-validation leave-more-out procedure using five cancellation groups randomly selected. With a small number of cancellation groups, the resulting training sets are very different, and the measure of the predictive ability is not optimistic, possibly pessimistic (CASALE *et al*., 2008). Moreover, the Ratio Performance Deviation (RPD) value was calculated. RPD is defined as the ratio between the standard deviation of the response variable and RMSECV. RPD values below 1.5 indicate that the calibration is not useful. When the RPD value is higher than 2, quantitative predictions are possible. Between 1.5-2.0, the algorithm has the possibility to distinguish between high and low values (WILLIAMS and NORRIS, 1987). The best model calibrations were selected based on minimizing the RMSECV and maximizing the RPD.

3. RESULTS AND DISCUSSIONS

The average spectra of both olives and pastes showed three main peaks: around the 670 nm band, corresponding to the chlorophyll absorption peak (MCGLONE *et al*., 2002); around the 730 nm band, equal to the maximum reflectance peak; and the 780 nm band, representing the third overtone of OH bond stretching (CLEMENT *et al.*, 2008).

Changes in spectra reflected modifications in chemical parameters. For a better visualization, two arbitrary classes based on oil content (a, Level 1 ≤ 358.3 g kg^{-1}; and Level 2 > 358.3 g kg^{-1}) and on moisture content (b, Level 1_m ≤ 53.5 %; and Level 2_m > 53.5 %) were created to show changes in the optical data. The average vis/NIR spectra acquired on intact olives are grouped into two classes by oil and moisture content, and are shown in Figures 2a and 2b, respectively.

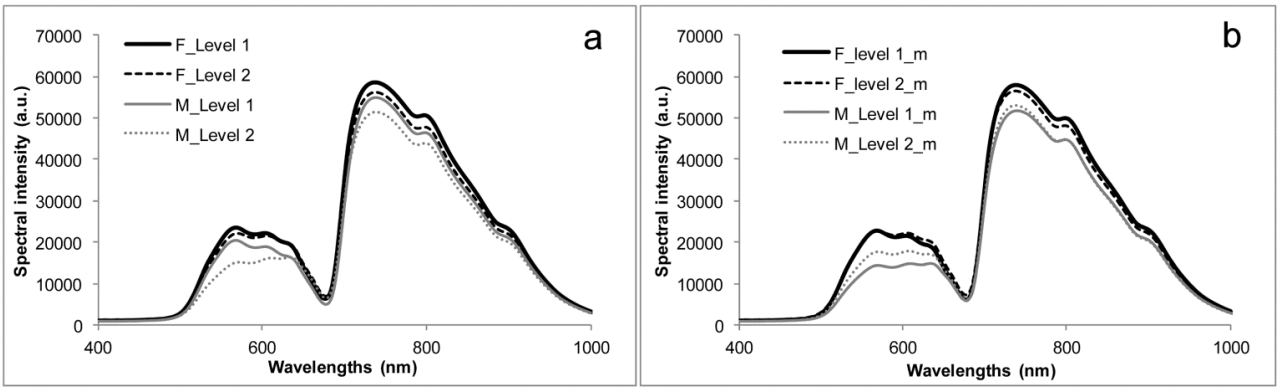


Figure 2. Average vis/NIR smoothed spectra of intact olives, Frantoio (F) and Moraiolo (M) cultivar, grouped in two classes of oil content (a, level 1 ≤ 358.3 g kg^{-1}; level 2 > 358.3 g kg^{-1}) and in two classes of moisture content (b, level 1_m ≤ 53.5 %; level 2_m > 53.5 %).

Vis/NIR spectra exhibited differences for both the cultivars among the two classes, with relevant changes particularly in the visible range occurring from Level 1 to Level 2 for Moraiolo cultivar. The spectra of Moraiolo cultivar showed higher absorption in the visible range compared to Frantoio cultivar. This is linked to anthocyanin pigmentation during ripening from green berries to the completely black-pigmented olives, which leads to a strong decrease in reflectance in the visible band associated with the anthocyanin absorption peak centered on 540 nm. This different behavior in the spectral reflectance is confirmed by the maturity index (UCEDA and FRIAS, 1975). This parameter is based on the subjective evaluation of the progressive pigmentation of olive skin and flesh. The index is the main reference utilized by the olive oil chain to characterize the olive ripeness degree at the mill or on the tree (GARCÍA *et al.*, 1996), and by scientists to identify the ripeness levels of olives for harvesting all over the world, e.g. in Israel (DAG *et al.*, 2011) in Spain (GUTIÉRREZ *et al.*, 1999); BELTRÁN *et al.*, 2005) in Tunisia (MRAICHA *et al.*, 2010), and in Italy (SINELLI *et al.*, 2008). When the olives are fully ripe, the MI reached values equal to 6 on Barnea and Souri cultivars (DAG *et al.*, 2011) and 7 on Chemlali cultivar (MRAICHA *et al.*, 2010). Instead, for the two analyzed cultivars, the MI achieved at harvest maximum values of 3.39 for Moraiolo cv and 2.31 for Frantoio cv. Frantoio remains substantially green even when fully ripe, hence the spectra in the visible range not showing evident changes between the two classes considered.

In Figure 2, as expected, an opposite trend can be noticed between spectra grouped by oil content and by moisture content; the oil accumulation in the fruit caused greater absorption in the spectra of both cultivars, which results in lower average values of reflectance in the whole spectra of the class Level 2. In particular, this behavior is evident for the Moraiolo cv, due to an increase of oil content and a simultaneously external pigmentation of the berries. This leads to a decrease in reflectance in the visible band associated with the anthocyanin absorption peak centered around 540 nm.

Conversely, the spectra of the berries richer in water (Level 2_m) showed slightly higher values of reflectance, especially in the visible spectral range for the Moraiolo cv. Similar behavior, as shown in Figure 3, can be noticed for the average spectra of olive pastes grouped by the same two classes of oil and moisture content. In particular, the spectra of pastes richer in water (Level 2_m) showed the highest values of reflectance. Also, in this case, the behavior is more evident for the Moraiolo cv.

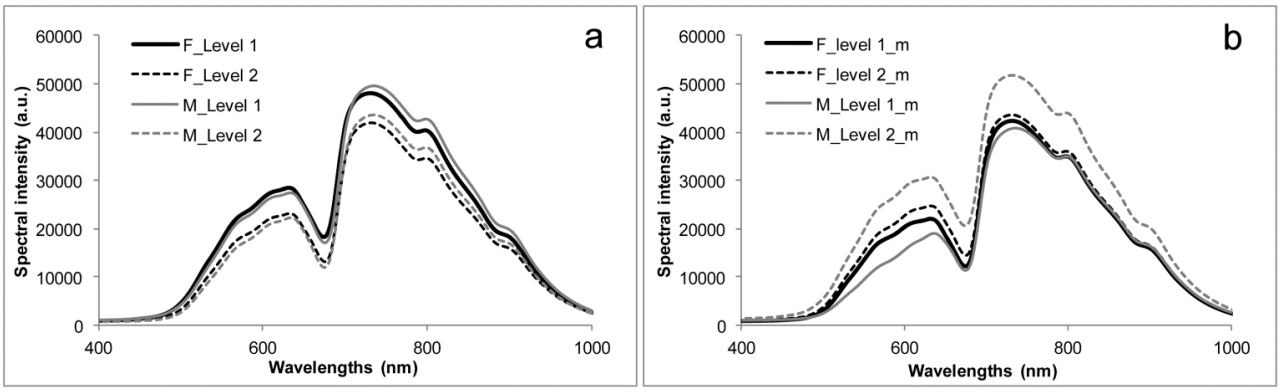


Figure 3. Average vis/NIR smoothed spectra of olive pastes, *Frantoio* (F) and *Moraiolo* (M) cultivar, grouped in two classes of oil content (a, level 1 ≤ 358.3 g kg^{-1}; level 2 > 358.3 g kg^{-1}) and in two classes of moisture content (b, level 1_m ≤ 53.5 %; level 2_m > 53.5 %).

PLS regression models were built for each parameter measured. In Table 2, the results of the PLS regression models arising from spectra on 30 intact olives for the predictions of moisture, oil and sugars content, of yield point force, total deformation energy, and MI are shown.

Regarding textural parameters the possibility to use the reference data on a single berry allowed us to obtain acceptable results for the prediction of indices usually difficult to predict in an optical non-destructive way. Interesting results were obtained for the prediction of the yield point force. Similar results were obtained for firmness prediction on intact olives by KAVDIR *et al.* (2009) and by BEGHI *et al.* (2013), using FT-NIR spectroscopy in the wavelength range 780-2500 nm in reflectance mode and vis/NIR spectroscopy (400-1000 nm), respectively. Instead, regarding the prediction of the total deformation energy, results are not satisfactory and similar to those obtained in a previous study by GIOVENZANA *et al.* (2015): R^2 in cross-validation equal to 0.58 for vis/NIR range and equal to 0.33 for NIR range.

The application of vis/NIR and NIR spectroscopy for the analysis of textural parameters often encounters considerable difficulties, which was highlighted elsewhere (ZUDE *et al.*, 2006; NICOLAÏ *et al.*, 2008). This difficulty is usually due to several factors: first, the extreme variability of this parameter among berries; the high instrumental error of the penetrometer; and the difficulty of calibrating a model for the estimation of an index not directly associable with a chemical species (and consequently the absorption bands of those chemical bonds).

Encouraging results were also obtained for chemical parameters, in particular for the prediction of moisture and oil content, with RPD values of about 2.

Table 2. Descriptive statistics and statistics of the PLS models elaborated on vis/NIR spectra of intact olives for the prediction of chemical, maturity and textural parameters

					Calibration models		Cross-Validation models		
				Chemical parameters					
Sample	**n**	**Media**	**DS**	**LVs**	**R^2_{cal}**	**RMSEC**	**R^2_{cv}**	**RMSECV**	**RPD**
					Moisture (%)				
All samples	48	53.32	3.97	8	0.87	1.39	0.75	1.89	2.10
Frantoio cv	28	51.81	3.33	7	0.89	1.05	0.79	1.43	2.33
Moraiolo cv	18	55.42	4.07	5	0.91	1.13	0.74	2.12	1.92
					Oil content (g kg $^{-1}$)				
All samples	44	371 110	49.93	9	0.85	18.67	0.74	25.85	1.93
Frantoio cv	28	361.34	49.88	2	0.67	28.01	0.67	31.12	1.60
Moraiolo cv	18	390.71	50.87	3	0.88	16.97	0.81	22.3	2.28
					Sugar content (g kg $^{-1}$)				
All samples	46	38.87	5.69	7	0.65	3.31	0.42	4.35	1.31
Frantoio cv	24	35.91	3.76	10	0.95	0.77	0.75	2.03	1.85
Moraiolo cv	18	44.18	4.2	10	0.95	0.88	0.38	3.38	1.24
					Maturity Index				
Sample	n	Media	DS	LVs	R^2_{cal}	RMSEC	R^2_{cv}	RMSECV	RPD
					MI				
All samples	47	0.85	1.02	3	0.93	0.24	0.92	0.26	3.92
Frantoio cv	28	0.32	0.46	5	0.87	0.15	0.7	0.22	2.09
Moraiolo cv	18	1.71	1.08	3	0.96	0.21	0.94	0.28	3.86
					Textural parameters				
Sample	n	Media	DS	LVs	R^2_{cal}	RMSEC	R^2_{cv}	RMSECV	RPD
					Yield point force (N)				
All samples	1410	41.26	17.26	9	0.63	12.32	0.62	12.44	1.39
Frantoio cv	918	52.83	22.29	10	0.63	13.51	0.61	13.82	1.61
Moraiolo cv	486	43.6	17.81	9	0.74	8.04	0.72	8.31	2.14
					Total deformation energy (mJ)				
All samples	1373	450.35	103.69	10	0.42	78.83	0.4	79.96	1.30
Frantoio cv	876	467.11	106.65	10	0.54	71.96	0.52	73.59	1.45
Moraiolo cv	408	426.98	81.63	3	0.33	66.67	0.31	67.93	1.20

A similar study was performed by SALGUERO-CHAPARRO *et al.* (2013) for the evaluation of moisture and fat content in intact olive fruits from 50 varieties using an NIR instrument in the range 400-2500 nm. They achieved for PLS models on moisture, fat content and acidity RPD values of 2.32, 2.08 and 1.70, respectively. CAYUELA *et al.* (2009) obtained slightly worse results using an AOTF-NIR on intact olives in the range 1100-2300 nm for the prediction of oil content (R^2 equal to 0.65) and moisture (R^2 ranged 0.35-0.78) compared with those obtained by the authors of this study for the same parameters. Moreover, similar results were achieved by FERNÁNDEZ-ESPINOSA (2016) using an on-line AOTF-NIR system (1000-2300 nm) on intact olives for the estimation of the oil content (R^2 0.76), while better results were obtained by the same author for the prediction of the moisture content (R^2 0.88).

Excellent results were obtained for the estimation of the MI with RPD about 4. This result may have interesting applicative implications, since the MI requires time for measuring

and sample preparation. GUZMÁN *et al.* (2015) classified intact olives based on MI using image analysis, obtaining positive predictive values of about 90%.
In Table 3, the results for the PLS regression models arising from spectra on olive pastes are shown. In this case, the considered parameters were obviously only the chemical ones and the MI. Slightly better results were obtained compared to those arising from the models calculated using spectra on intact olives. Also in this case, better results were achieved for the prediction of MI, in particular for the Moraiolo cultivar (RPD = 4.15). BENDINI *et al.* (2007) applied FT-NIR for in-process monitoring of different cultivar pastes in diffuse reflectance mode, obtaining models with R^2 equal to 0.92 and 0.91 for the prediction of oil content and moisture, respectively.

Table 3. Descriptive statistics and statistics of the PLS models elaborated on vis/NIR spectra of olive pastes for the prediction of chemical and maturity parameters.

					Calibration models		Cross-Validation models		
				Chemical parameters					
Sample	**n**	**Media**	**DS**	**LVs**	**R^2_{cal}**	**RMSEC**	**R^2_{cv}**	**RMSECV**	**RPD**
					Moisture (%)				
All samples	45	53.4	3.99	9	0.86	1.44	0.75	2	2.00
Frantoio cv	26	51.79	3.16	4	0.74	1.56	0.55	2.11	1.50
Moraiolo cv	18	55.42	4.07	4	0.86	1.42	0.79	1.91	2.13
					Oil content (g kg $^{-1}$)				
All samples	46	378.23	50.98	5	0.78	23.46	0.69	28.64	1.78
Frantoio cv	26	372.19	44.83	2	0.68	24.56	0.7	27.32	1.64
Moraiolo cv	18	390.71	50.87	3	0.88	16.66	0.85	19.76	2.57
					Sugar content (g kg $^{-1}$)				
All samples	45	51.68	7.3	4	0.61	4.5	0.51	5.2	1.40
Frantoio cv	28	48.67	5.72	4	0.6	3.53	0.51	4.42	1.29
Moraiolo cv	13	55.83	4.4	6	0.98	0.59	0.83	1.86	2.37
				Maturity Index					
Sample	n	Media	DS	LVs	R^2_{cal}	RMSEC	R^2_{cv}	RMSECV	RPD
					MI				
All samples	46	0.96	1.04	4	0.88	0.33	0.85	0.39	2.67
Frantoio cv	25	0.4	0.51	4	0.79	0.21	0.58	0.31	1.65
Moraiolo cv	18	1.71	1.08	7	0.98	0.12	0.95	0.26	4.15

Similar prediction performances were obtained starting from the spectra acquired on intact olives or on pastes. RPD values for the considered chemical parameters were 1.31–2.10 and 1.40–2.00 for the models calculated for intact olives and pastes, respectively. This result is very interesting with a view to future applications, as the possibility to perform optical analysis directly on the fruits before the process could be envisaged, without any sample preparation.
Regarding the MI, better results were obtained as expected starting from intact olives, due to the high correlation between ripeness and peel pigmentation; the evolution of MI values is driven by color evolution during ripening, which is mainly influenced by the external color of the fruits. The Moraiolo cultivar gave the best results overall. This is probably due to the evident evolution of external pigmentation of the fruits during the ripening process

that helps the correlation with the vis/NIR spectra, especially due to the contribution of the visible range.
Predictive models are usable for the monitoring of operative parameters in different steps of the milling process, i.e. crushing, malaxation, and extraction using decanter, for the enhancement of extraction oil yield and the control of semi-finished products of the process.

4. CONCLUSIONS

The olive oil sector is interested in new user-friendly systems for rapid analysis that can be performed directly on-line on the milling plant with the objective of using information from sensors to manage the product better, and to preserve consumers' expectations of high-quality extra virgin olive oil, closely related to the composition of phenols and of volatile compounds. Increasing demand for rapid, cost-effective and non-invasive measurement of texture remains a challenge for the oil extraction process. This study has interrogated the applicability of vis/NIR spectroscopy as a rapid technique for the analysis of olives directly on the tree or at the mill just before the oil extraction process, and the olive paste, for the monitoring of crucial parameters for the enhancement of extraction oil yield (moisture, oil, sugar content, MI and textural indices). Our results were encouraging for chemical, texture and MI parameters. Regression models could be used for real-time prediction of crucial indices to support specific requirements of the process, considering the technological characteristics of the different olives or olive pastes, in order to diversify quickly the oil production.
Investigation of wavelength bands in order to highlight and select the most informative ones is desirable in order to design a simple and inexpensive device to classify olives entering the mill based on technological requirements, and to monitor the operative parameters during the process. The olive oil sector could be provided with pre- and post-harvest methods and sorting systems for olive fruits and olive paste for quick evaluation of the essential features to optimize the oil-making process.

REFERENCES

Armenta S., Moros J., Garrigues S. and Guardia M. 2010. The use of near-infrared spectrometry in the olive oil industry. Crit. Rev. Food Sci. Nutr. 50:567-582.

Beghi R., Giovenzana V., Civelli R., Cini, E. and Guidetti R. 2013. Characterisation of olive fruit for the milling process by using visible/near infrared spectroscopy. J. Agric. Eng. 44:56-61.

Bellincontro A., Taticchi A., Servili M., Esposto S., Farinelli D. and Mencarelli F. 2012. Feasible application of a portable NIR-AOTF tool for on-field prediction of phenolic compounds during the ripening of olives for oil production. J. Agric. Food Chem. 60:2665-2673.

Beltrán G., Aguilera M., Rio C., Sanchez S. and Martinez L. 2005. Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. Food Chem. 89:207-215.

Beltrán G., Del Río C., Sánchez S. and Martínez L. 2004. Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. J. Sci. Food Agric. 84:1783-1790.

Bendini A., Cerretani L., Di Virgilio F., Belloni P., Lercker G. and Toschi T. 2007. In-process monitoring in industrial olive mill by means of FT-NIR. European J. Lipid Sci. Tech. 109:498-504.

Caponio F., Gomes T. and Pasqualone A. 2001. Phenolic compounds in virgin olive oils: influence of the degree of olive ripeness on organoleptic characteristics and shelf-life. Europ. Food Res. Technol. 212:329-333.

Casale M., Casolino C., Ferrari G. and Forina M. 2008. Near infrared spectroscopy and class modelling techniques for the geographical authentication of Ligurian extra virgin olive oil. J. Near Infrared Spectrosc. 16:39-47.

Cayuela J., García J., García J., Caliani N. and Caliani N. 2009. NIR prediction of fruit moisture, free acidity and oil content in intact olives. Grasas Y Aceites 60:194-202.

Cecchi L., Migliorini M., Cherubini C., Giusti M., Zanoni B., Innocenti M. and Mulinacci N. 2013. Phenolic profiles, oil amount and sugar content during olive ripening of three typical Tuscan cultivar to detect the best harvesting time for oil production. Food Res. Int. 54:1876-1884.

Cherubini C., Migliorini M., Mugelli M., Viti P., Berti A., Cini E. and Zanoni B. 2009. Towards a technological ripening index for olive oil fruits. J. Sci. Food Agric. 89:671-682.

Clement A., Dorais M. and Vernon M. 2008. Nondestructive measurement of fresh tomato lycopene content and other physicochemical characteristics using visible-NIR spectroscopy. J. Agric. Food Chem. 56:9813-9818.

Dag A., Kerem Z., Yogev N., Zipori I., Lavee S. and Ben-David E. 2011. Influence of time of harvest and maturity index on olive oil yield and quality. Scientia Horticulturae 127:358-366.

Diraman H. and Dibeklioglu H. 2009. Characterization of Turkish virgin olive oils produced from early harvest olives. J. Amer. Oil Chemists Soc. 86 (7):663-674.

Fernández-Espinosa A.J. 2016. Combining PLS regression with portable NIR spectroscopy to on-line monitor quality parameters in intact olives for determining optimal harvesting time. Talanta 148:216-228.

Gallardo L., Osorio E. and Sanchez J. 2005. Application of near infrared spectroscopy NIRS for the real-time determination of moisture and fat contents in olive pastes and wastes of oil extraction. Alimentación Equipos y Tecnologia 24:85-89.

García J., Gutiérrez F., Castellano J., Perdiguero S., Morilla A. and Albi M. 1996. Influence of storage temperature on fruit ripening and olive oil quality. J. Agric. Food Chem. 44:264-267.

García Sánchez A., Ramos Martos N. and Ballesteros E. 2005. Comparative study of various analytical techniques NIR and NMR spectroscopies, and Soxhlet extraction for the determination of the fat and moisture content of olives and pomace obtained from Jaén Spain. Grasas y Aceites 56:220-227.

Giovenzana V., Beghi R., Civelli R., Marai S. and Guidetti R. 2015. Postharvest characterization of olive oil fruits texture by NIR and vis/NIR spectroscopy. Chem. Eng. Transactions 44:61-66.

Gutiérrez F., Jimenez B., Ruiz A. and Albi M. 1999. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. J. Agric. Food Chem. 47:121-127.

Guzmán E., Baeten V., Pierna J.A.F. and García-Mesa J.A. 2015. Determination of the olive maturity index of intact fruits using image analysis. J. Food Sci. Tech. 52(3):1462-1470.

Hermoso M., Uceda M., Garcia-Ortiz A., Jimenez A. and Beltran G. 1999. Preliminary results of NIR "on-line" measure of oil content and humidity in olive cakes from the two phases decanter. Acta Hortic. 474:717-720.

Kavdir I., Buyukcan M., Lu R., Kocabiyik H. and Seker M. 2009. Prediction of olive quality using FT-NIR spectroscopy in reflectance and transmittance modes. Biosys. Eng. 103:304-312.

Marsilio V., Campestre C., Lanza B. and De Angelis M. 2001. Sugar and polyol compositions of some European olive fruit varieties Olea europaea L suitable for table olive purposes. Food Chem. 72:485-490.

McGlone V., Jordan R. and Martinsen P. 2002. Vis/NIR estimation at harvest of pre- and post-storage quality indices for 'RoyalGala' apple. Postharvest Bio. Tech. 25:135-144.

Morello J., Motilva M., Tovar M. and Romero M. 2004. Changes in commercial virgin olive oil cv Arbequina during storage, with special emphasis on the phenolic fraction. Food Chem. 85:357-364.

Mraicha F., Ksantini M., Zouch O., Ayadi M., Sayadi S. and Bouaziz M. 2010. Effect of olive fruit fly infestation on the quality of olive oil from Chemlali cultivar during ripening. Food Chem. Toxic. 48:3235-3241.

Nicolaï B.M., Verlinden B.E., Desmet M., Saevels S., Saeys W., Theron K., Cubeddu R., Pifferi A. and Torricelli A. 2008. Time-resolved and continuous wave NIR reflectance spectroscopy to predict soluble solids content and firmness of pear. Postharvest Bio. Tech. 47:68-74.

Salguero-Chaparro L., Baeten V., Abbas O. and Peña-Rodríguez F. 2012. On-line analysis of intact olive fruits by vis–NIR spectroscopy: Optimisation of the acquisition parameters. J. Food Eng. 112(3):152-157.

Salguero-Chaparro L., Baeten V., Fernández-Pierna J. and Peña-Rodríguez F. 2013. Near infrared spectroscopy NIRS for on-line determination of quality parameters in intact olives. Food Chem. 139:1121-1126.

Sinelli N., Casiraghi E., Tura D. and Downey G. 2008. Characterisation and classification of Italian virgin olive oils by near- and mid-infrared spectroscopy. J. Near Infrared Spectrosc. 16:335-342.

Uceda M. and Frıas L. 1975. Harvest dates evolution of the fruit oil content, oil composition and oil quality. Proceedings of the Segundo Seminario Oleicola Internacional 125-128.

Williams P. and Norris K. 1987. Near-infrared technology in the agricultural and food industries. St Paul, Minn, USA, American Association of Cereal Chemists.

Yousfi K., Cert R. and García J. 2005. Changes in quality and phenolic compounds of virgin olive oils during objectively described fruit maturation. Eur. Food Res. Technol. 223:117-124.

Zanoni B., Bertuccioli M., Rovellini P., Marotta F. and Mattei A. 2005. A preliminary approach to predictive modelling of extra virgin olive oil stability. J. Sci. Food Agric. 85:1492-1498.

Zude M., Herold B., Roger J.M., Bellon-Maurel V. and Landahl S. 2006. Non-destructive tests on the prediction of apple fruit flesh firmness and soluble solids content on tree and in shelf life. J. Food Eng. 77:254-260.

Paper Received July 20, 2016 Accepted February 6, 2017

SHORT COMMUNICATION

FATTY ACIDS METHYL AND ETHYL ESTERS BEHAVIOUR DURING OLIVES PROCESSING BY MEANS OF TECHNOLOGICAL COADJUVANTS

G. SQUEO, R. SILLETTI, C. SUMMO, V.M. PARADISO, A. PASQUALONE and F. CAPONIO*

University of Bari Aldo Moro, Department of Soil, Plant and Food Sciences, Food Science and Technology Unit, Via Amendola 165/A, I-70126, Bari, Italy

*Corresponding author. Fax: +39 0805443467

E-mail address: francesco.caponio@uniba.it

ABSTRACT

Recently, the quantification of fatty acids alkyl esters has become mandatory for the extra virgin olive oil classification. However, the behaviour of such metabolites, during olives processing, is not yet well understood. Thus, the present paper aims to point out the influence of the use of calcium carbonate on the fatty acids alkyl esters content of the oils. The results showed that the content of fatty acids alkyl esters was significantly influenced by the technological coadjuvant. The use of calcium carbonate led to a general reduction of fatty acids alkyl esters compared to the untreated samples. Methyl esters of fatty acids were more susceptible to the use of processing aid than the ethyl esters.

Keywords: alkyl esters, extra virgin olive oil, olives processing

1. INTRODUCTION

According to the EC Regulation (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2001), virgin olive oils are "oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using coadjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds". Such statement does not take into account the quality of the raw material that, instead, represents one of the main factors, together with the extraction process, affecting the final product quality.

To classify virgin olive oils, several parameters must be checked, some of which have very good correlation with the raw material features (SALVADOR *et al.*, 2001; KOPRIVNJAK *et al.*, 2010). The EU Regulation 61/2011 (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2011) added the determination of methyl and ethyl esters of fatty acids, generally recognised as fatty acids alkyl esters (FAAE), to the list of parameters to be checked for classifying the quality levels of virgin olive oils. Later, the EU Regulation 1348/2013 (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2013) stressed attention only on the fatty acids ethyl esters (FAEE). Alkyl esters originate from the esterification of fatty acids and low molecular weight alcohols, methanol and ethanol, respectively arisen from the progressive pectin degradation during the olive ripening and/or by the bad and/or prolonged storage of drupes (BIEDERMANN *et al.*, 2008; Pérez-Camino *et al.*, 2008). Thus, the presence of FAAE in oils became an established marker of the quality of the raw material employed.

The knowledge about the FAAE is continuously growing. Indeed, several researches have been carried out, focused on the correlation between FAAE and the raw material quality (CERT, 2006; MARIANI and BELLAN, 2008); the virgin oil sensory characteristics (BIEDERMANN *et al.*, 2008; GÓMEZ-COCA *et al.*, 2012); the olive pomace oil storage-related changes (RUIZ-MÉNDEZ and RAMOS-HINOJOSA, 2003); the olive storage conditions (JABEUR *et al.*, 2015) and, recently, on the oil storage temperature and substrate availability (GÓMEZ-COCA *et al.*, 2016) and on the influence of the washing process (ALCALÁ *et al.*, 2016).

Virgin olive oil producers try to reach the maximum yield during the extraction process while saving, at the same time, the nutritional, functional and organoleptic features that distinguish virgin olive oil from other oils. Several attempts have been done aiming to the improvement of the extraction yield, mostly on those cultivars, which give the so-called "difficult pastes" (DI GIOVACCHINO and MASCOLO, 1988). The most common strategies adopted regard: i) malaxation time and temperature increase (STEFANOUDAKI *et al.*, 2011); ii) use of technological coadjuvants able to break down the water-oil emulsions (CAPONIO *et al.*, 2016a; CAPONIO *et al.*, 2016b; SQUEO *et al.*, 2016).

As far as we know, no information are available about the evolution of the FAAE as a consequence of the use of processing aids, such as calcium carbonate, during the extraction process. Considering that the "extra" quality virgin olive oils have the highest price on the market and thus even the profit of the producers and farmers are linked to the compliance with the current legal limits, it is clear that such information could be very useful for both the operators as well as the lawmakers. Hence, the aim of this work was the assessment of the ethyl esters and methyl esters of fatty acids in extra virgin olive oils in regard to the use of calcium carbonate during the malaxation step.

2. MATERIALS AND METHODS

2.1. Sampling and experimental plans

All the experimental trials were carried out on olives (Coratina cultivar) milled within 24 hours after the harvest in industrial olive mills.
For studying the effect of the calcium carbonate, two olive lots, having 0.51 (lot A) and 1.40 (lot B) pigmentation index (*Pi*), calculated as reported in SQUEO *et al.* (2016), were divided in 14 homogeneous batches of about 300 kg. Two batches were processed without any treatment (controls) while the others (two batches per each treatment) were processed by using two different types of calcium carbonate (average particle size of 2.7 μm, Ca2, and 5.7 μm, Ca5, respectively) at three percentages of addition respect to the olives paste weight (1-2-4%). The full experimental plan was reported in Table 1.
Calcium carbonate was kindly furnished by Omya Spa (Milan, Italy). After being weighted, the coadjuvant was directly and gradually added into the malaxer at the begin of the malaxation stage without stopping the machine.

Table 1. Experimental plan for olive lots A and B.

Coadjuvant typology	Level of addition (%)	Trial name*
None	None	C
Calcipur®2	1	Ca2-1%
Calcipur®2	2	Ca2-2%
Calcipur®2	4	Ca2-4%
Calcipur®5	1	Ca5-1%
Calcipur®5	2	Ca5-2%
Calcipur®5	4	Ca5-4%

*Each trial was repeated twice.

2.2. Alkyl esters analysis

The analyses of the methyl and ethyl esters of fatty acids were carried out according to the official method (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2011). The gas chromatographic system was made up of a 7890B Agilent Technologies (Santa Clara, CA, USA) chromatograph equipped with a flame ionization detector (FID). The column used was a capillary fused silica DB-5HT (length 15 m, i.d. 0.32 mm, film thickness 0.10 μm). The operating conditions were as follows: oven temperature, 80 °C for 1 min and then increased at 20 °C min^{-1} to 140 °C, then increased at 5 °C min^{-1} to 335 °C and maintained for 20 min. The detector temperature was 350 °C. Helium was used as the carrier gas, with a flow through the column of 2 mL min^{-1} in splitless mode.

2.3. Statistical analysis

ANOVA and Tukey post-hoc test for multiple comparisons were carried out on the experimental data by means of Minitab 17 software (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSIONS

All the virgin olive oils obtained in the industrial trials were classified as extra virgin olive oils according to the EU Regulation 1348/2013 (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2013) (data not shown).
Fig. 1 reports the average amounts of alkyl esters in the samples under study. The contents found were in accordance with those reported in literature (BIEDERMANN *et al.*, 2008; MARIANI and BELLAN, 2008; Pérez-Camino *et al.*, 2008) and lower than law limits. Overall, a gradual decrease of FAAE than controls (C) was observed as the percentage of calcium carbonate increased. A good discrimination was observed in particular between the samples of the 4% addition trial and those without (C) or with the lowest additions (1%). Moreover, the FAEE content was generally higher than the amount of fatty acids methyl esters (FAME).

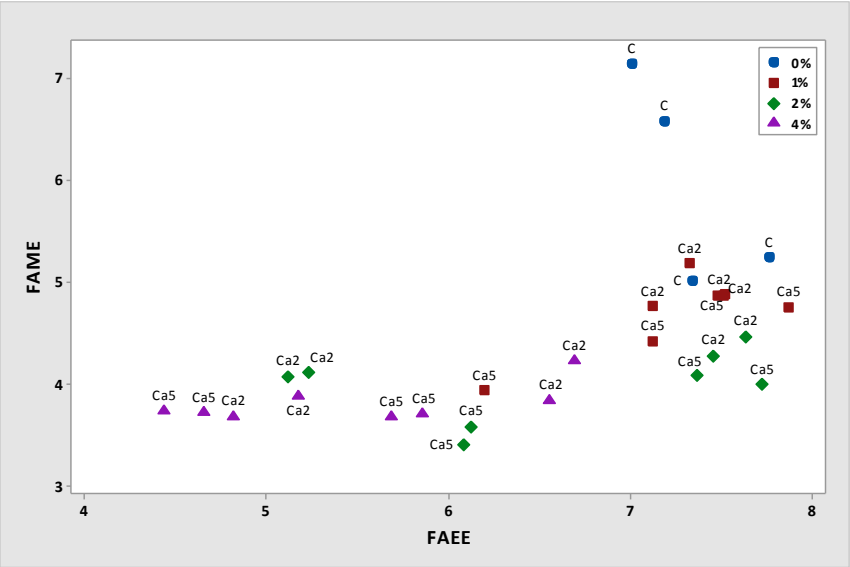


Figure 1. Fatty acids methyl esters (FAME) versus fatty acids ethyl esters (FAEE) (mg kg^{-1}) detected in the samples. Olive processed without calcium carbonate addition (C, control) and with the addition of 1%, 2%, and 4% of calcium carbonate. Ca2, Calcipur®2 with particle size of 2.7 μm; Ca5, Calcipur®5 with particle size of 5.7 μm (Squeo *et al.*).

The differences observed are clearly shown by Fig. 2, in which the differences of means, and the respective 95% confidence intervals obtained by the Tukey post-hoc test, are reported in regard to the variables *percentage of addition* and *type of coadjuvant*. Overall, the differences of means between the treatments and the control shown that the treated oils were poorer in FAAE (Fig. 2 A-D).
Considering the percentages of addition (Fig. 2 A-B), the differences between treatments and control were significant in all cases for the FAME, while only 4% of addition led to significantly lower amounts of FAEE compared to control. Among the treatments, significantly lower amounts of FAME were found using 2% and 4% of the coadjuvant respect to 1%. No significant differences have been highlighted between 2% and 4% addition (Fig. 2A). As regards the FAEE, a significant difference was reported for the 4%, as compared to 1% of addition (Fig. 2B).
The coadjuvant granulometry (Fig. 2 C-D) induced significant differences only for the FAME, with a more noticeable effect of the Ca5 than the Ca2 respect to the control. No

statistical difference was highlighted between the different types of calcium carbonate adopted for both the FAME and the FAEE. Overall, our findings underline that the use of calcium carbonate in the olive oil mill causes a reduction of the amounts of FAAE, that seems to be proportional to the amount of processing aid employed, whereas the type of coadjuvant did not show any significant effect.

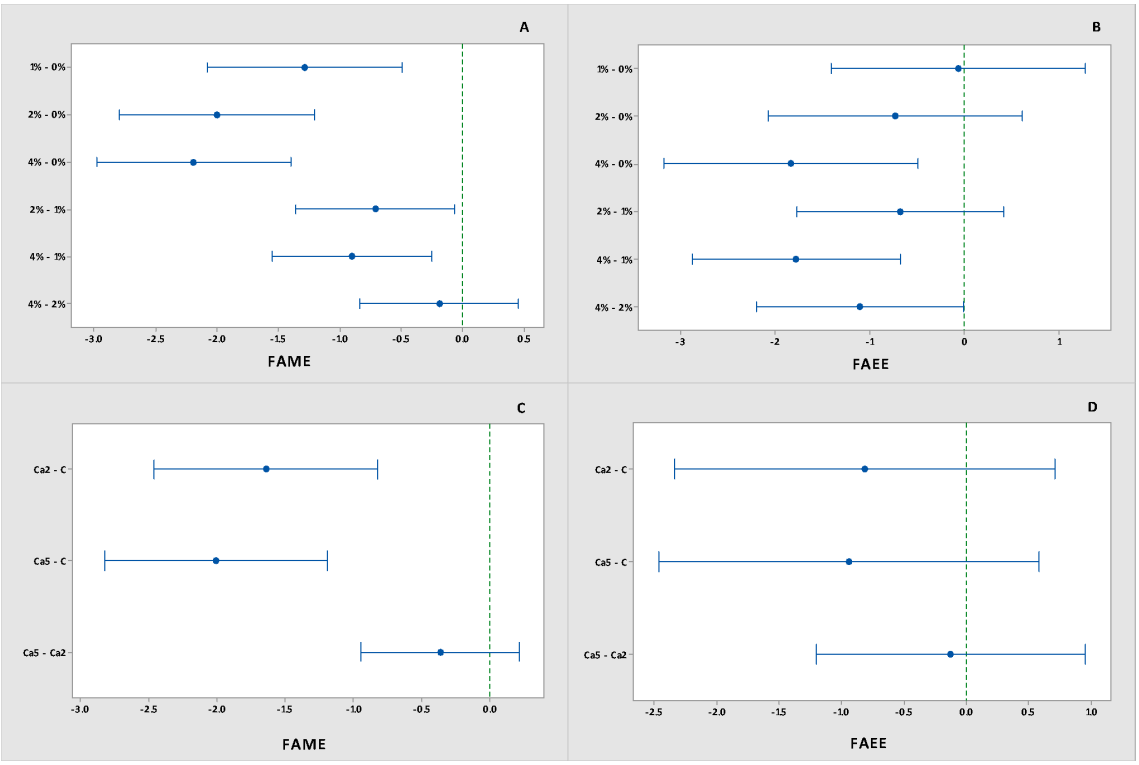


Figure 2. Tukey 95% Confidence Intervals for the differences of means for the calcium carbonate trials for both the olives lots. A) means differences in FAME as a function of the percentages of addition; B) means differences in FAEE as a function of the percentages of addition; C) means differences in FAME as a function of the type of coadjuvant; D) means differences in FAEE as a function of the type of coadjuvant (Squeo *et al.*).

It is known that the synthesis of the alkyl esters occurs in an acid environment and is catalysed by enzymes and temperature (PÉREZ-CAMINO *et al.*, 2003). In such conditions, methanol and ethanol react with fatty acids (mainly oleic acid) giving rise to the FAAE. The lowering in the content of these metabolites, observed in the treated oils, might be due to the interference on the enzymatic activity exerted by processing aids during the malaxation step or, more simply, by an absorption of the low weight alcohols, more evident for the methanol.

4. CONCLUSIONS

Our findings showed that the content of the fatty acids alkyl esters could be affected by the technological strategies adopted during olives processing into oil. In particular, the use of technological coadjuvants, aimed to increase the extraction yield, influences the amount of such compounds, bringing to a reduction. Anyway, this is more evident for the methyl esters, while a weaker influence has been observed on the ethyl ester amount. Further

studies should be carried out in order to confirm these preliminary results and understanding deeply the FAAE behaviour in the olive oil matrix. As regards the economic point of view, the use of such processing aid will be easily faced by the producers considering a cost increase of about 0.40 € kg^{-1} of oil in the face of an extraction efficiency increase of about 4%.

ACKNOWLEDGEMENTS

This research was supported by Regione Puglia, project Qu.Al.E.olio.

REFERENCES

Alcalá S., Ocaña M.T., Cárdenas J.R., Miquel M.A., Vilar J., Espínola F. and Moya M. 2016. Alkyl esters content and other quality parameters in oil mill: A response surface methodology study. Eur. J. Lipid Sci. Technol. 118:DOI: 10.1002/ejlt.201600026.

Biedermann M., Bongartz A., Mariani C. and Grob K. 2008. Fatty acid methyl and ethyl esters as well as wax esters for evaluating the quality of olive oils. Eur. Food Res. Technol. 228:65.

Caponio F., Squeo G., Difonzo G., Pasqualone A., Summo C. and Paradiso V.M. 2016a. Has the use of talc an effect on yield and extra virgin olive oil quality? J. Sci. Food Agric. 96:3292.

Caponio F., Squeo G., Summo C., Paradiso V.M. and Pasqualone A. 2016b. Talc effect on the volatiles of virgin olive oil during storage. Ital. J. Food Sci. 28:705.

Cert A. 2006. Meeting of chemists to study methods of analysis olive oils and olive pomace oils. International Olive Oil Council, Madrid, T20/Doc. No. 53-3.

Di Giovacchino L. and Mascolo A. 1988. Incidenza delle tecniche operative nell'estrazione dell'olio dalle olive con il sistema continuo. Nota II. Riv. Ital. Sostanze Grasse 65:283.

Gómez-Coca R.B., Fernandes G.D., Pérez-Camino M.C. and Moreda W. 2016. Fatty acid ethyl esters (FAEE) in extra virgin olive oil: a case study of a quality parameter. LWT-Food Sci. Technol. 66:378.

Gómez-Coca R.B., Moreda W. and Pérez-Camino M.C. 2012. Fatty acid alkyl esters presence in olive oil vs. organoleptic assessment. Food Chem. 135:1205.

Jabeur H., Zribi A., Abdelhedi R. and Bouaziz M. 2015. Effect of olive storage conditions on Chemlali olive oil quality and the effective role of fatty acids alkyl esters in checking olive oils authenticity. Food Chem. 169:289.

Koprivnjak O., Dminić I., Kosić U., Majetić V., Godena S. and Valenćić V. 2010. Dynamics of oil quality parameters changes related to olive fruit fly attack. Eur. J. Lipid Sci. Technol. 112:1033.

Mariani C. and Bellan G. 2008. Detection of low quality oils in extra virgin olive oils. Riv. Ital. Sostanze Grasse 85:3.

Official Journal of the European Communities. 2001. Council Regulation No. 1513/2001, N. L. 201 of July 26th, Publications Office of the European Union, Bruxelles.

Official Journal of the European Union. 2011. Commission Regulation No. 61/2011, N. L. 23 of January 1st, Publications Office of the European Union, Bruxelles.

Official Journal of the European Union. 2013. European Community Regulation No. 1348/2013, N. L. 338 of December 17th, Publications Office of the European Union, Bruxelles.

Pérez-Camino M.C., Cert A., Romero-Segura A., Cert-Trujillo R. and Moreda W. 2008. Alkyl esters of fatty acids a useful tool to detect soft deodorized olive oils. J. Agric. Food Chem. 56:6740.
Pérez-Camino M.C., Moreda W., Mateos R. and Cert A. 2003. Simultaneous determination of long-chain aliphatic aldehydes and waxes in olive oils. J. Chromatogr. A 983:283.

Ruiz-Méndez M.V. and Ramos-Hinojosa A.E. 2003. Fatty acid esters with short-chain alcohols in two-phase olive pomace oils. Eur. J. Lipid Sci. Technol. 105:346.

Salvador M.D., Aranda F. and Fregapane G. 2001. Influence of fruit ripening on Cornicabra virgin olive oil quality. A study of four successive crop seasons. Food Chem. 73:45.

Squeo G., Silletti R., Summo C., Paradiso V.M. Pasqualone A. and Caponio F. 2016. Influence of calcium carbonate on extraction yield and quality of extra virgin oil from olive (*Olea europaea* L. cv. Coratina). Food Chem. 209:65.

Stefanoudaki E., Koutsaftakis A. and Harwood J.L. 2011. Influence of malaxation conditions on characteristic qualities of olive oil. Food Chem. 127:1481.

Paper Received January 9, 2016 Accepted February 6, 2017

GUIDE FOR AUTHORS

Editor-in-Chief: **Paolo Fantozzi**
Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università di Perugia, Via S. Costanzo, I-06126 Perugia, Italy - Tel. +39 075 5857910 - Telefax +39 075 5857939-5857943 - e-mail: paolo.fantozzi@ijfs.eu

IJFS has a publication charge of € 350.00 each article

Publication Ethics and Publication Malpractice

Italian Journal of Food Science is committed to upholding the highest standards of publication ethics and takes all possible measures against any publication malpractices. All Authors submitting their works to Italian Journal of Food Science for publication as original articles attest that the submitted works represent their Authors' contributions and have not been copied or plagiarized in whole or in part from other works. The Authors acknowledge that they have disclosed all and any actual or potential conflicts of interest with their work or partial benefits associated with it. In the same manner, Italian Journal of Food Science is committed to objective and fair Editor(s) review of the submitted for publication works and to prevent any actual or potential conflict of interests between the editorial personnel and the reviewed material. Any departures from the above-defined rules should be reported directly to the Editor-in-Chief, who is unequivocally committed to providing swift resolutions to any of such a type of problems.

1. Manuscript Submission

Manuscripts must submitted as an electronic version to
http://www.chiriottieditori.it/ojs/index.php/IJFS/index
All the Authors must be registered with initals, surname and e-mail. The word processor used to generate the file should be indicated and the files should be saved in format "Text only"; **graphs, pictures and diagrams must be saved in JPG format**. Manuscripts must be typed single-space, using Times New Roman 12 pt as the advised font; employs italics, rather than underlining (except with URL addresses); **lines must be numbered**. A single file must contain the manuscript and all the tables and figures inserted at the appropriate points.

English is the official language. The Editor-in-Chief and/or Co-Editors reserve the right to make literary corrections and to make suggestions to improve brevity, but **the paper must be previously revised for English by the authors**. If English is not the mother tongue of authors, they **must seek** help from one of the following agencies (or other similar official agencies):
WWW.JOURNALEXPERTS.COM
WWW.SCIENCEDOCS.COM
WWW.INTERNATIONALSCIENCEEDITING.COM
WWW.WRITESCIENCERIGHT.COM

When the paper will be accepted, copy of the English Translator Certificate of the revised version must be uploaded.

2. Manuscript Preparation

(1) The paper should be divided under the following headings in this order:
Title. Informative of the content of the article (<50 characters + spaces).
Author(s). Initials and Surname, omit professional and official titles. The institute and address where the research was carried out and the current address of each author should be given on the title page.
Abstract. Clearly state the objective of the study, give a concise description of experiment(s), observations, results and conclusions. No references should be cited. **Do not exceed 100 words**.
Key words. Up to six words, in alphabetical order, which describe the document must be given to aid data retrieval and indexing.
Introduction. Review pertinent previous work and cite appropriate references. State the purpose of the investigation.
Materials and Methods. Indicate apparatus, instruments, reagents, etc., giving sufficient detail to allow the work to be repeated.
Results and Conclusions. Results and Conclusions may be presented together or separately. Concisely present results using tables and figures to help justify conclusions (do not present the same information in both forms). Use statistical analysis when appropriate. Unsupported hypotheses should be avoided. Conclusions should point out the significance of the findings and, if possible, relate the new findings to some problem in Food Science and Technology.
Acknowledgments. Acknowledgments of assistance are appropriate provided they are not related to analyses or other services performed for a fee. Financial support, thanks for assistance, article number or thesis fulfilment may be included.
Units. A list of units particular to the paper may be included.
References. References in the Reference list should be arranged alphabetically (initials of first name, only), and, for the same author, should be arranged consecutively by year, typed double-spaced. Each individual reference should begin flush left (no indentation). Refer to attached examples taken from "Style Guide for Research Papers" by the Institute of Food Technologists (Chicago - I1linois - USA). Literature citations in the text should be referred to by Surname and year in parentheses. If there are more than two authors, give the surname of the first author and add et al. and the year in parentheses. Examples: (SMITH, 2007), (SMITH and JONES, 2008) (SMITH et al., 2008).

(2) Tables should be as few and as simple as possible and include only essential data. Each table must be saved within the text at the appropriate points, and have an Arabic number, e.g. Table 4 NOT Tab. 4. Legends must be self-explanatory. Use lower-case letters for footnotes in tables and explain below the table in the order in which they appear in the table.

(3) Figures must be prepared and saved in **JPEG format,** and inserted in the manuscript at the appropriate points. They should be prepared so that on 50% reduction, lines, figures and symbols will be clearly legible and not overcrowded. All figures must be given Arabic numbers, e.g. Fig. 3. Legends for figures must be self-explanatory.

(4) Standard Usage, Abbreviations and Units. The Concise Oxford and Webster's English Dictionaries are the references for spelling and hyphenation. Statistics and measurements should always be given in figures, e.g. 10 min, except when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelled out unless it is 100 or greater. Abbreviations should be used sparingly, only when long or unwieldy names occur frequently, and never in the title; they should be given at the first mention of the name. International Standard abbreviations should generally be used except where they conflict with current practice or are confusing. For example, 3 mm rather than $3x10^{-3}$ m. Abbreviations should be defined the first time they are used in the text and they should be used consistently thereafter. Temperatures should be expressed in the Celsius (centigrade) scale. Chemical formulae and solutions must specify the form used, e.g. anhydrous or hydrated, and the concentration must be in c1early defined units.
Common species names should be followed by the Latin binomial (italics) at the first mention. For subsequent use, the generic name should be contracted to a single letter if it is unambiguous.

3. Editorial and Review Policy

Scientific contributions in one of the following forms may be submitted:
Reviews –They can be submitted directly to the Editor-in-Chief or articles can be requested directly by the Editor-in-Chief.
Short Communications, Surveys and Opinions – They do not need to have the formal organization of a research paper; they will receive priority in publication; maximum of five pages allowed.
Papers – The paper must follow the guidelines as specified under the section Manuscript Preparation.

Reviews, Papers, Short Communications and Surveys will be **subjected to critical review by referees.**
(1) Manuscripts will be processed in the order received. The Editor-in-Chief will select papers to enter into the reviewing system based on originality and innovation. A letter will be sent to the authors acknowledging receipt of the manuscript along with a Declaration form stating that it has NOT been previously published, accepted or submitted for publication elsewhere and agreeing to the page charges upon acceptance of the paper. On receipt of the signed Declaration form, the Editor-in-Chief will send the manuscript to a Co-Editor and/or referees for evaluation.
(2) Referees may not be from the same institution as the author. Referees should make their comments and questions in detail and return the paper to the Editor-in-Chief and/or Co-Editor as soon as possible, usually within three weeks. The identity and report of the referees are made know to the Editor-in-Chief, but only the anonymous referee report is sent to the author(s). If all referees recommend acceptance or rejection, the decision stands. If the opinions of the referees tie, the Editor-in-Chief and/or Co-Editors have the freedom to decide upon acceptance or rejection of the paper.
(3) The results of the refereeing process, accompanied by a letter from the Editor-in-Chief or the Co-Editor, will be sent to the author(s). **Papers needing revision must be returned to the Co-Editor within four weeks, otherwise the paper will be considered as withdrawn**. A letter announcing acceptance of the manuscript will be sent to the author(s) upon acceptance by the referees.
(4) The authors **will receive galley proofs** of the manuscript along **with the invoice** for the article charge which **must be paid in order to allow for publication**. The

proofs will be sent to the corresponding author as a PDF file by e-mail, only.
Once the page charge is paid, the DOI will be sent to the Author.

REFERENCE EXAMPLES

EXAMPLES of use in a Reference list are given below. The bold-faced parenthetical type of citation above the example is indicated ONLY for information and is NOT to be included in the reference list.

(Anonymous)
Anonymous. 1982. Tomato product invention merits CTRI Award. Food Technol. 36(9): 23.

(Book)
AOAC. 1980. "Official Methods of Analysis" Association of Official Analytical Chemists, Washington, DC.
Weast, R.C. (Ed.). 1981 "Handbook of Chemistry" 62nd ed. The Chemical Rubber Co. Cleveland, OH.

(Bulletin, circular)
Willets C.O. and Hill, C.H. 1976. Maple syrup producers manual Agric. Handbook No. 134, U.S. Dept. of Agriculture, Washington, DC.

(Chapter of book)
Hood L.F. 1982. Current concepts of starch structure. Ch. 13. In "Food Carbohydrates". D.R. Lineback and G.E. Inglett (Ed.), p. 217. AVI Publishing Co., Westport, CT.

(Journal)
Cardello A.V. and Maller O. 1982. Acceptability of water, selected beverages and foods as a function of serving temperature. J. Food Sci. 47: 1549.
IFT Sensory Evaluation Div. 1981a. Sensory evaluation guide for testing food and beverage products. Food Technol. 35 (11): 50.
IFT Sensory Evaluation Div. 1981b. Guidelines for the prepaation and review of papers reporting sensory evaluation data. Food Technol. 35(4): 16.

(Non-English reference)
Minguez-Mosquera M.I., Franquelo Camacho A, and Fernandez Diez M.J. 1981. Pastas de pimiento. Normalizacion de la medida del color. Grasas y Aceites 33 (1): 1.

(Paper accepted)
Bhowmik S.R. and Hayakawa, K. 1983. Influence of selected thermal processing conditions on steam consumption and on mass average sterilizing values. J. Food Sci. In press.

(Paper presented)
Takeguchi C.A. 1982. Regulatory aspects of food irradiation. Paper No. 8, presented at 42nd Annual Meeting of Inst. of Food Technologists, Las Vegas, NV, June 22-25.

(Patent)
Nezbed R.I. 1974. Amorphous beta lactose for tableting U.S. patent 3,802,911, April 9.

(Secondary source)
Sakata R., Ohso M. and Nagata Y. 1981. Effect of porcine muscle conditions on the color of cooked cured meat. Agric. & Biol. Chem. 45 (9): 2077. (In Food Sci. Technol. Abstr. (1982) 14 (5): 5S877).

(Thesis)
Gejl-Hansen F. 1977. Microstructure and stability of Freeze dried solute containing oil-in-water emulsions Sc. D. Thesis, Massachusetts Inst. of Technology, Cambridge.

(Unpublished data/letter)
Peleg M. 1982. Unpublished data. Dept. of Food Engineering., Univ. of Massachusetts, Amherst.
Bills D.D. 1982. Private communication. USDA-ARS. Eastern Regional Research Center, Philadelphia, PA.

CONTRIBUTORS

Gratitude is expressed to the following entities for contributing to the realization of the Journal by being supporting subscribers for 2017.

ASSOCIATIONS and COMPANIES

Associazione Italiana di Tecnologia Alimentare (A.I.T.A.)
Parma
Fax +39-0521-230507
www.aita-nazionale.it

Società Italiana di Scienze e Tecnologie Alimentari (S.I.S.T.Al)
Perugia
Fax +39-075-5857939
www.sistal.org

RESEARCH INSTITUTES

Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali (DI.VA.P.R.A.), Sezione Microbiologia ed Industrie Agrarie, Università di Torino
Grugliasco
Fax +39-011-6708549

ITALIAN JOURNAL OF FOOD SCIENCE
Rivista Italiana di Scienza degli Alimenti
DIRETTORE RESPONSABILE: Alberto Chiriotti
AUTORIZZAZIONE: n. 3/89 in data 31/1/1989
del Tribunale di Perugia

ISSN 1120-1770

CHIRIOTTI EDITORI srl - 10064 Pinerolo - Italy

publishes the technical magazines:

VOLUME XXIX IJFS No. 2, 2017

CONTENTS