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PAPER

EFFECT OF ROSEMARY POWDER ON THE QUALITY OF DRY EWE SAUSAGES

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ABSTRACT

The aim of this study was to evaluate the effect of rosemary powder on the quality of dry ewe sausages. Color parameters (L, a* and b*), pH and microbiological profile were evaluated over the 6-day drying period. Sausages with 4 % rosemary powder added showed the lowest values of total viable counts and total coliforms. pH values of dry ewe sausages with rosemary powder decreased significantly during drying. Redness (a*) decreased significantly during the drying of control dry sausages against a stability in sausages added with rosemary powder. Finally, incorporating rosemary significantly affected dry sausages acceptability.

Keywords: dry ewe sausage, microbiological quality, physicochemical quality, rosemary powder, sensory quality

1. INTRODUCTION

Many countries have meat products whose tradition people wish to preserve as part of their history and culture. Dry ewe sausages are one of the oldest known traditional meat products in Tunisia. In the past, they were often homemade for religious celebrations. Nowadays, most dry ewe sausages are produced all year round both in butcher's shops and sausage manufacturing companies. The ewe meat and fat are finely ground and seasoned with salt and other spices. Chemical additives are not used. The mixture is then stuffed into natural casings. Sausages are dried naturally at ambient temperature until reaching the desired dehydration, and they are usually consumed without cooking. Despite, their short shelf life (not exceeding 2 weeks at 4°C), traditional dry cured ewe sausages are considered safe due to several factors such as reduced water activity and the addition of salt and spices. Sensorial attributes such as color and texture are linked to drying conditions (temperature and relative humidity).

Rosemary (*Rosmarinus officinalis L.*) is one of the most well-known Mediterranean *Lamiaceae*. Like other aromatic herbs and spices used in Mediterranean cuisine, it is used not only to improve or modify the flavor of foods, but also for its antioxidant and antimicrobial effects (LIU *et al*., 2009). The antioxidant activity of rosemary has been related with the presence of some phenolic diterpenes such as carnosic acid and carnosol (PIZZOCARO *et al*., 1994; GEORGANTELIS *et al*., 2007). In addition, several authors have reported that some compounds present in rosemary extract possess antimicrobial effects (DEL CAMPO *et al*., 2000; MCCARTHY *et al*., 2001; BALENTINE *et al*., 2006; LUND *et al*., 2007).

The widespread availability of the rosemary plant in Tunisia makes it easy to use as a preservative in meat products. Many studies have documented the effects of rosemary essential oils on the quality of meat products (STEPHANIE *et al*., 2002; MIELNIK *et al*., 2003; AHN *et al*., 2007; GARCÍA-DIEZ *et al*., 2016); however, few have focused on the effects of rosemary powder.

The aim of this work was to study the effect of rosemary powder on physicochemical, microbiological and sensorial characteristics of traditional dry ewe sausage.

2. MATERIALS AND METHODS

2.1. Plant material

The leaves of *Rosmarinus officinalis* L. were collected from Ouesslatia, (semi-arid bioclimatic zone of Tunisia) in June. Specimens of the plant were submitted to the herbarium division of the National Institute of Research on Rural Engineering, Water and Forests where identification was confirmed in the Laboratory of Forest Ecology. Rosemary leaves were dried in an oven (Ecocell Drying Oven, MMM Med center, Germany) at 60°C, ground with a grinder (Moulinex, France) and then stored in a moisture-proof container until use.

2.2. Meat sampling and sausage preparation

The meat used was taken from adult fat-tail Barbarine ewes. Ewes were fed oat hay and concentrate until slaughter at 48 kg. The carcasses were cut into leg, lumbar region, flank, thoracic region, neck, and shoulder following the procedure of COLOMER-ROCHER *et al*. (1972). The shoulders were dissected into muscle, fat and bone; the muscles were conserved at -20°C until sausage preparation.

The sausage mixture was prepared according to the following formulation: 80 % muscle, 20 % tail fat, 3 % salt and 1 % paprika (Kamy, Tunisia). Muscle and tail fat were minced and mixed in a rotating bowl meat cutter (Rowenta, Universo, Germany). The sausage mixture was divided into 3 batches. To the first and the second batches, 2 and 4 % (levels are results of sensorial analysis) of rosemary powder were added, respectively, corresponding to the samples labeled RP2 and RP4. The third batch, without rosemary powder, acted as a control (C).
Natural salted casings were purchased from a local market in Tunis and soaked in water prior to use. Subsequently, the sausage mixture was manually stuffed into casings (20 cm of length and about 4 cm of diameter) at approximately 25 g each and then dried at ambient temperature for 6 days. Relative humidity and temperature averaged 70 % and 18°C respectively. For sampling, five sausages were taken from each batch at days 0, 3 and 6 of drying for pH, color determination and microbiological analysis. Chemical composition and sensorial analysis were done after 6 days of drying.

2.3. Sausage color and pH analysis

The pH was measured on sausages with a penetrating electrode connected to a portable pH-meter (HANNA instruments, Romania) after calibration with two buffers (7.00 and 4.00).
A Minolta CM-2006 d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) was used to measure color directly on the surface. Color coordinates were calculated in the CIELAB space (CIE, 1986). The lightness (L*), redness (a*) and yellowness (b*) parameters were directly recorded (Hunt *et al*., 1991). The hue angle (H*) and chroma (C*) indices were calculated as $H^* = \tan^{-1} (b^*/a^*) \times 57.29$, expressed in degrees and $C^* = (a^{*2}, b^{*2})^{1/2}$. H* is the attribute of a color perception denoted by blue, green, yellow, red, purple, etc. C* is related to the quantity of pigments and high values represent a more vivid color and denote lack of greyness (MILTENBURG *et al*., 1992).

2.4. Microbiological analysis

For the microbiological analysis, 10 g of meat were collected aseptically from the center of each sausage, and diluted with 90 ml of sterile peptone water (Accumix, Belgium) using a Stomacher 80 Biomaster. Serial 10-fold dilutions were prepared in sterile peptone water. Appropriate dilution samples (1 or 0.1 ml) were poured or spread in duplicate on different growth media. Total viable counts were determined on Plat Count Agar (PCA) (Accumix, Belgium) after 48 h of incubation at 30°C; yeasts and molds on Sabouraud Agar (Accumix, Belgium) after 5 days of incubation at 25°C and coliforms on Desoxycholate Agar (Accumix, Belgium) after 24 h at 37°C for total coliforms and 44 °C for fecal coliforms (Guiraud, 1998).

2.5. Chemical composition

Sausage samples were dried by lyophilization (CHRIST, BETA 1-8 LD plus); samples of dry matter were ground (1 mm screen) and used for subsequent analyses. Total protein was determined using the Kjeldahl method (ID 942.01) and ash content was determined by ashing at 600°C for 8 h (ID 942.05) according to AOAC (1990). Water activity (a_w) of the sausage samples was determined at 25°C using a Thermoconstanter A_w Sprint Novasina TH500 (Switzerland).

2.6. Sensory analysis

After 6 days of drying, sausages were cooled at room temperature, then cut into 1x1 cm pieces. Each piece was then coded and served in random order to a sensory panel, which consisted of 13 panelists with experience in sensorial evaluation. Each parameter in the sensorial analysis was evaluated on a scale of 1 to 9 (1- low intensity; 9- high intensity). Color, odor, flavor, tenderness and overall acceptance were scored; then, mean values were calculated for each parameter.

2.7. Statistical analyses

Each parameter was measured three times and then averaged. All data were analyzed using a General Linear Model ANOVA with treatment (rosemary powder incorporation) and time as factors. Duncan's multiple range test was used to determine any significant difference between mean values, and evaluations were based on a significance level of $P < 0.05$. All statistical analyses were done using SAS (2002) Version 8.2.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

Moisture, protein and ash contents of the ewe sausages after drying are presented in Table 1. Results showed that incorporating rosemary powder significantly increased ($P < 0.05$) moisture in the traditional ewe sausages. Our results are in agreement with of those of JUNG *et al*. (2015). Indeed, rosemary powder appeared to improve water retention due to its antioxidant activity (ESTEVEZ and CAVA, 2006). The antioxidant activity of rosemary powder for protein degradation moderated the loss of sulfhydryl groups and the generation of carbonyl compounds and then maintained water holding ability (JUNG *et al*., 2015). Other studies have reported that moisture of dried sausages could be affected by both processing method and processing time (DALMIS and SOYER, 2008). The addition of rosemary powder did not affect protein and ash content. The protein content of dried ewe sausages was the same as that found by KOVAČEVIĆ *et al*. (2010) who showed that protein content of traditional dry sausages ranged between 26 and 53%. Water activity (Table 1), an important factor for microorganism growth, was not affected by the addition of rosemary powder, which contrasted with the results found by JUNG *et al*. (2015).

Table 1. Effect of rosemary powder incorporation on chemical composition of dry ewe sausages.

Chemical composition	C	RP2	RP4	S.E.M	P-Value
Moisture (%)	15.7	18.5	20.7	1.22	0.001
Protein (% DM)	39.7	40.4	41.2	5.48	0.977
Ash (% DM)	7.8	7.7	8.3	0.47	0.590
Aw	0.75	0.72	0.78	0.01	0.450

C: control sausage; RP2: sausage added with 2 % of rosemary powder; RP4: sausage added with 4 % of rosemary powder; S.E.M: standard error of means.

3.2. Microbiological analysis and pH

Results of the microbiological analysis of the sausages during drying are presented in Table 2. Counts of total viable and coliform increased significantly during drying and were significantly affected ($P < 0.05$) by the addition of rosemary powder. At the end of the drying period, sausages with 4 % rosemary powder added showed the lowest value of total viable counts (4.17 log cfu/g) and total coliforms (2.18 log cfu/g). Since there is no significant difference in water activity values between samples of dry ewe sausages, this result confirmed the antimicrobial effect of rosemary, widely seen when added directly to meat and meat products (AHN *et al*., 2007; ANGIONI *et al*., 2004; GEORGANTELIS *et al*., 2007; JUNG *et al*., 2015). The antimicrobial properties of rosemary, especially on Gram positive bacteria are related to some non-polar components such as phenolic diterpenes. Furthermore, phenolic diterpenes could inhibit Gram negative bacteria when in combination with factors which can disturb cell membrane permeability and/or integrity such as pH values and NaCl concentrations (Liu *et al*., 2009).
Yeast, mold and fecal coliform counts were not affected either by the drying period or by the addition of rosemary powder.

Table 2. Populations of microbial groups (cfu/g) during the drying of traditional ewe sausages.

	Batch	Days of drying		
		0	3	6
Total Viable Counts	C	4.39 ± 0.352^{aX}	5.35 ± 0.387^{aX}	6.07 ± 0.889^{bX}
	RP2	3.98 ± 0.18^{aY}	4.78 ± 0.94^{bY}	5.38 ± 0.98^{cY}
	RP4	3.77 ± 0.27^{aY}	3.74 ± 0.47^{aY}	4.17 ± 0.95^{bY}
Molds & yeasts	C	3.38 ± 0.74^{aX}	4.14 ± 0.89^{aX}	5.27 ± 0.72^{bX}
	RP2	3.58 ± 0.83^{aX}	5.37 ± 0.74^{bY}	5.36 ± 0.98^{bX}
	RP4	3.26 ± 0.46^{aX}	3.51 ± 0.85^{aX}	5.22 ± 0.65^{bX}
Total coliforms	C	3.81 ± 0.32^{aX}	4.03 ± 0.24^{abX}	4.31 ± 0.85^{bX}
	RP2	4.29 ± 0.46^{aY}	3.23 ± 0.63^{bY}	3.11 ± 0.51^{bY}
	RP4	4.16 ± 0.59^{aY}	2.43 ± 0.20^{aZ}	2.18 ± 0.76^{bZ}
Fecal coliforms	C	2.61 ± 0.68^{aX}	2.37 ± 0.99^{aX}	2.44 ± 0.51^{aX}
	RP2	2.81 ± 0.72^{aX}	2.74 ± 0.62^{aX}	2.95 ± 0.30^{aY}
	RP4	2.38 ± 0.23^{aX}	2.12 ± 0.37^{aX}	2 ± 0.56^{aX}

C: control sausage; RP2: sausage added with 2 % of rosemary powder; RP4: sausage added with 4 % of rosemary powder; values are the mean of three replicates; a, b et c: means in the same line for the same treatment with a different letter differ significantly ($P<0.05$); X, Y et Z: means in the same column for the same day with a different letter differ significantly ($P<0.05$).

The potential of hydrogen (pH) is usually ranked among the technological characteristics because it greatly influences meat processing and conservation. Table 3 shows that incorporating rosemary significantly affected pH values every day of drying except the first. For the control samples, the pH remained stable between Day 0 and Day 3 of drying, then significantly increased to a value of 5.83 on Day 6 ($P < 0.05$). This rise can be attributed to the increase of total viable counts and total coliforms, which cause protein and amino acid degradation resulting in ammonia formation and consequently an increase in pH (GEORGANTELIS *et al*., 2007). For RP2 and RP4 sausages, pH values significantly decreased during the drying period ($P < 0.05$). These lower values are probably due to

sausage acidification, although no sugar was added to the mixture. Rosemary is an herb rich in carbohydrates (Deef, 2007) and during the drying process, these sugars are used by bacteria to produce lactic acids responsible for the drop in pH. This was not the case in the control sausage because no rosemary powder was added.

3.3. Sausage color

Table 3 shows that L* values, expressing brilliance in color, decreased significantly during the drying period ($P<0.05$). KOVAČEVIĆ *et al*. (2010) reported that this loss of clarity could be attributed to drying time. This decrease in L* values was in fact due to water loss (SANABRIA *et al*., 2004). The results indicated that adding rosemary did not have a significant effect on the lightness of dry sausages.

Table 3. Effect of rosemary powder incorporation on the pH and color parameters of dry ewe sausages.

Parameters	Batch	Days of drying		
		0	3	6
pH	C	5.59 ± 0.13^{aX}	5.62 ± 0.06^{abX}	5.83 ± 0.07^{bX}
	RP2	5.58 ± 0.01^{aX}	5.47 ± 0.12^{bXY}	5.41 ± 0.1^{abY}
	RP4	5.55 ± 0.06^{aX}	5.48 ± 0.16^{abY}	5.44 ± 0.07^{bY}
L	C	47.93 ± 4.82^{aX}	38.36 ± 5.04^{bX}	37.68 ± 5.02^{bX}
	RP2	46.29 ± 3.65^{aX}	38.37 ± 5.6^{bX}	38.11 ± 4072^{bX}
	RP4	43.51 ± 3.66^{aX}	36.36 ± 3.83^{bX}	36.12 ± 4041^{bX}
a*	C	17.98 ± 3.22^{aX}	15.21 ± 2.84^{abX}	12.08 ± 1.94^{bX}
	RP2	7.19 ± 1.74^{aY}	7.37 ± 1.4^{aY}	7.03 ± 1.37^{aY}
	RP4	5.83 ± 1.49^{aY}	3.51 ± 0.22^{bY}	3.82 ± 0.31^{bZ}
b*	C	27.66 ± 3.53^{aX}	25.76 ± 2.71^{abX}	21.15 ± 2.78^{bX}
	RP2	28.63 ± 3.19^{aX}	23.51 ± 2.68^{bX}	22.82 ± 3.39^{bX}
	RP4	23.43 ± 3.95^{aY}	22.11 ± 3.18^{bY}	19.12 ± 1.78^{aX}
C	C	33.06 ± 4.04^{aX}	30.14 ± 4.04^{abX}	24.71 ± 3.58^{bX}
	RP2	29.59 ± 2.63^{aX}	24.71 ± 3.06^{bY}	23.92 ± 3.13^{bX}
	RP4	24.27 ± 2.6^{aY}	19.46 ± 3.49^{bZ}	22.44 ± 1.75^{aX}
H	C	56.96 ± 4.15^{aY}	60.21 ± 4.5^{aZ}	59.81 ± 5.34^{aZ}
	RP2	75.80 ± 3.71^{aX}	72.79 ± 4.54^{aY}	72.57 ± 3.71^{aY}
	RP4	75.87 ± 3.48^{aX}	79.71 ± 2.65^{aX}	80.12 ± 1.18^{aX}

C: control sausage; RP2: sausage added with 2 % of rosemary powder ; RP4:sausage added with 4 % of rosemary powder; values are the mean of three replicates; a, b, c: means in the same line for the same treatment with a different letter differ significantly ($P<0.05$); X, Y, Z: means in the same column for the same day with a different letter differ significantly ($P<0.05$).

Redness (a*) decreased significantly during the drying of control sausages, probably due to the oxidation of myoglobin to metmyoglobin (BALENTINE *et al*., 2006). Sausages with 2 % rosemary powder (RP2) showed no change in redness over time ($P > 0.05$), versus a decrease in sausages RP4 between Day 0 and Day 3 followed by stability until Day 6 of the drying process. This stability may be attributed to the phenolic components of rosemary, which exert an antioxidant effect inhibiting the oxidation of myoglobin and thus maintaining the red color of samples (BALENTINE *et al*., 2006; SMETI *et al*., 2013).

LIU *et al*. (2009) found that adding rosemary powder to fresh chicken sausage increased a* and decreased L values. Similarly, GEORGANTELIS *et al*. (2007) explained that many factors such as differences in the oxidation pattern of myoglobin under conditions of reduced enzymatic activity, storage temperatures, packaging methods, muscle type and light intensity and differences in the meat spices studied might contribute to the variations in rosemary color retention efficiency between different studies.
Results showed that values of b* decreased significantly for all sausage samples during drying ($P < 0.05$), but values stayed relatively high (>19). KOVAČEVIĆ *et al*. (2010) reported that the higher b* values in the sausages are probably related to the presence of yellow carotenoids (ß-carotene and cryptoxanthin) from paprika. With their combined polyene chain, which acts as a chromophore, carotenoids are often responsible for the bright colors of certain fruits and vegetables. This chain is also responsible for the instability of the carotenoids against oxidation, light and heat, which may in turn. This instability of the carotenoids may be the cause of a decrease yellowness index during drying (CHANFORAN, 2010). On the other hand, rosemary powder did not affect the b* values of sausages.
On Day 0, not all types of sausages exhibited the same degree of saturation; the control samples had the highest Chroma when compared to samples RP2 and RP4. Thereafter, the control samples saturation level dropped throughout the drying period. This continuous decline was not observed in sausages with rosemary added, thus demonstrating the effectiveness of rosemary by-products against myoglobin oxidation (CAMO *et al*., 2008).

3.4. Sensory analysis

Color is one of the most important points in sensory evaluation, helping the consumer accept or reject particular foods. Figure 1 showed that rosemary powder significantly affected sausage color ($P < 0.001$). Sausages with rosemary powder were darker, which could increase consumer sensory preferences. Our results differed from those of LIU *et al*. (2009) who found that the addition of rosemary decreased color preferences of fresh chicken sausages during refrigerated storage.

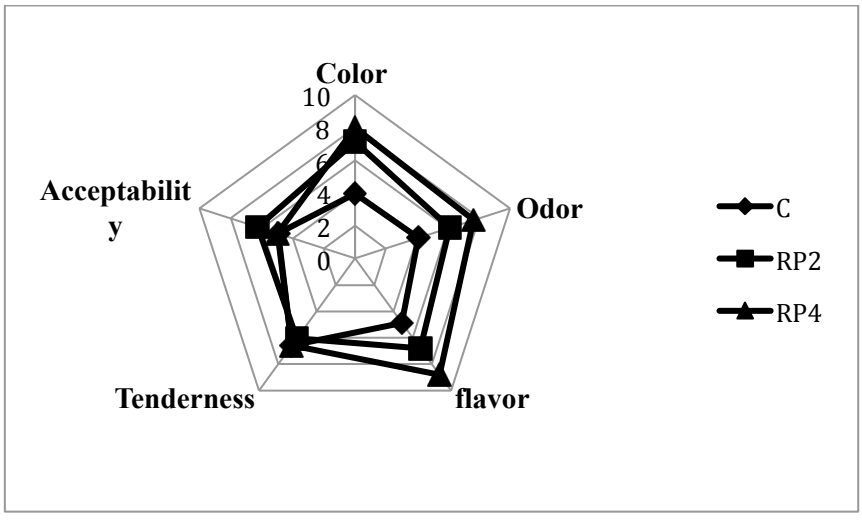

Figure 1. Sensory evaluation of dry ewe sausages.
C: control sausage ; RP2: sausage added with 2 % of rosemary powder ; RP4: sausage added with 4 % of rosemary powder.

Figure 1 shows that incorporating rosemary significantly affected the odor of the sausages ($P < 0.05$). In fact, aromatic substances allowed tasters to distinguish between samples. Similarly, it has been documented that certain compounds found in rosemary (verbenone, borneol, camphor) may give food a specific odor, even at low concentrations (CARRILLO *et al*., 2006). Likewise, incorporating rosemary significantly affected sausage flavor ($P < 0.05$). However, sausage tenderness was similar between groups ($P > 0.05$). In addition, rosemary significantly affected the acceptability of dry sausages ($P < 0.05$) in favor to RP2 samples, which were the most appreciated by panelists. These results agreed with previous investigations showing a beneficial effect of rosemary by-products on the sensory quality of ewe meat products (DJENANE *et al*., 2002; DJENANE *et al*., 2003; BALENTINE *et al*., 2006).

4. CONCLUSIONS

The effect of rosemary powder on the microbiological, chemical and sensory qualities of dry ewe sausages was investigated. The results obtained showed that rosemary powder added at 2 and 4 % improved microbiological quality by decreasing total viable and total coliform counts. Similarly, moisture and pH were affected by adding rosemary powder. Therefore, sausages added with 2 % of rosemary powder were the most appreciated by the panelists. This level of inclusion could be of interest for ewes' sausage preparation.

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PAPER

MYOSIN HEAVY CHAIN ISOFORMS, FATTY ACID COMPOSITION, SENSORY EVALUATION AND QUALITY OF *CINTA SENESE* PIG MEAT

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ABSTRACT

The aims of this study were to examine the effects of myosin heavy chain (MHC) isoforms on Cinta Senese meat and sensory quality. The research was carried out on 65 pigs and muscle samples characteristics such as MHC isoform, meat quality, fatty acid composition, and sensory were evaluated. The results demonstrated that MHC slow isoform content was significantly correlated with pH_{24h} ($r=0.25$, $P<0.05$) and drip loss ($r=-0.31$, $P<0.005$), whereas the content of MHC isoforms was only weakly correlated with fatty acids. Sensory evaluation was done by a trained panel test and the results shown that the MHC fast/slow ratio was correlated with the juiciness ($r=-0.32$, $P<0.005$), off-flavor ($r=0.33$, $P<0.01$), and tenderness attributes ($r=-0.42$ to -0.46). We therefore conclude that the content of MHC isoforms can be one of the most important factors for examination of overall quality of Cinta Senese pigs.

Keywords: fatty acid, fiber, myosin, quality, tenderness

1. INTRODUCTION

To date, studies have demonstrated that muscle fibre composition plays an important role in meat quality traits (DAI, 2009). Skeletal muscle is composed of various types of fibers. Muscle fiber types have different biochemical characteristics, including oxidative and glycolytic capacities, contraction speed, fiber size, myoglobin, and glycogen content (SCHIAFFINO and REGGIANI, 1996). Muscle fiber type I has slow-twitch, oxidative metabolic characteristics, and a low glycogen content. Type IIA is a fast oxidative-glycolytic fiber. On the other hand, type IIB has fast-twitch, glycolytic metabolic characteristics, and high glycogen content (VELOTTO, 2012). Another study has indicated that during postmortem period, some glycolysis enzymes might be the candidate predictors for meat discoloration (Wu *et al*. 2015). Therefore, during the postmortem period, muscle fiber type composition may impress metabolite content (FERNANDEZ, 1995). The composition of type IIB fiber has a positive correlation with meat lightness and drip loss (RYU and KIM, 2006), whereas type IIB fiber has a negative correlation with juiciness and flavor (TAYLOR, 2004). There is a clear relationship between meat quality to consumer satisfaction and flavor, texture, juiciness, tenderness and meat palatability (BEHRENDS, 2005; CALKINS and HODGEN, 2007). Moisture content in fresh and cooked meat influence on the Juiciness of meat and also intramuscular fat (IMF) content contributes to the perceived juiciness (FORTIN, 2005). Glycolytic rate during the postmortem period is related to myosin heavy chain (MHC) isoforms content, therefore MHC isoforms content can influence ultimate meat quality traits (GIL, 2003). Our objective was to investigate the effects of MHC isoforms to postmortem meat quality traits, fatty acid composition, and sensory evaluation in *Cinta Senese* pigs. The *Cinta Senese* pig has existed for much longer than any of the other white breeds in Northern Europe: the Large White, the Yorkshire and the Landrace. The renewed interest in the breeding of the *Cinta Senese* is quite recent. The battle to safeguard the breed is in progress and is succeeding. Following the line of these perspectives, we aimed the examination of the effects of MHC isoforms on Cinta Senese meat and sensory quality.

2. MATERIALS AND METHODS

2.1. Preparation of muscle samples

65 *Cinta Senese* pigs, one year old, (25 gilts and 40 castrated male pigs) were investigated. In order to follow the recommendation of the National Research Council (NRC, 1998), we fed the pigs with a commercial diet. Animals were treated according to the guidelines of the European Community on the treatment of experimental animals (Reg. EC 1/2005). The slaughterhouse had EEC mark with reference to rules 852/853/854/2004; 2076/2005. Pigs were anesthetized by an intramuscular injection of ketamine and sumianxin (rules120/2008/CEE). Samples of longissimus dorsi muscles were recovered from the carcasses at the 7-8th thoracic vertebra, 48 h after slaughter and were prepared for analysis. The slaughtering process was based on a traditional mechanized slaughter line. This method has the processes of vat scalding (62°C), dehairing, singeing/flaming, and polishing. The muscle samples were cut into 0.4×0.4×1.5 cm pieces, frozen in liquid nitrogen and stored at -80°C until analysis for MHC isoforms. The pork loins (the 10-13th thoracic vertebrae) were taken for meat quality assessment after 24 h of chilling at 4°C and then instantly stored at -20°C without chopping until measurements of IMF content, fatty acid composition, and sensory quality were made.

2.2. Myosin heavy chain isoform content

Eight frozen sections of each muscle sample were prepared and placed in 1.5-ml tubes. Myofibrils were prepared according to the proposed method of the Talmadge and Roy (1993), and Bradford's method (BRADFORD, 1976) was used to determination of the protein concentration of each sample. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze MHCs (TALMADGE and ROY, 1993) and separated into slow and fast isoforms. The MHC bands were identified by Coomassie Brilliant Blue staining. These findings were evaluated using an image analysis system for quantitative measures (Leica Application Suite Interactive measurement). The percentage of each MHC isoforms was taken from the ratio of the density of each MHC band to the all MHC band densities (slow and fast), and the MHC fast/slow ratio was obtained from the ratio of the density of fast MHC bands to the density of slow MHC bands within each sample (CHOI, 2010).

2.3. Meat quality measurements

Muscle pH was obtained at 24 h postmortem (pH_{24h}) using a spear-type portable pH meter (Hanna instruments, pH 210). Meat color parameters was assessed after exposing the surface to air at 4°C for 30 min by the lightness (L*), redness (a*), and yellowness (b*) system (C.I.E., 1978) using a Minolta chromameter (CR-300, Minolta Camera Co., Japan). The mean value of three replicates conducted on each sample and also color (1=pale pink is gray to white; 6=dark purplish-red) and marbling (1=1.0% IMF; 10=10% IMF) were assessed visually (NPPC, 2000). Three parameters including, drip loss, filter-paper fluid uptake (FFU), and cooking loss were used to evaluate the Water holding capacity (WHC). According to HONIKEL (1987), driploss was calculated after weighing (70 gr). The sample was placed in a net surrounded and then hung in an inflated plastic bag for 48 h at 4°C. The sample weighed again after 48 h and drip loss was calculated as percent change in weight. in order to measurement of FFU (KAUFFMAN, 1986), filter paper (Whatman #2, 42.5 mm in diameter) was weighed, then putted on the surface of the sample to absorb fluids (<2s) and then was re-weighed. FFU was expressed as milligrams of exudates absorbed by the filter-paper (EIKELENBOOM *et al*., 1996). This was how we measured the cooking loss. Following 24 h of chilling, loin sections were placed in thin-walled polyethylene bags and were then putted in a continuously heated water bath (80°C). Samples were cooked to 71°C internal temperature that measured using a thermometer with a handheld probe; (TES-1300, TES Electrical Electronic Co., Taiwan) and were then held in ice water for 15 min. finally, the samples were removed from ice water and therefore were taken from the polyethylene bag, blotted dry, and weighed. Cooking loss was expressed as a percentage of the initial sample weight (HONIKEL, 1987). For measurement of Warner-Bratzler shear force (WBS), loin sections were cut into 2 cm thick chops and therefore, cooked meat samples for WBS were prepared as described for cooking loss samples. After cooking we took eight to ten cores (1.25 cm diameter) from the steak parallel to the longitudinal orientation of muscle fibers. WBS was examined by an Instron Universal Testing Machine (Model 1011, Instron Corp., USA) equipped with a Warner-Bratzler shearing device using a crosshead speed of 200 mm/min and a load capacity of 10 kN. Samples were sheared perpendicular to the long axis of the cores.

2.4. Intramuscular fat content and fatty acid composition

IMF content was analysed using the Soxhlet method with a solvent extraction system (AOAC, 2000). For fatty acid composition analysis, the fat was extracted following the

procedure described by FOLCH (1957). Homogenized meat (1.5 gr) was blended with an extraction solvent of chloroform/methanol (2:1, v/v) twice, filtered, and then placed in a separator funnel and mixed with saline solution (0.9% NaCl). After phase separation, chloroform lipid fraction was washed using extraction solvent, whereas the aqueous methanol fraction was discarded. Lipid extracts were concentrated using a rotary evaporator and were then placed in test tubes for subsequent gas chromatographic analysis. Before the gas chromatograph analysis, methylation of lipids was performed by adding 2 ml of sodium methoxide, distilled water, and heptane. Gas chromatograph analysis was carried out using a Gas Chromatography-Mass Selective Detector (GC, Agilent 7890N, USA; MSD, Agilent 5975A, USA) equipped with a HP-INNOWAX column (length of 30m, internal diameter of 0.25mm, film thickness of 0.25 μm). Operating conditions included a helium flow rate of 0.7 ml/ min, a FID setting of 260°C, a split-splitless injector setting of 220°C with an injection rate of 120 ml/min, and an injection volume of 1 μl. The temperature program was composed of an initial hold at 140°C for 4 min, ramping to 220°C at 4°C/min. Retention time and area of each peak were computed using Agilent software. Individual fatty acid peaks were identified by comparing the retention times with those of known mixtures of standard fatty acids (FAME, Sigma-Aldrich CO, USA). Fatty acid composition was expressed as the percentage of total methylated fatty acid. Data were initially recorded and listed as the percentage of individual fatty acids in each sample. Total saturated fatty acid (SFA) was computed as the sum of C12:0, C14:0, C16:0, C17:0, and C18:0. Monounsaturated fatty acid (MUFA) included C16:1, C18:1n-9, C18:1n-7, and C20:1n-9, whereas total polyunsaturated fatty acid (PUFA) was calculated as the sum of C18:2n-6, C18:3n-6, C18:3n-3, C20:2n-6, C20:3n-3, C20:4n-6, C20:5n-3, and C22:6n-3. The ratios of MUFA to SFA and PUFA to SFA were then calculated.

2.5. Sensory evaluation

Eleven panelists were selected by standing external descriptive panel. 65 pork samples were evaluated in three replications. The sensory analysis took place in 6 weeks sessions of up to 1.5 h each. According to the American Meat Science Association (AMSA, 1995) and published procedures (MEILGAARD *et al*., 1991), we performed formal trainings for the panelists. Samples were thawed overnight at 4°C, and then cooked without salt or spice in a humid heat oven (MCS312CF4, Electrolux, Sweden) set at 180°C until they reached an internal temperature of 70°C, which was measured using a thermometer (TES-1300, TES Electrical Electronic Co., Taiwan). They were then immediately sliced into 1.3×1.3×1.3 cm^3 pieces. Samples were held in a water bath (54°C) until presented to the panelists simultaneously in a compartmented plate and three-digit codes were used to name them and served one at a time in random order. In order to eliminate the taste from the previous sample, the evaluators were served distilled water (30°C) and about 3 to 5 min elapsed before evaluation of the next samples. Sensory properties including softness, initial tenderness, juiciness, flavor intensity, off-flavor intensity, chewiness, rate of breakdown and amount of perceptible residue were evaluated.

2.6. Statistical analysis

SAS PC software (SAS Institute, 2004) was used to analysis the content of MHC isoforms in terms of means and standard deviations. Means, standard deviations, and overall ranges are presented as results. Correlations among data obtained were calculated using Pearson's correlation coefficient (r).

3. RESULTS AND DISCUSSION

3.1. Myosin heavy chain isoform content in pig muscle

Table 1 illustrates the results of means and standard deviations for MHC isoform contents of the longissimus dorsi muscle in *Cinta Senese* pigs. Mean values of the MHC slow and fast isoforms were 4.88% and 84.02%, respectively, and the fast/slow ratio was 23.21. According to information obtained, differences in the MHC isoform composition were observed among muscles.

Table 1. Content of the Myosin Heavy Chain (MHC) isoforms of the Longissimus Dorsi muscle in Cinta Senese pigs.

Parameter	μ	SD
MHC slow isoform (%)	4.88	2.28
MHC fast isoform (%)	84.02	2.28
MHC fast/slow ratio (%)	23.21	20.30

3.2. Meat quality

Table 2 shows the correlations between the content of MHC isoforms and meat quality measurements. This experiment found a significant correlation between muscle pH_{24h} and content of MHC slow (r=0.25, P<0.05), fast (r=-0.25, P<0.05) isoforms and fast/ slow ratios (r=-0.30, P<0.01). The results showed that the MHC fast/slow ratio indicated a positive correlation with drip loss (r=0.36, P<0.001) and FFU (r=0.24, P<0.05). Whereas MHC slow isoforms content had an opposite tendency.

Table 2. Correlation coefficients between the content of myosin heavy chain (MHC) isoforms and meat quality measurements of the Longissimus dorsi muscle in Cinta Senese pigs.

	MHC isoform		
Parameter	**Slow isoform**	**Fast isoform**	**Fast/slow ratio**
Muscle pH_{24h}	0.25[1]	-0.25[1]	-0.30[2]
Lightness (L*)	0.04	-0.04	-0.05
Redness (a*)	0.12	-0.12	-0.10
Yellowness (b*)	0.24[1]	-0.24[1]	-0.17
Drip loss	-0.31[2]	0.31[2]	0.36[3]
Filter-paper fluid uptake	-0.221	0.22[1]	0.24[1]
Cooking loss	0.12	-0.12	-0.9
Warner-Bratzler shear force	0.03	-0.03	-0.03
Color	0.23[1]	-0.23[1]	-0.12
Marbling	0.005	-0.005	-0.02

Statistically different values ([1]P<0.05; [2]P<0.01; [3]P<0.001).

3.3. Fat content

Table 3 shows the correlation between content of MHC isoforms and fatty acid composition. The results revealed that the MHC isoform contents were not related to lipid content and marbling score. In the present study, limited relationships emerged from the content of MHC isoforms with individual fatty acids (data not shown) as well as SFA, MUFA, PUFA, and ratio of PUFA and SFA.

Table 3. Correlation coefficients between the content of Myosin Heavy Chain (MHC) isoforms, Intramuscular Fat (IMF) and fatty acid composition of the Longissimus dorsi muscle in Cinta Senese pigs.

	MHC isoform		
Parameter	**Slow isoform**	**Fast isoform**	**Fast/slow ratio**
IMF	-0.079	0.079	0.11
SFA	0.17	-0.17	-0.9
MUFA	0.03	-0.03	0.04
PUFA	-0.10	0.10	0.005
MUFA+PUFA	-0.17	0.17	0.9
MUFA: SFA	-0.12	0.12	0.16
PUFA: SFA	-0.12	0.12	0.03

3.4. Sensorial features

Analyzing all quality parameters panelists were asked to leave comments for each attribute if they felt the need. Particularly they found pig meat good for softness and initial tenderness but they didn't consider it excellent. Table 4 illustrates a correlation between the content of MHC isoforms and the sensory quality of cooked pork.

Table 4. Correlation coefficients between the content of Myosin Heavy Chain (MHC) isoforms and sensory evaluation of cooked meat of the Longissimus dorsi muscle in Cinta Senese pigs.

	MHC isoform		
Parameter	**Slow isoform**	**Fast isoform**	**Fast/slow isoform**
Softness	0.25[1]	-0.25[1]	-0.46[3]
Initial tenderness	0.25[1]	-0.25[1]	-0.43[3]
Juiciness	0.18	-0.18	-0.32[2]
Flavor intensity	0.23[1]	-0.23[1]	-0.19
Off-flavor intensity	-0.25[1]	0.25[1]	0.33[2]
Chewiness	0.21[1]	-0.21[1]	-0.42[3]
Rate of breakdown	0.23[1]	-0.23[1]	-0.44[3]
Mouth coating	-0.02	0.02	0.17
Amount of perceptible residue	0.26[1]	-0.26[1]	-0.42[3]

Score distributions: softness, soft to hard; initial tenderness, tender to tough; chewiness, tender to chewy; rate of breakdown, fast to slow; juiciness, not juicy to extremely juicy; flavor intensity, no pork flavor to full pork flavor; off-flavor intensity, none to strong off-flavor; mouth coating, none to very much; amount of perceptible residues, none to abundant. .Statistically different values ([1]$P<0.05$; [2]$P<0.01$; [3]$P<0.001$).

In this study, a significant correlation was found between content of MHC isoforms and tenderness attributes including softness, initial tenderness, chewiness, rate of breakdown, and amount of perceptible residue. A positive correlation was shown between the content of MHC slow isoform and tenderness characteristics, whereas a negative correlation was found between the fast/slow ratio and softness ($r=-0.46$, $P<0.001$), initial tenderness ($r=-0.43$, $P<0.001$), chewiness ($r= -0.42$, $P<0.001$), rate of breakdown ($r=-0.44$, $P<0.001$), and amount of perceptible residue ($r=-0.42$, $P<0.001$). The results showed that, there was no significant correlation between juiciness and content of MHC slow and fast isoforms, whereas there was a negative correlation between juiciness and the fast/slow ratio ($r=-0.32$, $P<0.01$). We observed that there are positive and negative correlations between flavor intensity and MHC slow isoform content ($r=0.23$, $P<0.05$) and MHC fast isoform ($r= -0.23$, $P<0.05$), respectively, even if there weren't great differences. On the other hand, off-flavor intensity had inverse relation to flavor and there were positive and negative correlations between off-flavor intensity and MHC fast isoform ($r= 0.25$, $P<0.05$) and MHC slow isoform content ($r= -0.25$, $P<0.05$), respectively.

4. DISCUSSION

The aim of this study was to investigate the effects of MHC isoforms to postmortem meat quality traits, fatty acid composition, and sensory evaluation in *Cinta Senese* pigs. According to information obtained, differences in the MHC isoform composition were observed among muscles.

Scientists (SAZILI *et al*., 2005) detected a higher MHC fast isoform content in the longissimus dorsi and tensor fasciae latae muscles in comparison with the supraspinatus, semitendinosus, and trapezius muscles. However, in crossbred pigs (Yorkshire x Landrace x Duroc), the MHC slow and fast isoform contents of the longissimus dorsi muscle were 6.61% and 93.38%, according to CHOI *et al*. (2006). Differences seen in glycogen content and enzyme activities between muscles may be related to fiber type composition and the physical activity level of the muscle **(**GRANLUND, 2011). CHOI and KIM (2009) reported that the MHC fast isoform have a fast ATPase and high anaerobic capacity, whereas the MHC slow isoform have a slow ATPase and a high aerobic capacity in single muscle fibers. In contrast with our results MHC 1 isoform content was negatively correlated with the lightness and glycolytic enzyme activity, and was positively correlated with oxidative enzyme activity (CHOI, 2009). These demonstrated how the composition of MHC isoforms leads to a decline in the rate and extent of pH caused by lactate overproduction. Our results show a significant correlation between muscle pH_{24h} and content of MHC slow, fast isoforms and fast/ slow ratios. According to RYU and KIM (2006), muscles harboring a higher percentage of MHC fast isoform tend to show a more rapid pH decline than muscles harboring a higher percentage of MHC slow isoform. Genetic and pre-slaughter factors that influence postmortem rate of glycolysis and pH decline are used to determine the occurrence of PSE (pale, soft, exudative) meat (KAZEMI, 2011). Protein denaturation occurs when in high temperature of the muscle, rapid rate of postmortem glycolysis leads to a rapid pH fall (BENDALL and SWATLAND, 1988; KAUFFMAN *et al*., 1998). The denaturation of myofibrillar proteins and particularly myosin is related to the low water holding capacity of PSE meat (OFFER and KNIGHT, 1988; OFFER, 1991). Muscles with a higher extent of protein denaturation show a higher percentage of MHC fast isoforms and higher degrees of fluid loss by exudation than muscles with a lower extent of protein denaturation (CHOI, 2010). Our results showed that the MHC fast/slow ratio indicated a positive correlation with drip loss.

According to PETER *et al* (1972), type I fiber contains greater amount of lipid, some of which presumably serves as a source of aerobic metabolic fuel; in contrast, type IIB contains greater amounts of glycogen and glucose (HINTZ *et al*., 1984), and also glucose uses as fuel (CHOI, 2009). Our results however revealed that the MHC isoform contents were not related to lipid content and marbling score. Lipid composition is one of the main characteristics related to meat quality affected by muscle fiber type. According to LESEIGNEUR-MEYNIER and GANDEMER (1991), total phospholipids (PLs) and PUFA of PLs in pork are influenced by muscle fiber type. Variations in the IMF content are mainly due to changes in the triglyceride content, therefore, high IMF contents imply a high level of triglycerides (RUIZ-CARRASCAL, 2000).

In this study, a significant correlation was found between content of MHC isoforms and tenderness attributes including softness, initial tenderness, chewiness, rate of breakdown, and amount of perceptible residue. A positive correlation was shown between the content of MHC slow isoform and tenderness characteristics, whereas a negative correlation was found between the fast/slow ratio and softness, initial tenderness chewiness, rate of breakdown, and amount of perceptible residue. Proteins and structures that bind and entrap water, specifically the myofibrillar are responsible for the mechanism of water-holding capacity (WHC). It was earlier demonstrated that there is a direct effect of pH, ionic strength, and oxidation on the ability of myofibrillar protein and myofibrils and muscle cells to entrap water (HUFF-LONERGAN, 2005). Glycolysis causes a more rapid pH decline, and muscle contraction, thereby, resulting in a high drip loss (ZELECHOWSKA, 2012). It has also been declared that pH can affect meat tenderness (ZHENG *et al*., 2017). It is generally accepted that after cooking, muscles with a higher WHC are more tender and juicy meat than muscles with a lower WHC (JEONG, 2010; KANG, 2011). Fiber type composition affects the sensory quality of cooked (KANG, 2011; NAM, 2009). In the present study, a positive correlation exists between content of MHC slow isoform and two parameters including pH and WHC. Moreover a high ratio MHC fast/slow isoforms and a high content of the MHC slow isoform are associated with tough and tender meat, respectively. According to OURY (2009), a positive correlation exists between the content of the MHC slow isoform and the initial tenderness of rectus abdominis muscle from Charolais heifers. In our study we noticed, as previously asserted, that there was a major percentage of fast-twitch glycolytic fibers, so that the WHC was low, consequently *Cinta Senese* pig had high drip loss and low cooking loss. Probably this feature was associated with the muscle chosen. There is a positive correlation between the proportions of type I fibers and sensory assessed tenderness in beef (MALTIN *et al*., 1998) and pork (OCKERMAN *et al*., 1984) has been found. It was earlier demonstrated that effect of muscle fibre traits on meat tenderness are not uniform (ORZECHOWSKA, 2008). According to TOTLAND (1988), the superficial regions in bovine semitendinosus muscle contained a high percentage of type IIB fibers and were more tender than the deeper muscle layers, with a high percentage of type I fibers. There is a negative correlation between the percentage of type IIB fibers and toughness in pigs (KARLSSON, 1993), whereas there is a positive correlation between the percentages of type I fibers and meat toughness in bulls. STRYDOM (2000) reported that sensory tenderness shows a positive and negative relationship with the percentage of type I and IIB fibers, respectively.

On the other hand, we demonstrated that, there was no significant correlation between juiciness and content of MHC slow and fast isoforms, whereas there was a negative correlation between juiciness and the fast/slow ratio. According to HUFF-LONERGAN (2002), lipid content can influence the sensory features including texture, tenderness, flavor, and juiciness. Also DALL AASLYNG (2008) reported that water holding capacity (WHC) of the meat might can influence the juiciness. In this study, the higher WHC in muscles with a lower fast/slow ratio compared to muscles with a higher fast/slow ratio

probably explains the relationship between juiciness and content of MHC. Taste and aroma were defined as flavor (MOODY, 1983), which is based on taste-active compounds, flavor enhancers and aroma components with over 880 compounds presently identified in cooked beef alone (MACLEOD, 1998). Tenderness, juiciness and flavor are as components of the palatability of meat (MUCHENJE, 2008). Fatty components cause different flavors among beef, pork, chicken, turkey, and lamb and also fatty tissues give them specific flavor profiles. When the fat acts as one of the flavor agent heated, they are combined with amino acids from proteins and other components and therefore, they release (DINH TRAN NHAT THU, 2006). There is about twice as much phospholipid than glycolytic ones in oxidative muscles. Muscle fiber type composition may influence flavor through the phospholipids (LEFAUCHEUR, 2006) as major determinants of cooked meat flavor (MEYNIER and GANDEMER, 1994). Particularly phospholipids have an important role in the development of flavor intensity (MOTTRAM and EDWARDS, 1983). High content of type I fiber is related to juiciness and flavor, whereas high type IIB fiber content tends to be associated with tougher meat (CHOI and KIM, 2009). According to our results, the off-flavor intensity had inverse relation to flavor and there were positive and negative correlations between off-flavor intensity and MHC fast isoform and also with the MHC slow isoform content respectively.

5. CONCLUSIONS

The present study revealed that the composition of MHC isoforms influences the sensory quality of cooked pork, including tenderness features. A notable point that should be considered is about the type of the MHC isoform; muscles having a higher content of the MHC slow isoform shows good meat quality and tenderness compared to muscles having a higher ratio of MHC fast/slow isoforms. We correlated different parameters to fibers content in order to obtain more information about pig meat quality and particularly the *Cinta Senese* pig. Local breed of pigs shows a high potential for composition and ultrastructure of muscle that could impact pork quality. The results of our study represent the starting point for increasing knowledge about a local breed as *Cinta Senese* pig and the muscle properties that affect meat quality.

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ULTRASOUND USE FOR *LISTERIA MONOCYTOGENES* ATTACHED CELLS REMOVAL FROM INDUSTRIAL BRINE INJECTION NEEDLES

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ABSTRACT

This work aimed at studying methods for *Listeria monocytogenes* attached cells removal from food industry equipment. In order to verify possible contamination points, a tracking was carried out in food products and equipment surfaces (brine injection needles) by swab. Brine injection needles were defined as a study due to their higher contamination potential. *L. monocytogenes* adhesion after 6 h contact with needles was observed. Minimum bactericidal concentration (MBC) of peracetic acid on *L. monocytogenes* attached cells was 0.06 and 0.24% with 6 and 24 h contact with bacteria, respectively. 75% power (700 W) and 14 min exposure ultrasound treatment showed a 3.25 Log CFU microbial load reduction in injection needles. The combination of ultrasound and peracetic acid showed an MBC reduction from 0.24 to 0.03%, hence, it may be recommended for meat industry application.

Keywords: *L. monocytogenes*, biofilms, sanitizer, ultrasound, food industry

1. INTRODUCTION

Brine application with or without seasoning (marination), is a process used to improve the physical and sensory characteristics of meat. This process involves immersing the meat in a brine solution to increase its fluid retention, to improve its sensory attributes, color, texture, flavor, protein addition, water binding capabilities, as well as meat safety (RUST and KNIPE, 2014). The application can be performed in different ways, either by immersion, injection, or massage (dynamic). Curing brine injection into marinated products or into pieces of meat is performed using injection needles. The needles shapes varies depending on the product to be injected.

However, the cleaning and sanitization of the injection system, especially the brine injection needles, is a hard process due to the difficulty for disassembling and carry out the complete sanitization and/or immersion. The industrial scale injection system cleaning is done by manual and/or mechanical scrubbing with alkaline detergent and subsequently, peracetic acid for sanitization. Scrubbing removes organic matter, while sanitization removes microorganisms.

The injection needles mounted onto the equipment, having their internal diameter reduced when only sanitized by the pressure drag system. This presents a potential for biofilm formation (or cell adhesion), due to the difficulty in total organic material removal, as sanitization does not cover the entire internal surface of the needle.

Biofilms are characterized by increased biomass accumulation of microorganisms and extracellular materials on a solid surface, detrimental to both human health and industrial processes (HORI and MATSUMOTO, 2010; BAN and KANG, 2015). BRANDA *et al.* (2005) defined biofilm as a structured bacterial cells comunity embedded in an exopolysaccharides matrix produced by the adhered cells themselves on either an abiotic or biotic surface. The cells presented greater resistance to antimicrobial agents and detergents when biofilm is formed. This resistance could be partially related to the passage through the extracellular matrix due to cells phenotypic changes in the biofilm structure and on the resistance mechanism generated by the three-dimensional structure.

Studies show that washing and sanitizing used by the food industry do not always guarantee complete biofilms elimination (BANACH *et al.*, 2015; MALHEIRO and SIMÕES, 2017). Adhered cells are highly resistant to acid disinfection, as they also show tolerance/resistance to disinfectants' lethal concentrations due to exopolysaccharides production that protects against chemical agents (CARPENTIER and CERF, 1993; MYSZKA and CZACZYK, 2011).

Bacterial adherence to stainless steel, glass, and polypropylene surfaces is a potential source of contamination, which may lead to disease transmission (CHAVANT *et al.*, 2007). One of the most serious food-borne diseases is listeriosis, caused by *L. monocytogenes*, due to its sequelae degree and high lethality index (20 to 30%) (BAN and KANG, 2015). *L. monocytogenes* may persist in industrial equipment and installations due to its high adhesion potential and biofilm forming capacity at low temperatures (CARPENTIER and CERF, 2011; BELTRAME *et al.*, 2016).

Among the sanitizers used in the food industry against food-borne pathogens, peracetic acid is excellent for Gram-positive and Gram-negative bacteria, filamentous fungi and yeasts, viruses, and bacterial spores. In addition, it attacks the proteins' cell wall and migrates into the cell, breaking internal components (MCDONNELL and RUSSELL, 1999). However, in order to be effective, it requires direct contact with the microorganism for a period of time, which varies depending on the sanitizer concentration.

Ultrasound is another widely investigated method, responsible for thinning bacteria cell walls, making them more susceptible to rupture and inactivation. Ultrasound can be combined with other treatments in the food industry, such as heat and chemicals to

inactivate bacteria (BETTS *et al.*, 2014). In addition, it has been reported that this process could be effective in biofilm removal (SREY *et al.*, 2013).
Ultrasound presents a cavitation effect, which is cavities or bubbles formed in the liquid medium, resulting in a certain amount of gas that can lead to structural or functional changes on cells due to molecular bonds disruption (JOSÉ and VANETTI, 2012). The energy generated by this process releases microorganisms from biofilms (JAY, 2005). Ultrasound hydrodynamic properties destabilize the biofilm structure, and higher power and longer exposure time were associated with improved bacteria elimination in biofilms (SCHERBA *et al.*, 1991).
Due to *L. monocytogenes* inherent risks in the food industry, it is necessary to identify and map out the most favorable sites for microbial adherence and provide effective methods for contaminant cells and biofilms elimination, avoiding cross contamination. In this sense, this work aims at tracking microbial contamination spots in a large meat product plant and evaluate the effects of using ultrasound alone and its combination with peracetic acid in the *L. monocytogenes* removal, previously adhered to brine injection needles under industrial conditions.

2. MATERIALS AND METHODS

2.1. Tracking contamination spots in a meat production plant

The study was carried out in a slaughterhouse located in Southern Brazil, which industrializes pork meat products, inspected by the Brazilian Inspection Body. The plant has a wide range of products such as seasoned and cold cuts, as well as fresh and smoked sausages.
Microorganisms presence in processed products was evaluated before freezing in order to map out possible microbial contamination spots in the plant. Analyses for *Salmonella* sp. and *L. monocytogenes* were performed after product handling in the following sectors: (A) cutting and (B) seasoning "traditional technique, used to tenderize and improve the meat flavor and succulence, similar to marinated products use".
After data evaluation for each product and sector, a surface swab analysis of the highly contaminated site was performed. The analyzed spots were drain/gutter, kick/mat, internal equipment, wall/floor, pallet/container, and roof.
Analyses were carried out on a weekly basis between January and September, in products and different surfaces, aiming at being indicative of production conditions.

2.2. *L. monocytogenes* adhesion on brine injection needles

L. monocytogenes (ATCC 7644) adhesion in brine injection needles was evaluated after identifying the main contamination spots.
For bacterial adhesion experiments, hypodermic needles were used (120 mm length x 3.0 mm external thickness x 1 mm internal thickness), which are commonly used in the industry for brine application by injection process. For this study, used needles were employed (worn out due to usage), aiming for the most favorable conditions for biofilms formation or cell adhesion.
The needles were washed with running water, then scrubbed with liquid neutral detergent, rinsed with running water, followed by distilled water and disinfected in distilled boiling water immersion (100°C), for 5 min.
After sanitization and disinfection, the needles were immersed in a Luria Bertani - LB broth (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) previously inoculated with 0.1

mL *L. monocytogenes* pre-inoculum (~ 1x10^{8} CFU/mL) and incubated at 35-37°C for 3, 6, 12, and 24 h contact with the needles to allow bacterial adhesion. After incubation, the needles were removed from the LB broth and rinsed with deionized water for 15 s, followed by three washes with sterile water (vortex 20 s). Bacterial adhesion was evaluated by external swab on the needle and standard counting in LB agar plates in accordance with Barbosa *et al.*, (2016) and Beltrame *et al.*, (2016).

2.3. *L. monocytogenes* removal on brine injection needles by ultrasound

An ultrasound probe (20-kHz QSonica, CT., USA) was used to evaluate the effects of *L. monocytogenes* removal on brine injection needles at different times (0, 3.5, and 14 min), with 30 s intervals in ice bath, at different powers (60, 75, and 90%/700 W). The times were chosen due to equipment limitation. Ultrasound application schematic representation on injection needles are shown in Fig. 1. The brine injection needles were previously subjected to adhesion (described above), ultrasound treatment was applied and then, the ultrasound-removed liquid (0.1% peptone water) was plated in LB agar medium.

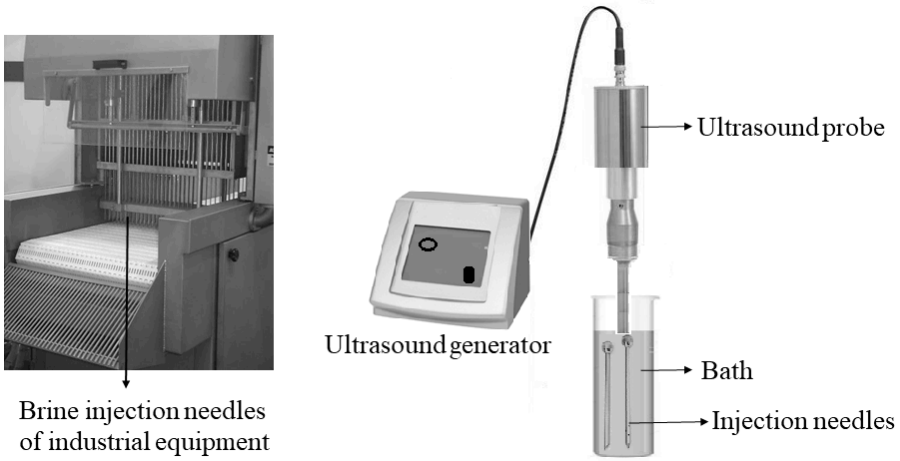

Figure 1. Representations of ultrasound treatment on brine injection needles.

Dilutions were performed in peptone water for surface plating on LB agar-containing plates, incubated at 35-37°C for 24 h, followed by colony counting. All analyses were performed in triplicate. The microbial counting was expressed in Log CFU, in relation to time 0 (control).

2.4. Minimal bacterial concentration determination

Assays were performed using peracetic acid in order to evaluate the MBC and all experiments are performed in triplicate. The needles were incubated with test microorganism for 6 and 24 h growth to simulate the times used in industrial sanitization. After incubation, three rinses with deionized water were performed, and then they were immersed in test tubes containing 30 mL chlorinated alkaline detergent solution (Easyfoam/Diversey) and/or a combination with acid detergent (Kalyclean 244) for 10 min, as specified by the manufacturer.
After detergent application, the needles were immersed in peracetic acid sanitizer (17% - ECOPER Quimica) at different concentrations (0.03, 0.06; 0.12, 0.24, and 0.30%). After

10 min contact with the sanitizer (as per manufacturer's recommendation), the needles were submitted to external swab and inoculated on LB agar (LB and agar 15 g/L), and incubated at 35-37°C for 24 h, followed by colony counting.
MBC was evaluated on needles after *L. monocytogenes* adhesion for 6 and 24 h, then rinsed with sterile water to remove unbound cells and sprinkled inside and outside with a 10 mL sanitizing solution. The industrial peracetic acid-PA sanitizer (17% - ECOPER Quimica) was evaluated at different concentrations (0.015, 0.03, 0.06, 0.12, 0.24, and 0.30 %) for application to previously incubated needles for 6 and 24 h. After 10 min contact with the needles, they were internally sprinkled with a 10 mL peptone water solution (peptone 10.0 g/L, hydrogen phosphate 9.0 g/L, NaCl 5.0 g/L, phosphate potassium 1.5 g/L) and 3% Tween-80 for residual peracetic acid inactivation. The liquid was plated by immersion in LB agar (LB and agar 15 g/L), incubated at 35-37°C for 24 h, followed by colony counting.

2.5. Effect of ultrasound and peracetic acid combination

The effect of ultrasound and peracetic acid combination on MBC reduction with sanitizer were evaluated through the best result for *L. monocytogenes* removal using ultrasonic method combined with different peracetic acid concentrations (0.03, 0.06, 0.12, 0.24, and 0.30%).

2.6. Microbiological analysis

L. monocytogenes: Presence/absence analyses in 25 g sample were carried out according to ISO 11290-1 (1996).
Salmonella sp.: The analysis was performed using VIDAS equipment, according to methodology described by AOAC (2016).

2.7. Statistical analysis

The ultrasound effect results were subjected to Tukey test at a 5% significance level for comparison between the means. All statistical analyses were performed using software SPSS Student version.

3. RESULTS AND DISCUSSION

3.1. Plant Contamination Tracking

The seasoned meat products of the studied industry were submitted to brine injection, both in the early and intermediate stages. In addition, the equipment was used to temper an array of products. The injection system was formed by needles block with an extremely small diameter. Thus, the injection step turns into a microbiological control point to optimize both hygiene and sanitation, due to possible cross-contamination inside the industrial plant.
L. monocytogenes presence may indicate a hygiene system failure that should be evaluated in order to identify the contamination source (HENRIQUES *et al.*, 2016). This reinforces the importance of an effective diagnosis to evaluate sanitation system by microbiological tests.
Table 1 shows results of L. *monocytogenes* and *Salmonella* sp. presence in different handling Sectors (A and B) of the industrial plant.

Table 1. Results in percentage of the presence of *L. monocytogenes* and *Salmonella* sp. in different sectors (A and B) of the industry, during the period between January and September.

Sectors	Presence of *L. monocytogenes* (%)	Presence of *Salmonella* sp. (%)
A (n=450 analysis)	0	3
B (n=315 analysis)	47	8

The products handled in sector B presented *L. monocytogenes*, contamination, while no contamination was observed in sector A. The *Salmonella* sp. contamination was higher in sector A, but with low indexes in relation to sector B. Considering the higher *L. monocytogenes* incidence in sector B, that site was choosen to continue the evaluations for that bacterium.

In order to track *L. monocytogenes*, contamination points in sector B, swab analyses were performed on surfaces in contact with the product. The points were mapped out and divided into the following categories; drain/gutter, kick/mat, internal equipment, wall/floor, pallet/container, and roof. The swab results after operational hygiene showed no presence of this bacterium at the different points analyzed.

Sector B processed food products were analyzed in order to confirm *L. monocytogenes* contamination. The results demonstrated contamination only in the products after brine injection process, since there was no detected contamination before injection in any products. In order to track the possible contamination points, the brine solution was subsequently evaluated, which showed no *L. monocytogenes* presence

Since all swab-tested injection equipment, points showed no contamination, the possible contamination may be in the needles, as the swab analysis was not performed due to their reduced internal diameter. Therefore, this point was chosen to study biofilm formation and removal, since there is a lack of brine injection needles microbial contamination studies, which are difficult to sanitize due to their shape.

3.2. *L. monocytogenes* adhesion on brine injection needles

The results presented in Fig. 2 demonstrated *L. monocytogenes* adhesion on brine injection needles. An initial fast growth can be observed up to 12 h, tending to stabilize in 18 and 24 h.

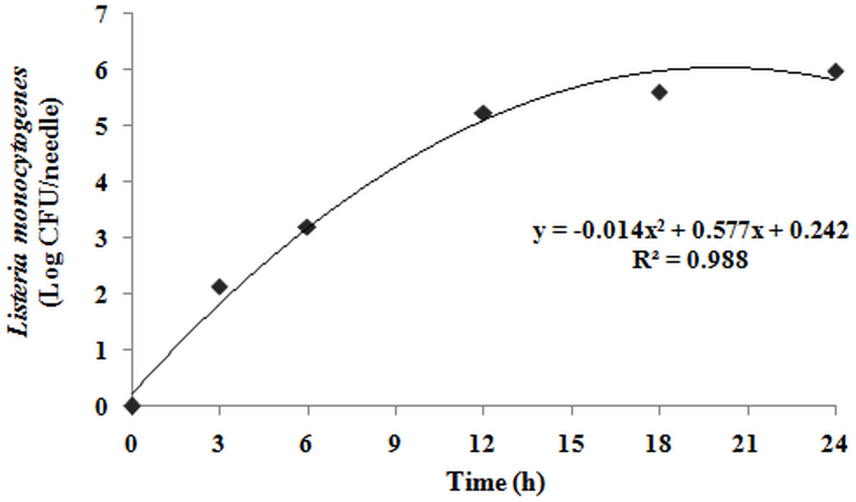

Figure 2. *L. monocytogenes* adhesion on brine injection needles.

Biofilm formation can occur in a short period of time and in relation to counting (bacterial cells adhesion) (BARBOSA *et al*., 2016). According to BARBOSA *et al.* (2016) and BELTRAME *et al.* (2016) a 3.0 log CFU or $1x10^3$ CFU adhering cell counting per square inch may be identified as biofilm formation. In this way, according to the above mentioned authors' criteria, *L. monocytogenes* attached cells occurred after 6 h contact with injection needles.

3.3. *L. monocytogenes* removal adhered to injection needles using ultrasound

Table 2 shows the *L. monocytogenes* counting reduction adhered to brine injection needles at different contact times (0, 3.5, and 14 min) and in different ultrasound powers (60, 75, and 90%/700 W), at 20 KHz.

Table 2. Reduction of the *L. monocytogenes* count (Log CFU/cm^2) as a function of contact time and ultrasound power. Results in Log CFU/cm^2.

Ultrasound power (20 KHz/700W)	Contact time (min)	*L. monocytogenes* initial count (CFU)	Reduction (Log CFU)	Reduction (%)
60%	0 (control)	$1.4x10^{6\ ab}±6.1x10^3$	-	0
60%	3.5	$9.3x10^{4\ bc}±8.3x10^2$	1.18	93.36
60%	14.0	$2.0x10^{4\ c}±8.9x10^2$	1.84	98.57
75%	0 (control)	$1.4x10^{7\ a}±8.6x10^3$	-	0
75%	3.5	$2.0x10^{5\ b}±3.1x10^3$	1.85	98.57
75%	14	$9.7x10^{3\ cd}±2.4x10^1$	3.25	99.93
90%	0 (control)	$1.3x10^{5\ b}±4.4x10^2$	-	0
90%	3.5	$4.3x10^{3\ d}±4.9x10^1$	1.48	96.69
90%	14.0	$1.8x10^{3\ d}±1.9x10^1$	1.86	98.61

Means (± standard deviations) followed by same letters on the column, represents no significant difference at 5% level (Tukey test).

It was observed that the increased exposure time caused a reduction in the microbial counting of 1.18 to 1.84 Log UFC with 60% power, 1.85 to 3.25 Log UFC with 75%, and 1.48 to 1.73 Log UFC with 90% ultrasound power (Table 2). This reduction could be explained by the microbial death mechanism generated by cell membrane wear, heating, and free radicals production (BRONDUM *et al.*, 1998; BUTZ and TAUSCHER, 2002).
Although at the highest power (90%), the initial microbial counting was lower than the one at 60% power, the Log UFC reduction after 14 min sonication was similar. However 3.5 min high-power sonication resulted in a higher reduction than the 60% power, indicating that the contact time and power influenced the microbial destruction of the attached cells. PIVASENA *et al.*, (2003) reported that exposure/contact time and ultrasonic waves amplitude affect microbial inactivation effectiveness.
In food processing, high-power ultrasound is able to cause cavitation, consequently causing microbial inactivation and elimination. The ultrasound waves create cavitation bubbles that pass through the solution creating a negative pressure, hence breaking both the cell wall and membrane structures (BILEK and TURANTAS, 2013).
The highest reduction (3.25 Log CFU) among treatments was observed at 75% power. This may be justified, since, according to ERRIU *et al.* (2014) potency and contact time may be effective for biofilm removal or may be a formidable bacterial viability enhancer.

Ultrasound effectiveness in biofilm removal is dependent on the bacteria being treated (PIVASENA *et al.* 2003). *L. monocytogenes* could be inactivated by ultrasound combined with other treatments. FERRANTE *et al.* (2007) observed that high-intensity ultrasound combined with mild heat treatment and natural antimicrobials was effective for *L. monocytogenes* inactivation in orange juice.

3.4. Peracetic acid MBC of peracetic acid on *L. monocytogenes*

The efficacy of different peracetic acid concentrations (0.03; 0.06; 0.12, 0.24, and 0.30%) was assessed by establishing the MBC. Such evaluation was performed to simulate industrial conditions of sanitizer application under pressure on the injection needles for 10 min until rinsing (due to needles disassembling difficulty). The MBC of peracetic acid was 0.24% (v/v) and 0.06% (v/v), respectively for 24 h and 6 h incubation with *L. monocytogenes* (Fig. 3).

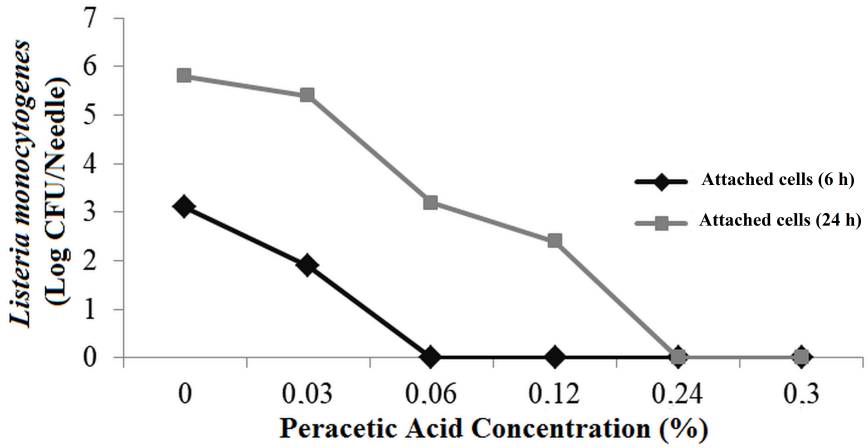

Figure 3. Bactericidal action of peracetic acid on *L. monocytogenes* on brine injection needles.

The data found in this work may be compared to the one found by POIMENIDOU *et al.* (2016), who found a MBC of peracetic acid on *L. monocytogenes* in food processing plants ranging from 115 to 2713 ppm (0.0115 to 0.2713%). The authors also observed higher MBC for longer incubation times. Therefore, incubation time is directly related to MBC, supporting a possible biofilm formation and increase resistance to sanitizers.
BELESSI *et al.* (2011) studying *L. monocytogenes* biofilm resistance under food processing conditions found that the number of surviving bacteria decreased as the contact time at 2% peracetic acid increased.

3.5. Effect of ultrasound and peracetic acid combination

MBC for the combination of ultrasound (14 min to 75%- 20 kHz) with peracetic acid concentrations (0.03; 0.06; 0.12, 0.24, and 0.30%) on *L. monocytogenes* removal from brine injection needles with (24 h contact) was evaluated. Ultrasound use at a 75% power for 14 min showed a 3.25 log CFU reduction, however; without total reduction, it was not possible to establish an ultrasound minimum bactericidal power/time. On the other hand, the combined method reduced the MBC of peracetic acid from 0.24% to 0.03% (Fig. 4),

demonstrating the combined method effectiveness with a low peracetic acid concentrations.

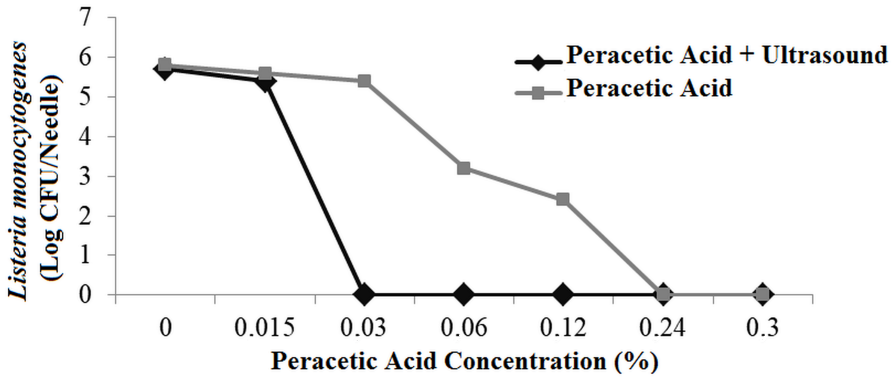

Figure 4. Effect of combined ultrasound and peracetic acid methods in the *L. monocytogenes* removal on brine injection needles.

The ultrasound process was studied as a "green", non-chemical technology in the industrial plant to improve meat quality and safety. Based on the *L. monocytogenes* removal results combining ultrasound and peracetic acid methods, this sanitization process could be suitablefor the food industry.

As mentioned in a number of literature studies, the combined method was effective in many quality criteria processes, such as tenderness, changes in proteins functional properties, shelf life improvement, microorganisms inactivation in meat and its products (TURANTAS *et al.*, 2015), knives sanitization used in the meat industrial plants (BRASIL *et al.*, 2017), raw salmon fillets (MIKS-KRAJNIK *et al.*, 2017), and fresh-cut bell pepper (LUO and OH, 2016). The combination of ultrasound and peracetic acid could potentially increase the microorganism inactivation rate (D value reduction) using the ultrasound cavitation process, either partially removing attached cells or facilitating sanitization.

4. CONCLUSIONS

It was observed a greater contamination with *L. monocytogenes* in relation to *Salmonella* sp. during the tracking. The tracking in the sector B indicated the brine injection needles as the source of contamination. The *L. monocytogenes* on the needles indicated cells adhesion after 6 hours of contact. The peracetic acid showed a MBC of 0.06 and 0.24% for attached cells with 6 and 24 h of contact, respectively. The application of 75% (700W) of the ultrasound power and 14 min of exposure time in the needles provided a microbial reduction of 3.25 Log CFU. The combined use of ultrasound (75%- 700W, 14 min) with peracetic acid showed a reduction of MBC from 0.24% to 0.03%. The results indicate that the combined use of ultrasound and sanitizer may increase food safety, removing the *L. monocytogenes*, and/or serve as a specific treatment when this bacterium occurs in the industry.

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PAPER

PASTA-MAKING PROPERTIES OF THE NEW DURUM WHEAT VARIETY *BIENSUR* SUITABLE FOR THE NORTHERN MEDITERRANEAN ENVIRONMENT

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ABSTRACT

Industrial pasta is commonly made from mixtures of semolina from different durum wheat varieties, and there is a very low market presence of mono-varietal pasta from local, short supply chains. In this work, dough rheological properties and pasta quality traits of the new durum wheat cv. *Biensur*, which has a high HMW/LMW-GS ratio, were evaluated with a view to developing short-chain, mono-varietal pasta production in NE Italy. Chemical and sensory analyses on short-cut pasta, *viz.* tubetti, made with semolina from cv. *Biensur* at two drying temperatures revealed that it has good technological characteristics and stability, excellent cooking and sensory properties, and is comparable to the high-quality commercial reference cv. *Aureo*. We conclude that *Biensur* provides farmers and traders with new market opportunities and offers improvements to the environmental and economic sustainability of the durum wheat chain.

Keywords: mono-varietal pasta, short-chain production, pasta texture, HMW and LMW GS, gliadins, technological characteristics

1. INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is one of the most important crops worldwide with an average annual production of 32.6 million tons (MMT), and is the most widely grown crop in the Mediterranean area with an annual production of about 18 MMT (INTERNATIONAL GRAIN COUNCIL, 2015). Italy is a major producer of durum wheat in the Mediterranean, with an average production of about 4.0 MMT/year, about 67% of which is grown in the south of Italy and used mainly for pasta production (D'EGIDIO, 2007). Pasta is made from the kneaded dough of semolina flour and water, which is shaped by a press and stabilised by drying. The pasta-making industry generally uses mixtures of different durum wheat varieties as blending yields semolina with high technological properties. As a consequence, there is very little mono-varietal pasta currently on the market, therefore it would be worthwhile studying the effects of individual cultivars on pasta quality (PADALINO *et al.*, 2014).

Protein content and gluten characteristics are the main factors influencing pasta quality (SISSONS *et al.*, 2005; CUBADDA *et al.*, 2007), but their relative importance depends on many factors, including genotype, and environmental and processing conditions, such as drying temperature (D'EGIDIO *et al.*, 1990; NOVARO *et al.*, 1993). The performance of durum wheat in pasta making is related, in particular, to the storage proteins of grains, i.e., glutenins and gliadins, which influence dough strength, extensibility and stability (SISSONS, 2008). Glutenins are large aggregates of sub-units of either low molecular weight (LMW; 31–51 kDa) or high molecular weight (HMW; 80-140 kDa) joined by disulphide bonds (VARZAKAS *et al.*, 2014). Gliadins are alcohol-soluble proteins, which fall into four groups, ω, γ and α/β, based on their electrophoretic mobility and molecular weight (45-25 kDa). Glutenins are mainly polymeric proteins responsible for dough elasticity, whereas gliadins are monomeric and determine characteristics related to extensibility.

Aside from the amounts of proteins and types of gluten proteins, the glutenin to gliadin and the HMW-GS to LMW-GS ratios are also directly related to the balance between dough strength and extensibility (SAMAAN *et al.*, 2006; SISSONS, 2008).

Drying temperature, one of the most important factors in the pasta production process, gives rise to highly aggregated proteins that are cross-linked via covalent bonds and disulphide bonds. A higher drying temperature intensifies polymerisation of the proteins into a protein network, which entraps the starch granules thereby preventing starch leaching during cooking and increasing the pasta's sensory properties and cooking quality (ZWEIFEL *et al.*, 2003; PADALINO *et al.*, 2016).

In recent years, the environmental impact of the entire pasta production cycle, from the cropping system in the field to semolina production techniques and packaging, has been reviewed (BEVILACQUA *et al.*, 2007). Moreover, under a recent Italian decree (LEGISLATIVE DECREE, 2017), it is now obligatory to declare the origin of the durum wheat grains used in pasta production.

In light of this, an advantageous strategy would be to promote local, short food supply chains in order to improve environmental and economic sustainability. In this regard, a recent study assessed the new cv. *Biensur* and found it offered a suitable combination of high grain yield [7.31 t/ha, 132% higher than the Italian mean (3.15 t/ha) and 37% higher than the Veneto regional mean (5.32 t/ha) (source: ISTAT, averages of the 12-year period 2006-2017)] and high quality semolina when grown under sustainable agronomic management on the edge of the cultivation area of this species in the Mediterranean (VISIOLI *et al.*, 2018).

The aims of this research, therefore, were: a) to evaluate the dough rheological properties and the quality of the pasta obtained from *Triticum durum* cv. *Biensur* cultivated in

Northern Italy with a view to its possible use in the production of mono-varietal pasta; b) to evaluate the effects on pasta quality of two different drying temperatures during industrial processing. Comparative analyses were made against a high-quality standard represented by cv. *Aureo*.

2. MATERIALS AND METHODS

2.1. Field experiment and grain production

A field experiment was carried out in a sandy loam soil at the Miana Serraglia farm (Mira, Venice, Italy), located close to the Venetian Lagoon, during the 2012-2013 growing season. The durum wheat cv. *Biensur* (Apsovsementi, Voghera, Italy) was grown in a 13.6 ha field. In accordance with local recommendations, 215 kg/ha of nitrogen fertilizer was applied: 200 kg N/ha to the soil as ammonium nitrate and 15 kg N/ha (UAN, urea-ammonium-nitrate) by foliar spraying at the flowering stage. The wheat was sown late in October and harvested early in July. Grain samples of 100 kg were collected to carry out dough tests and manufacture the pasta.

2.2. Gluten protein quantification and chemical composition

Grains of the cv. *Biensur* (*Triticum durum* Desf.) were ground in an experimental laboratory mill (Buhler MLU202 roller mill; Braunschweig, Germany) at the Scientific Technology Park of the Molise Region (Campobasso, Italy) in order to obtain fine semolina with a particle size similar to that of the reference control (200 to 350 μm). The reference control was a high-quality commercial semolina (cv. *Aureo*) used in industrial, mono-varietal pasta production. Protein, starch, fat, total fibre and ash contents were quantified in the semolina samples.

Total protein content was quantified by the Kjeldahl 2001.11 method (AOAC, 2000). In addition, relative quantification of the gliadin and the high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin (GS) fractions in the semolina of both *Biensur* and *Aureo* was carried out using the protein sequential extraction procedure (VISIOLI *et al.*, 2016) followed by quantification by Bradford assay (BRADFORD, 1976). Three technical replicates were performed for each variety. SDS-PAGE was performed on a Mini-PROTEAN Tetra Cell (Bio-Rad) on 8%, 12% and 15% acrylamide gel for the HMW-GS, LMW-GS and gliadin fractions, respectively, as previously described (VISIOLI *et al.*, 2016). Following gel staining and image acquisition, protein molecular weights (MW) were identified and relative quantification of the gliadin, LMW-GS and HMW-GS in each gel was carried out using the IMAGE lab 4.5.1 (Bio-Rad) software.

The total starch content in the semolina samples was determined according to the 996.11 method (AOAC, 2000), while the fat content was estimated according to the 2003.05 method (AOAC, 2000).

Total fibre content was measured according to the official 991.43 method (AOAC, 2000), and ash content according to the 942.05 method (AOAC, 2000). Chemical analyses were performed in triplicate and the results expressed on a dry matter basis.

Short-cut pasta (*tubetti*) made from both the *Biensur* and reference (*Aureo*) semolina samples (Fig. 1) was processed using a pilot system at the Pavan-Map Impianti factory (Galliera Veneta, Padua, Italy). Briefly, pasta samples were prepared in accordance with Italian legislation (PRESIDENTIAL DECREE n°187, 2001) by mixing water and semolina to form a dough with a 30% moisture content. The dough was driven through a vacuum system then extruded to mould the pasta. Samples of *Biensur* and *Aureo* pasta were dried

at two different temperatures, low (maximum temperature 60°C, for 9 h) or high (maximum temperature 85°C, for 5 h), to obtain a final moisture content of 11% DW. The pasta samples from the two wheat varieties dried at the low and high temperatures are henceforth referred to as Biensur LT, Biensur HT, Aureo LT and Aureo HT.

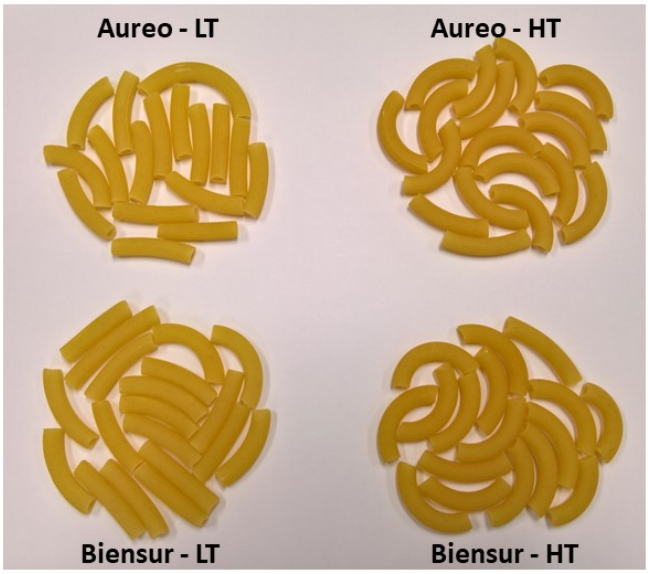


Figure 1. Appearance of the "Tubetti" pasta made from mono-varietal semolina of cv. *Biensur* compared with the reference cv. *Aureo* at two drying temperatures, 60°C for 9 h (LT) or 85°C for 5 h (HT).

2.3. Farinographic evaluation

The properties of the semolina samples were measured with a farinograph (T6, Promylograph, Max Egger, Austria) according to the approved 54-21 method (AACC, 2000). Farinograph tests allowed us to determine: (i) the water absorption (g water per 100 g of semolina) required to reach a dough consistency of 500 PU (promylograph units); (ii) dough stability, defined as the length of time the dough maintains its maximum consistency; (iii) dough weakening, defined as the reduction in dough consistency (as PU) after 20 minutes of mixing. The analyses were performed in triplicate.

2.4. Cooking properties

2.4.1 Determination of optimal cooking time

Pasta samples (50 g) were cooked in deionised boiling water (500 mL). Optimal cooking time (OCT), defined as "al dente", was determined by pressing the pasta between two glass slides at different times during cooking and observing the time it took the starchy white core of the pasta to disappear (ABÉCASSIS *et al.*, 1994).

2.4.2 Cooking loss and water absorption

The pasta samples were drained immediately at the OCT to halt the cooking process. Cooking loss was defined as the amount of solids lost in the cooking water (D'EGIDIO *et al*., 1990). In brief, the cooking water was collected in a beaker, placed in an air oven at 110°C and evaporated until dry. Cooking loss was the weight of the residue expressed as a percentage of the initial weight of the pasta (g solids per 100 g of dry pasta). Water absorption was measured as the increase in the weight of the pasta after cooking and expressed as a percentage of the weight of the uncooked pasta. Cooking losses and water absorption were determined with 3 individual measurements (replicates).

2.4.3 Texture analyses and colour determination

Pasta firmness, *viz.* the resistance to a bite with the incisors through the cooked pasta, and stickiness, i.e., the material adhering to the surface of the cooked pasta, were determined using a TA.XT plus Texture Analyser (Stable Micro Systems, UK) equipped with a 5 kg load cell. The firmness test was performed according to the AACC 16-50 method. A single *tubetto* (12 mm thick) was oriented perpendicularly to a knife probe, cut, then compressed at a speed of 0.5 mm/s. Firmness was measured as the maximum peak force curve (N) required to compress the pasta sample.
For the stickiness test, the pasta was oriented perpendicularly, as described above, to a rectangular probe. Excess water was removed before testing. The probe applied a compression force to the pasta sample (1 kg), was held in contact for 2 seconds then withdrawn at the same speed as above (0.5 mm/s). Stickiness was measured as the maximum peak force curve (g/s) required to withdraw the probe from the surface of the sample. The average value of five replicates was reported for each test.
The colour of cooked pasta was determined using a reflectance colorimeter (Minolta®, CR300, Japan) following the CIE-$L^*a^*b^*$ colour system, where the L* value (brightness) ranges from black (0) to white (100), the chroma a^* value ranges from green (-60) to red (+60), and the chroma b^* value ranges from blue (-60) to yellow (+60) (MINOLTA, 1993). Each colour data represents the mean of three measurements on different pasta samples.

2.4.4 Sensory evaluation of pasta

To assess acceptability of the pasta made from cv. *Biensur*, a sensory evaluation was carried out by 15 panel members (9 women, 6 men; ages ranging from 22 to 40 years) with experience in general food evaluation. The four pasta samples (*Biensur* HT and LT, and *Aureo* HT and LT) were cooked "al dente" without the addition of salt, drained and kept warm until serving in randomised order on plastic plates labelled with random 2-digit codes. Panellists were asked to evaluate colour, flavour and texture properties (firmness, stickiness) on a five-point scale from 1, low intensity, to 5, high intensity. They were also asked to score the overall quality of the product based on these same attributes using the same five-point scale. The attribute scores for each sample and panel member were subjected to a one-way analysis of variance (ANOVA) to obtain mean sensory scores for each of the 15 panel members.

2.5. Statistical analysis

Statistical analysis of the data was performed with the Statgraphics Centurion XIV software (StatPoint Technologies, Inc., Warrenton, VA, USA) and the results compared by

one-way ANOVA. Significant differences between treatments were determined by Tukey's test.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of the semolina and rheological properties of the dough

Table 1 shows a comparison of the compositions of the refined semolina from cv. *Biensur* and the commercial high-quality semolina from cv. *Aureo*, which is already used in Italy to produce the mono-varietal pasta Voiello® (Naples, Italy). Although *Biensur* had a lower protein content (138.8 *vs*. 146.8 mg/g DW), the levels were high in both compared with the minimum levels (105 mg/g DW) required by Italian legislation (Presidential decree n° 187, 2001) and were commercially acceptable. In pasta making, the quantity and quality of wheat storage proteins is important in determining essential dough properties, such as stability and firmness (SAMAAN *et al.*, 2006; SISSONS, 2008).

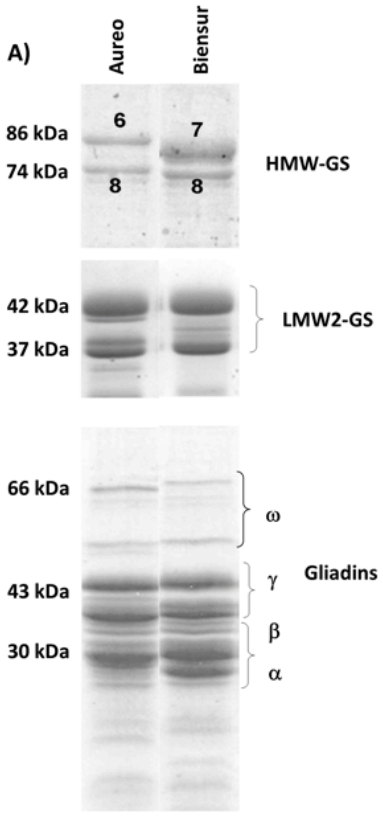
Table 1. Chemical and gluten protein composition of semolina samples (mg/g of DW) of cv. *Biensur* compared with the commercial reference cv. *Aureo*.

	Total protein[1]	Gli[2]	HMW-GS[2]	LMW-GS[2]	GS/Gli	HMW/LMW-GS	Moisture	Total fibre	Starch	Ash	Lipids
Biensur	138.8^{b}	64^{a}	12^{a}	24^{b}	0.56^{b}	0.51^{a}	14.11^{a}	3.17^{a}	74.9^{a}	0.79^{a}	1.73^{a}
Aureo	146.8^{a}	58^{b}	12^{a}	30^{a}	0.72^{a}	0.42^{b}	13.65^{a}	3.10^{a}	73.2^{b}	0.80^{a}	1.80^{a}

Within each parameter, different letters indicate significant differences (Tukey test, $P \leq 0.05$; n = 5).
[1]Kjeldahl method.
[2]Percentage of total gluten proteins, which were: *Biensur* 22.3±0.33, *Aureo* 24.2±0.06 mg/g semolina.

Biensur has been recently recognised as a high-yielding variety, with higher GS/gliadin and HMW/LMW-GS ratios than other Italian cultivars, and an optimal allelic GS configuration (Bx7 and By8) (Fig. 2), suggesting that high productivity can be combined with good quality through suitable breeding programmes (VISIOLI *et al.*, 2018).
The HMW-GS configuration is indicated as Bx7 and By8 for cv. *Biensur* and as Bx6 and By8 for cv. *Aureo*. The LMW-GS pattern in the two varieties is indicated as the LMW-2 protein group, which, in the modern cultivars, replaced the low quality LMW-1 protein configuration (D'OVIDIO and MASCI, 2004). The gliadin fractions ω, γ, α/β were indicated according to the molecular weight range in relation to molecular weight markers.
Besides protein content, the types of gluten proteins, and the ratios between glutenins and gliadins, and between HMW-GS and LMW-GS are known to be directly related to the balance between dough strength and extensibility (SAMAAN *et al.*, 2006; SISSONS, 2008). *Biensur* semolina had a lower total gluten protein content than *Aureo* (22.3±0.33 *vs*. 24.2±0.06 mg/g flour; $P \leq 0.05$), a lower percentage of the LMW-GS fraction and a higher gliadin fraction with respect to total gluten proteins (Table 1). However, our results confirm *Biensur* as having a higher HMW/LMW-GS ratio than *Aureo* (0.51 *vs*. 0.42) and an acceptable GS/Gli ratio (0.56), which are important parameters for gluten technological quality (Table 1). We also found differences between the varieties in the abundances of the HMW-GS x-type and y-type sub-units and the most common LMW-GS (42 and 37 kDa), as

previously reported (VISIOLI *et al.*, 2018). *Biensur* had higher proportions of x-type HMW sub-units and 37 kDa LMW-GS than *Aureo* (Fig. 2). Regarding the gliadin fractions, *Biensur* had a greater amount of α/β gliadins (rich in Cys residues) and a lower fraction of ω-gliadins (poor in Cys residues) than *Aureo,* although they had similar amounts of γ-gliadins, which are very rich in Cys residues. Gluten composition and the relative amounts of sub-units are known to contribute to the technological quality of semolina. *Biensur* also had more starch than *Aureo* (749 *vs*. 732 mg/g DW).



B)

	Gliadins			HMW-GS		LMW-GS	
	ω	γ	α/β	x-type	y-type	42 kDa	37 kDa
Aureo	18.8	37.5	43.7	47.3	52.7	73.5	26.5
Biensur	14.7	36.8	48.5	65.7	34.3	67.3	32.7

Figure 2. SDS-PAGE of HMW-GS, LMW-GS and gliadin sub-units extracted from cv. *Aureo* and *Biensur* semolina (A), and their relative abundances (%) obtained by densitometric analysis (B).

We compared samples of *Biensur* and *Aureo* for dough stability and weakening using a farinograph (Table 2) and found the two varieties to have very similar levels of dough stability, while cv. *Aureo* had better indices of dough weakening and water absorption. Although there were no significant differences between the two varieties in dough stability, after 20 minutes of mixing the *Biensur* dough was found to have a higher dough weakening index, meaning a lower tolerance to mechanical mixing.

Table 2. Farinographic indices (means; n = 3) of dough samples of cv. *Biensur* compared with the commercial reference cv. *Aureo*.

	Water absorption (%)	Dough stability (min)	Dough weakening (PU)
Biensur	52.4[b]	11.5[a]	35[a]
Aureo	55.7[a]	12.0[a]	25[b]

Within each parameter, different letters indicate significant differences (Tukey test, $P \leq 0.05$).

3.2. Physical and sensory characteristics of pasta

We looked at the most important quality indicators to properly compare the pasta obtained from the two varieties. Good quality pasta should meet the criteria of high water absorption, low cooking losses and good texture (CUBADDA *et al.*, 2007; BRUNEEL *et al.*, 2010). After cooking, it should be firm enough to resist surface disintegration and have no excessive stickiness.
The data regarding water absorption, cooking loss, firmness and stickiness of all pasta samples at the optimal cooking time are similar for the two durum wheat varieties (Table 3).

Table 3. Cooking properties [optimal cooking time (OCT), water absorption and cooking loss (n = 3)], firmness and stickiness measured by Texture Analyzer (n = 3), and colour indices of pasta samples of cv. *Biensur* compared with the commercial reference cv. *Aureo* dried at different temperatures (LT = 60°C for 9 h; HT = 85°C for 5 h).

		Cooking quality				Colour		
	OCT (min.sec)	Water absorption (%)	Cooking loss (%)	Firmness (N)	Stickiness (g/s)	L*	a*	b*
Biensur HT	9.0	111.90[a]	2.96[a]	5.70[a]	81.6[a]	57.10[a]	-1.15[a]	17.6[a]
Biensur LT	8.3	111.58[a]	3.00[a]	5.65[a]	82.3[a]	58.67[a]	-2.4[b]	18.0[a]
Aureo HT	9.0	111.90[a]	2.96[a]	5.80[a]	80.1[a]	60.57[a]	-1.9[ab]	18.4[a]
Aureo LT	8.3	111.70[a]	2.97[a]	5.71[a]	81.1[a]	59.40[a]	-2.41[b]	17.7[a]

Within the same parameter, values with the same letter are not significantly different from each other (Tukey test, $P \leq 0.05$).

Analyses of variance for these parameters did not reveal any significant differences between *Biensur* and *Aureo* pasta dried at the same temperature. This provides confirmation that *Biensur*, despite having a lower protein content than *Aureo*, has good gluten quality and is therefore suitable for high quality pasta production. There were also no significant differences between *Biensur* and *Aureo* pasta dried at different temperatures: in all cases the gluten network seems to provide similar shear resistance and equally restricts starch swelling and leaching. This was probably because the gluten quality of cv. *Biensur* has a better HMW-GS configuration (Bx7-By8) and a higher HMW/LMW-GS ratio than *Aureo*, as well as an acceptable GS/Gli ratio, as previously described (Table 1), which plays an important role in the formation of a strong protein network.
Moreover, we consider that the difference between the LT and HT drying temperatures is not so great as to affect the pasta structure. Indeed, only large increases in drying temperature would modify the pasta structure, with positive effects on the sensory

properties and cooking quality (PASINI *et al.*, 2015; PADALINO *et al.*, 2016), especially when total protein content is low (CUBADDA *et al.*, 2007). High drying temperatures, particularly >70 °C, are also known to lower protein digestibility (PETITOT *et al.*, 2009; STUKNYTE *et al.*, 2014).
In this trial, we detected slight improvements to pasta firmness and lower cooking losses in both varieties dried at the high temperature compared with the lower (85 °C *vs*. 60 °C), but they were not statistically significant. However, *Biensur* was slightly stickier than *Aureo*, probably due to its higher starch content, and the higher drying temperature seems to be effective in slightly reducing this effect.
The sensory properties of the cooked pasta, such as colour, flavour and texture (firmness and stickiness), play an essential role in determining consumer acceptability of the product, especially in traditional pasta-consuming countries (D'EGIDIO and NARDI, 1998).
Sensory evaluation of pasta made from the cv. *Biensur* and from the reference cv. *Aureo* showed there to be no significant differences between them for any of the parameters tested (Table 4), which is consistent with the texture analysis (Table 3) and hence shows good overall acceptability.
Texture and flavour appear to play a major role in sensory evaluation, but the initial impact is also highly influenced by colour. Similar brightness (L^*) and b^* values were observed for all cooked pasta samples. Differences between the HT and LT pasta samples were found, as indicated by a significant increase in the a^* value (redness) under the higher drying temperature (Table 3), which is known to be correlated with non-enzymatic browning (ANESE *et al.*, 1999).
Although it is difficult to compare results from different studies because of the different drying cycles and raw materials used, our results are in accordance with those of other authors who investigated the effects of drying temperatures and the role of gluten content in pasta quality (CUBADDA *et al.*, 2007; PADALINO *et al.*, 2016).

Table 4. Summary of the sensory properties (n = 15) of pasta samples of cv. *Biensur* compared with the commercial reference cv. *Aureo* dried at different temperatures (LT = 60°C for 9 h; HT = 85°C for 5 h).

	Colour	Flavour	Firmness	Stickiness	Overall acceptability
Biensur HT	2.7[a]	3.5[a]	4.4[a]	1.7[a]	3.0[a]
Biensur LT	2.5 [a]	3.5 [a]	4.1[a]	1.9 [a]	2.8 [a]
Aureo HT	3.0[a]	3.8[a]	4.8[a]	1.5[a]	3.5[a]
Aureo LT	2.6[a]	3.7[a]	4.5[a]	1.4[a]	3.4[a]

Each attribute was assessed on a 5-point scale from 1, low intensity, to 5, high intensity

4. CONCLUSIONS

To cultivate durum wheat in the northern latitudes of the Mediterranean region, greater attention needs to be paid to varietal choice and crop management, particularly nitrogen nutrition and pathogen control, as the climatic conditions are extreme for this species (lower temperatures, higher humidity). Currently, high quality semolina is mainly associated with high-protein cultivars, such as *Aureo*, the reference in our trial, although this variety commonly fails to reach high grain yield targets and may not be economically sustainable for farmers in the potentially high-yield, fertile soils of NE Italy.
One of many wheat cultivars, *Biensur* grown at the extreme northern edge of the Mediterranean region has been recently found to have high yield, appreciable protein

contents and a good gluten sub-unit configuration for pasta making (VISIOLI *et al.*, 2018). We therefore felt there was a need to assess whether the characteristics of this cultivar meet the requirements for producing high-quality pasta from large field cultivations, and whether the drying temperature may mitigate possible weaknesses during processing.
This study suggests that cv. *Biensur* is a good candidate to increase Italy's production of mono-varietal pasta by extending cultivation of it to the more fertile soils of the Po plain, given that the dough has high technological characteristics and the pasta very good sensory properties, comparable to well-established commercial mono-varietal semolinas. The effect of drying temperature (high or low) was minimal, suggesting that the intrinsic characteristics of individual varieties are of central importance, as reported by PADALINO *et al.* (2014), and that the most energy/economically sustainable technological processes can be selected without compromising pasta quality.
There is currently rising market demand for mono-varietal brands, a situation that could stimulate local cultivation of specific durum wheat cultivars to supply short-chain pasta production, thereby offering new market opportunities for farmers and traders, especially in light of the recent Italian decree (LEGISLATIVE DECREE, 2017) requiring the origin of the wheat to be indicated on the label. As all the steps in this project (cultivation, milling, pasta-making) were carried out on a large scale, we are confident that the results will be useful for future development of the chain in NE Italy. Furthermore, having demonstrated that essential sensory (mechanical) properties, such as firmness and stickiness, can be faithfully measured by a texture analyser and panellists' judgements, we are sure that consumers will find the quality of pasta made from cv. *Biensur* acceptable.

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PAPER

DEHYDRATION AND REHYDRATION CHARACTERISTICS OF PRETREATED PUMPKIN SLICES

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ABSTRACT

The influence of an alternative chemical pretreatment on dehydration and rehydration of an Italian ecotype pumpkin was investigated. The pretreatment consisted of soaking the slices in a diluted solution of trehalose, sucrose and NaCl. Hot air-drying was performed in a convective dryer at temperatures of 55, 60, 65 and 70°C. Samples treated prior to drying showed a shorter (about 1/4) drying time, less volume shrinkage and colour changes, but showed higher rehydration capacity compared to untreated ones, especially in the range 55-65°C. Moreover, the pretreatment was effective in retention of total phenolic content and antioxidant activity. The Midilli model was the most appropriate for describing drying behaviour, while the Weibull model for rehydration.

Keywords: drying, kinetic model, pretreatment, pumpkin, rehydration

1. INTRODUCTION

Pumpkin (*Cucurbita maxima*) belongs to *Cucurbitacae* family. Botanically it is a squash fruit, most commonly orange in colour when ripe, that has been used traditionally both as human and as animal feed (GUINÉ *et al.,* 2011). Its nutritive value is encouraging an increase in the consumption and its use for nutritional and technological applications. Pumpkin is rich in antioxidants and vitamins, which have an important health-protecting effect. It is also poor in total solids (AREVALO-PINEDO and MURR, 2006) and in calories, which means that it is adequate for low calories regimes and it is often recommended in diets (SHELKE *et al.,* 2015).

Fresh pumpkin should be stored at temperature between 10 and 13°C and relative air humidity between 50% and 70%. When stored at low temperature, unfavourable physiological processes occur. The above-mentioned processes cause chill damages. Therefore, it is desirable to use optimum methods of pumpkin preservation, appropriate for the specific final use of the fruit (SOJAK and GLOVACKI, 2010). Pumpkin is generally processed to obtain the juice, pomace, pickles, dried products in many countries worldwide.

Drying is an excellent method to preserve the pumpkin flesh that can add variety to meals and provide delicious and nutritious ready-to-eat crispy snacks. Dried pumpkin may be a finished product or a half-finished product, subject to further processing (SEREMET *et al.,* 2016). Dried and rehydrated pumpkins are key ingredients in dairy products, breakfast cereals, traditional foods (such as puddings, desserts, cakes, biscuits) and dietetic foods formulated for people suffering from physiological disorders or for healthy people with additional needs. Rehydration product behaviour must be known when a total or partial reconstitution is required (Contreras *et al.,* 2012).

Pumpkin slices are generally dried using the convective method (SOJAK and GLOVACKI, 2010), because of its simplicity and low cost. Using this technique, mass and heat transfer occur simultaneously (ADILETTA *et al.,* 2014). Hot air drying produces stable dehydrated products, but unfortunately their final quality (i.e. colour, texture) is drastically reduced when compared to the fresh product due to the high temperatures and longer times involved in the process (BRASIELLO *et al.,* 2017; BRASIELLO *et al.,* 2013; RUSSO *et al.,* 2013).

Drying combined with a pretreatment appears to be a cost-effective method of preservation. Several methods of pretreatment have been widely utilised: such as immersion in chemical solutions (ADILETTA *et al.,* 2016a), hot-water blanching (XIN *et al.,* 2015) and physical pretreatments (DI MATTEO *et al.,* 2000; SENADEERA *et al.,* 2014; ADILETTA *et al.,* 2016b). Pretreatments prior to drying have been reported to help reducing some of undesired changes such as antioxidant activity reduction, colour and textural changes. Also, they reduce drying time by relaxing tissue structure and yield good quality dried products (ADILETTA *et al.,* 2016a; SENADEERA *et al.,* 2014; ATKAS *et al.,* 2007; BRASIELLO *et al.,* 2011). In this way, when pretreatments are used, final products differ from those without pretreatment. The differences are evident in many properties, including also the rehydration capacity (PEREZ and SCHMALKO, 2009).

In the literature, several works have been reported on the effect of pretreatment on drying kinetics and quality of pumpkin. They include blanching, salt coating, osmotic dehydration, SO_2 or citric acid treatments. Many researchers have focused on novel anti-browning agents to replace sulphites (ADILETTA *et al.,* 2016a; FALADE and SHOGAOLU, 2010; MAYOR*ET al.,* 2011).

WORKNEH *et al.* (2014) studied the effect of two pretreatments on the quality of dried pumpkin slices: blanching (60°C for 1 min) and 10 % of salt solution (room temperature for 10 min). Salted pumpkins that were subjected to oven-drying at 60°C required shorter

drying time than those blanched to attain 10 % moisture content. Moreover, salted pumpkin slices showed higher total soluble sugars, ascorbic acid, sugar to acid ratio, pH value, lower total acidity and shorter time of drying (18 h) with respect to the untreated pumpkin slices (28.3 h).

FALADE and SHOGAOLU (2010) investigated the effect of three pretreatments on air-drying pattern and colour of pumpkin slices. Untreated, sulfited (1,000 ppm), blanched (100°C for 3 min) and osmotically pretreated pumpkins (40, 50 and 60°Brix) were air dried at 50-80°C. At 60°C fresh (untreated) pumpkin slices showed the higher initial drying rate compared to the pretreated ones. Moreover, sulfited and blanched pumpkin slices showed higher drying rate than the osmotically pretreated ones due to water removal during osmotic pretreatment of pumpkin. Similar trends were observed by air-drying at 50, 70 and 80°C. The difference in the drying rates of osmotically and un-osmotically treated pumpkin could be related to the increase in internal resistance to water movement caused mainly by shrinkage and solid uptake during the osmotic step.

Mayor *et al.* (2011) studied the changes in volume, density, porosity and shape factors of pumpkin tissue during osmotic dehydration carried out with solutions of sucrose, sodium chloride and mixtures of both solutes at different temperatures and air drying conducted at 70°C. The osmotic dehydration experiments were conducted with sodium chloride solutions (5, 10 and 20 kg/100 kg at 25, 38 and 12°C, respectively) and with binary solutions 3.75% NaCl-58% sucrose and 7.5% NaCl-45% sucrose (25°C). The authors observed a linear decrease of volume with water loss during osmotic dehydration: this decrease is more accentuated in the case of NaCl solutions, followed by sucrose solutions and NaCl/sucrose solutions.

In the studies reported above, blanching at high temperature and osmotic solutions of sugar and salt exceeding 10% for long contact time were used. The blanching pretreatment is used to: inactivate the enzymes, maintain the freshness, colour, stabilize the texture and nutritional quality, expel the air between the cells and destroy the microorganisms to some extent. However, blanching treatment causes undesirable changes in the quality properties of food such as the loss of soluble nutrients (i.e. sugars, minerals and vitamins) (XIN *et al.*, 2015). Moreover, it causes loss of aroma and negatively impacts the sensory properties associated with texture (LESPINARD *et al.*, 2009).

Dipping of plant tissue in solutions containing 15–30% sugars or salts for a long time results in shortening of the drying process and, hence, in lower energy requirements. Osmotic dehydration offers high retention of initial food characteristics, such as colour and flavour (SHELKE *et al.*, 2015). On the other hand, osmotic dewatering adversely affects reconstitution properties of dry material and causes softening of the tissue (LEWICKI, 2006).

In this framework, the aim of this work was to investigate the influence of an alternative pretreatment, by dipping pumpkin slices in a diluted solution of trehalose, NaCl and sucrose, on drying behaviour, rehydration capacity and some physico-chemical properties (i.e. colour, total phenolics, antioxidant activity, shrinkage) of pumpkin. The drying kinetics at four temperatures 55, 60, 65 and 70°C were fitted with different kinetic models found in literature. The effective moisture diffusivities were then estimated by using Fick's second law of diffusion for the present operating conditions. Also, the rehydration kinetics were fitted with empirical models.

2. MATERIALS AND METHODS

2.1. Sample preparation

The pumpkin used in this study is a *C. maxima* ecotype known as "di Teggiano" which is cultivated in Campania region, Italy. Fresh whole pumpkin was washed, peeled, sliced. Cylindrical slices with a diameter of 30 mm and thickness of 6 mm were prepared using a suitable steel mould. The zone near the peel (<10 mm) was removed because of its different texture. The initial moisture content was 0.93 g H_2O /g of sample (or on dry basis (db) 15.23±0.05 g H_2O /g db) (AOAC, 1990).
Two types of samples were used: (1) without pretreatment (UTR), with pretreatment (TR). The pretreatment was carried out by soaking the samples in an aqueous solution of 0.8% (w/v) trehalose, 0.1% (w/v) NaCl and 0.2% (w/v) sucrose for 5 min at 25°C. Following submersion, the samples were removed from the bath and blotted with tissue paper.

2.2. Drying kinetics: experiments

The drying experiments were conducted at constant temperatures of 55, 60, 65 and 70°C using a convective dryer (Zanussi FCV/E6L3) with a constant air flow rate of 2.3 m/s, until they reached a moisture content under 5% (wet basis) as suggested by GUINÉ *et al.* (2011). During drying at fixed times, pumpkin samples were withdrawn from the dryer and their weight was measured by a digital balance (mod. Gibertini E42, Italia). Drying tests were replicated three times at each temperature.
The results were reported in terms of moisture ratio:

$$M_R = (M_t - M_e)/(M_0 - M_e) \qquad (1)$$

where M_t is the actual moisture content (g H_2O/g db), M_0 is the initial moisture content (g H_2O/g db), M_e is the final moisture content at the end of process (g H_2O/g db) until no measurable weight change was observed, which is assumed equivalent to the equilibrium moisture content. All samples obtained at different drying temperatures were then characterized.

2.3. Drying kinetics: modelling procedure

Empirical models that are commonly applied for vegetables were here adopted (Table1) (HENDERSON and PABIS, 1961; PARK *et al.*, 2002; DOYMAZ, 2007; KASHANINEJAD and TABIL, 2004; MIDILLI *et al.*, 2002; HENDERSON, 1974).

Table 1. Mathematical models applied to drying curves.

Model name	Eqution	Reference
Henderson and Pabis	$M_R = aexp^{(-kt)}$	Henderson and Pabis (1961); Park *et al.* (2002)
Page	$M_R = exp^{(-kt)}$	Doymaz (2007); Kashaninejad and Tabil (2004)
Midilli	$M_R = aexp^{(-kt)} + bt$	Midilli *et al.* (2002)
Two term	$M_R = a_1exp^{(-k_1t)} + a_2exp^{(-k_2t)}$	Henderson (1974)

The empirical constants for the drying models were determined from normalized drying curves (M_R vs time) at each drying temperature. Non-linear least square regression

analysis was used to evaluate the parameters of the selected model with the Levenberg-Marquardt procedure.
The goodness of fit for each model was evaluated based on the statistical parameters: R^2, RMSE, χ^2.These parameters were calculated from the following equations:

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(M_{R,pre,i} - M_{R,exp,i}\right)^2\right]^{1/2} \quad (2)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(M_{R,pre,i} - M_{R,exp,i}\right)^2}{N-z} \quad (3)$$

where $M_{R,exp,i}$ and $M_{R,pre,i}$ are experimental and predicted dimensionless moisture ratios, respectively, N is the number of observations, and z is the number of constants. R^2 was used as the primary comparison criteria for selecting the best model to fit the experimental data. Its value should be higher and close to one. Also, a model is considered better than another if it has a lower value of RMSE and χ^2.
The continuous decrease in moisture ratio with increase in drying time shows that the results can be interpreted by using Fick's second law of diffusion. Considering pumpkin cylindrical slice to be infinite cylinder, the solution of Fick's diffusion equation was as follows (CRANK, 1975; SENADEERA *et al.*, 2003):

$$M_R = \frac{(M_t - M_e)}{(M_0 - M_e)} = \sum_{n=1}^{\infty}\frac{4}{\beta_n^2} exp\left[-\frac{\beta_n^2 D_{eff} t}{r_c^2}\right] \quad (4)$$

where, M_R is the dimensionless moisture ratio, M_t is the moisture content on dry basis at time t (g H_2O/g db), M_0 is the initial moisture content (g H_2O/g db), M_e is the equilibrium moisture content (g H_2O/g db), β is the roots of the Bessel function, D_{eff} is the effective diffusion coefficient (m^2/s), r_c is the cylinder radius and n is the positive integer.
The aforementioned equation is based on the following assumptions: i) isothermal drying conditions, ii) constant effective diffusivity, iii) negligible shrinkage, iv) uniform initial moisture content, v) negligible external resistance. For long drying times (M_R< 0.6), when r_c is small and t is large, limiting form of equation is obtained for cylindrical geometry by considering only the first term in the series expansion.
Then Eq. (4) can be written as Eq. (5):

$$M_R = \frac{(M_t - M_e)}{(M_0 - M_e)} = \frac{4}{\beta_1^2} exp\left[-\frac{\beta_1^2 D_{eff} t}{r_c^2}\right] \quad (5)$$

A general form of Eq (5) can be written in logarithmic form (Eq. 6):

$$\ln M_R = A - Bt \quad (6)$$

where, the constant B is $\frac{\beta_1^2 D_{eff}}{r_c^2}$. The slope B is calculated by plotting ln M_R versus time according to Eq. (6). The effective diffusivity is the derived from the slope B.
The dependence of the effective moisture diffusivity on temperature is generally described by the Arrhenius equation as given in Eq. (7).

$$D_{eff} = D_0 exp\left(-\frac{E_a}{RT}\right) \quad (7)$$

Where D_0 (m^2/s) is the temperature-independent constant, E_a (J/mol) the activation energy, R (8.314 J/mol K) the universal gas constant and T (K) the absolute temperature. Activation energy was calculated by plotting the natural logarithm of D_{eff} against the reciprocal of the absolute temperature.

2.4. Chemical and physical characterization

2.4.1 Colour parameters

Pumpkin slices colour was determined by two readings on the two different symmetrical faces of the slices in each replicate, using a Minolta Chroma Meter II Reflectance CR-300 colorimeter (Minolta, Osaka, Japan), calibrated with a white standard tile. It was recorded using CIE L*a*b* uniform colour space (CIE-Lab). The colour coordinate L* measures the whiteness value of a colour and ranges from black at 0 to white at 100. The chromaticity coordinate a* measures red when positive and green when negative, and the chromaticity coordinate b* measures yellow when positive and blue when negative (BERNS, 2000). Also, the overall colour difference (ΔE) (Eq.8), was calculated from L*, a* and b* values and used to describe the colour change during drying.

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (8)$$

2.4.2 Total phenolic content and antioxidant activity (EC50)

Total phenolic content (TPC) was extracted from fresh and dried samples with solution MeOH:H_2O=80:20 for three times following the method described by DINI *et al.* (2013). All extractions were performed in triplicate. The concentration of TPC was measured using the Folin Ciocalteu reagent (ADILETTA *et al.*, 2017). The absorbance was evaluated after 90 min at room temperature at λ = 760 nm using a spectrophotometer (Lambda Bio 40; Perkin Elmer, Waltham, MA, USA). Quantification was based on a standard curve generated with gallic acid.
Total phenolic content of extracts were then expressed as mg gallic acid equivalents per gram of dried basis (mg GAE/g db) that was derived from a calibration curve.
The free radical scavenging capability of the extract was determined using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Dini *et al.*, 2013). Aliquots (200 mL) of extract solutions were added to 3 mL of DPPH solution ($6 \cdot 10^{-5}$ mol/L). Then, the absorbance of DPPH without antioxidant (control sample) was used for baseline measurements. The scavenging activity was expressed as the 50% effective concentration (EC_{50}), which was defined as the sample concentration (mg) necessary to inhibit the DPPH radical activity by 50% during a 60-min incubation.

2.5. Shrinkage

The initial pumpkins volume (V_0) was calculated by measuring for each sample (about 10 slices) diameter and thickness by means of a digital Vernier caliper (0.01 mm accuracy). At given times during drying experiments for the same slices, the diameter and the height (or thickness) of the sample were measured and the volume (V) was calculated. In order to reduce the measurement error during drying, both dimensions were measured at different positions of the sample and their average value was considered. For the evaluation of shrinkage during drying, the mean volume shrinkage (V/V_0) was reported as a function of the relative moisture ratio M_R(ADILETTA *et al.*, 2014).

2.6. Rehydration tests

Rehydration curves were obtained by soaking the dried samples at room temperature in distilled water. Approximately 1 g of dried samples was added to 100 mL distilled water. The samples were removed, dried off with tissue paper and weighed at regular intervals. Weights of dried and rehydrated samples were measured by using an electronic digital balance (mod. Gibertini E42, Italia). The measurements were repeated three times.
The curves were reported in terms of moisture ratio (M_t/M_d) versus time, where M_t is the actual and M_d is the initial moisture content on dry basis of the dried sample.
The degree of structural disruption after the process was evaluated by means of the coefficient of rehydration defined as follows:

$$COR = \frac{m_{rh}(100 - X_0)}{m_{dh}(100 - X_{dh})} \quad (9)$$

where m_{rh} is the mass of the rehydrated sample (kg), m_{dh} is the mass of dried sample prior to the rehydration test (kg), X_0 is the moisture percentage of the sample before drying (% wet basis), and X_{dh} is the moisture percentage of the dried sample prior to the rehydration test (% wet basis) (MCMINN and MAGEE, 1997). Each test was performed in triplicate and the reported data were average of these three tests.

2.7. Rehydration kinetics: modelling

The rehydration kinetics were described by the models reported in Table 2 (PELEG, 1988; GOULA and ADAMOPOULOS, 2009). The Peleg equation is a two-parameter, non-exponential, empirical model for the description of moisture sorption curves. This model has been widely used due to its simplicity and has been reported to adequately describe the hydration of various foodstuffs (PELEG, 1988; GARCÍA-PASCUAL*et al.*, 2006; MOREIRA *et al.*, 2008).
In the Peleg equation t is the time (min), A_1 is a kinetic parameter (Peleg rate constant), A_2 is a parameter related to the equilibrium moisture content (Peleg capacity constant), M_t is the moisture content at time t, and M_d is the moisture content of dried sample used for rehydration test.
Furthermore, for the rehydration process, a power law equation based on the probabilistic Weibull model was used. In the Weibull equation A, β and α are kinetic constants of the model. The Weibull distribution has found wide application in food processing, and has been suggested for food rehydration process by several authors (GOULA and ADAMOPOULOS, 2009; MARABI *et al.*, 2003; VEGA-GÁLVEZ *et al.*, 2009).

Table 2. Mathematical models applied to rehydration curves.

Model name	Equation	Reference
Peleg	$M_t/M_d = 1 + (t/A_1 + A_2 t)$	Peleg (1988)
Veibull	$M_t/M_d = A + (1 - A)exp^{\left[-\left(\frac{t}{\beta}\right)^{\alpha}\right]}$	Goula and Adamopoulos, (2009)

Non-linear least square regression analysis was used to evaluate the parameters of the selected model with the Levenberg-Marquardt procedure. Fit quality of the models used on the experimental data was evaluated by means of statistical tests: linear regression

coefficient (R^2), root mean square error (RMSE) (Eq. 2), chi-square (χ^2) (Eq. 3) and the mean relative error, MRE (Eq. 10), which indicates the relative error of the predictions, and values below 10% are indicative of a reasonably good fit for most practical purposes (GOULA and ADAMOPOULOS, 2009). Therefore, the lowest values of MRE, RMSE and χ^2, together with the highest values of R^2, were selected as optimum criteria to evaluate fit quality of the models used.

$$MRE = \frac{100}{N}\sum_{i=1}^{N}\left|\frac{M_{R,pre,i}-M_{R,exp,i}}{M_{R,exp,i}}\right| \quad (10)$$

2.8. Statistical analysis

The means and standard deviations of experimental results were calculated from three replicates. One-way ANOVA (analysis of variance) at the level of significance $p < 0.05$ using Tukey's HSD test was performed for comparison of means in the case of colour, total phenolic content and antioxidant activity.

3. RESULTS AND DISCUSSION

3.1. Drying kinetics and empirical models

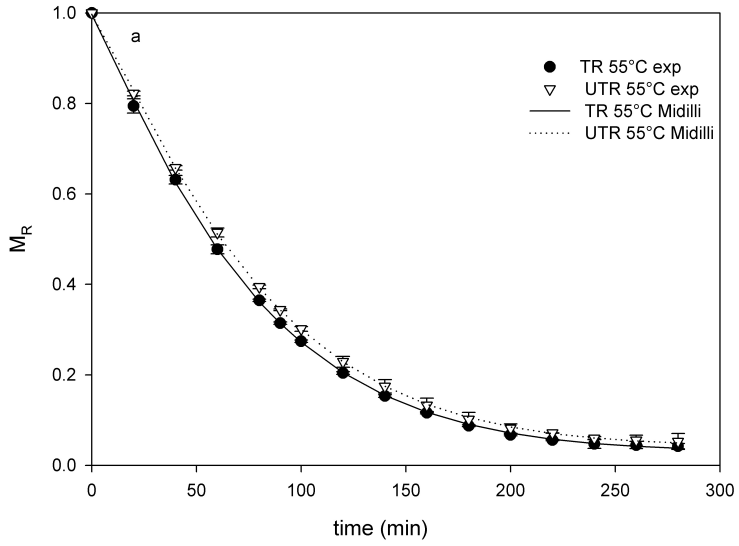
The average moisture content of fresh samples was 15.23±0.05 g water/g db (93.84% wb). Pumpkin slices were dried up to the final moisture of 0.60±0.10 g water/g db (about 4% wb) using different air temperatures (55, 60, 65 and 70°C) at 2.3 m/s.

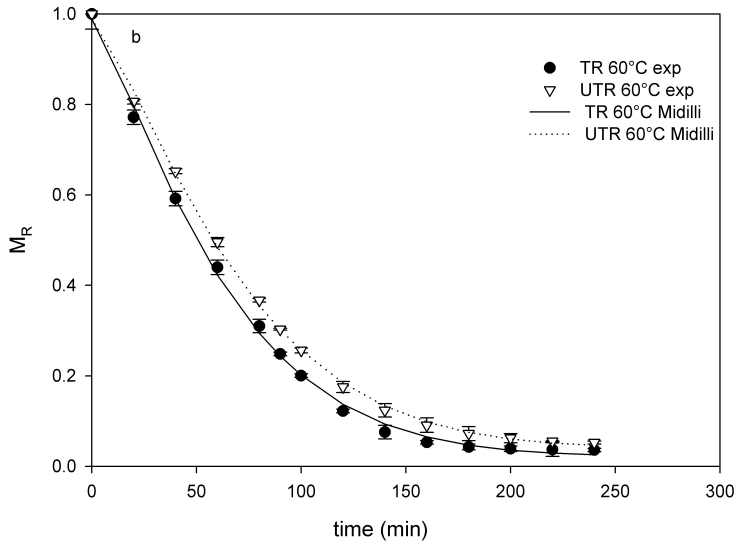
The effect of air temperature and pretreatment on the drying kinetics (M_R versus drying time) of pumpkin slices is shown in Fig. 1a-d. It can be observed that after an initial constant rate drying period, the drying process for treated/untreated pumpkins occurred in the range of the falling-rate period. In this latter stage the diffusion is the dominant mechanism governing moisture transport inside the sample. This result is in agreement with the drying behaviour of various vegetables and also for pumpkin (GUINÉ *et al.*, 2011; DOYMAZ, 2007; AGRAWAL and METHEKAR, 2017; ONWUDE *et al.*, 2016).

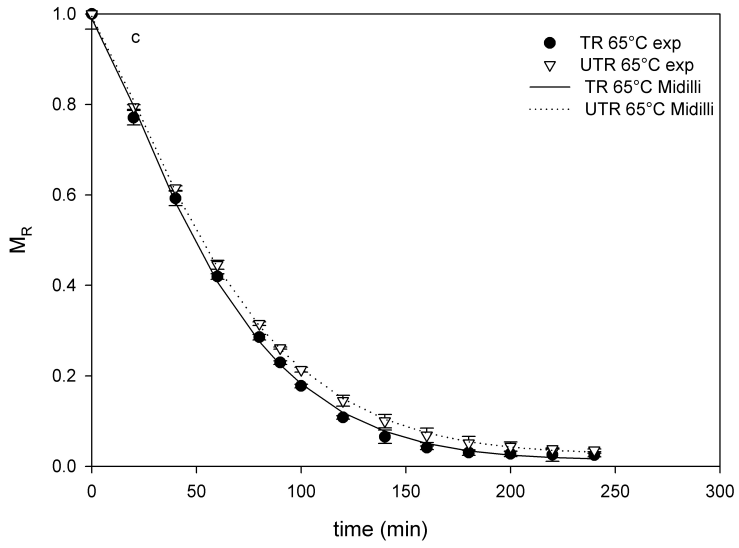
By analysing the Fig. 1, the plateau value of M_R (<0.05) for the UTR samples was obtained at the following times: 260, 220, 180 and 140 min at the air-drying temperatures of 55, 60, 65 and 70°C, respectively.

Pretreatment affects significantly drying time. Samples dipped prior to drying in sodium chloride, sucrose and trehalose solution (at low concentration) had a shorter drying time compared to control samples: at each temperature, the treated samples reached the plateau of M_R in a shorter time. It was equal to 200, 160, 140 and 120 min at 55, 60, 65 and 70°C, respectively. These results show that pretreatment solution contributed to reduce the internal mass transfer resistance that moisture encounters during drying.

Such behaviour is probably due to sugars, which effect on cell components results mainly from protecting functionality of proteins and stabilising the three-dimensional structure of protein (LEWICKI, 1998).


a
TR 55°C exp
UTR 55°C exp
TR 55°C Midilli
UTR 55°C Midilli
M_R
time (min)


b
TR 60°C exp
UTR 60°C exp
TR 60°C Midilli
UTR 60°C Midilli
M_R
time (min)


c
TR 65°C exp
UTR 65°C exp
TR 65°C Midilli
UTR 65°C Midilli
M_R
time (min)

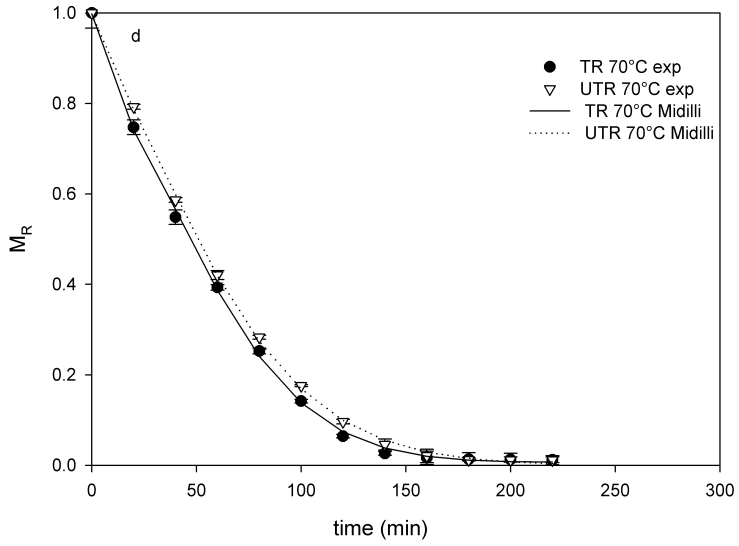


Figure 1. Experimental (symbols) and predicted (lines) drying curves of untreated (UTR) and pretreated (TR) samples at (a) 55°C, (b) 60°C, (c) 65°C and (d) 70°C.

Disaccharides such as trehalose or sucrose maintain general protein structure in the dry state, hence, the membrane is protected and upon rehydration its functionality is restored. With regard to the effect of NaCl, CURRY *et al.* (1976) suggested that NaCl penetrates tissue as ions and reassociates upon drying to form crystals. During rehydration the crystals of NaCl dissociate and form concentrated spots of Na and Cl ions. Solvation of the ions results in faster and better rehydration, as described in paragraph 3.6.

The moisture ratio data of pumpkin slices dried at different temperatures with and without pretreatment were fitted with the four models listed in Table 1. The values of R^2, χ^2, RMSE are summarized in Table 3.

Nonlinear regression was used to obtain each parameter value of every model. The best model describing the thin-layer drying characteristics of pumpkin slices was chosen as the one with the highest R^2 values and the lowest χ^2 and RMSE values. The values of parameters for each model were reported in Table 4.

The results in Table 3 show that all R^2 values are greater than 0.988, indicating a good fitting. The Midilli model gave the highest R^2 value, which varied from 0.9999 to 0.9982 for both UTR and TR samples in the experimental conditions considered in this study. The values of correlation coefficients RMSE and χ^2 for the Midilli model were the lowest for all the models considered. From the Tables 3-4, it is obvious that the Midilli model represents the drying characteristics of pretreated and untreated pumpkins better than the other models (Henderson and Pabis, Page or Two Term) considered in this study. These results confirm previous findings where Midilli model was found to be suitable in describing the drying kinetics of fruits and vegetables such as apple slices (ZAREIN *et al.*, 2013), chili (MIHINDUKULASURIYA *et al.*, 2013), mango slices (CORZO *et al.*, 2011) and pumpkin slices (AKPINAR, 2006).

Comparison between experimental data and best-fitting model results were reported in Fig 1 (a-d).

Table 3. Correlation coefficients (R^2, RMSE, χ^2) of the drying models.

Model name	Temperature (°C)	Treated			Untreated		
		R^2	RMSE	χ^2	R^2	RMSE	χ^2
Henderson and Pabis	55	0.9983	0.0116	0.0002	0.9975	0.0144	0.0002
	60	0.9931	0.0255	0.0008	0.9931	0.0251	0.0007
	65	0.9920	0.0284	0.0009	0.9942	0.0234	0.0006
	70	0.9896	0.0359	0.0015	0.9883	0.0391	0.0018
Page	55	0.9992	0.0084	0.0001	0.9988	0.0106	0.0001
	60	0.9978	0.0142	0.0002	0.9979	0.0139	0.0002
	65	0.9985	0.0117	0.0002	0.9991	0.0097	0.0001
	70	0.9972	0.0174	0.0004	0.9989	0.0113	0.0001
Midilli	55	0.9998	0.0039	0.0000	0.9999	0.0027	0.0000
	60	0.9982	0.0125	0.0002	0.9999	0.0095	0.0001
	65	0.9988	0.0106	0.0002	0.9996	0.0057	0.0001
	70	0.9993	0.0083	0.0001	0.9995	0.0069	0.0001
Two Term	55	0.9983	0.0116	0.0002	0.9975	0.0144	0.0003
	60	0.9931	0.0255	0.0009	0.9931	0.0251	0.0009
	65	0.9920	0.0284	0.0011	0.9942	0.0234	0.0008
	70	0.9896	0.0359	0.0019	0.9883	0.0391	0.0023

Table 4. Parameters of the Midilli model for drying kinetics.

Model name	Temperature (°C)	Parameter	Sample	
			Treated	Untreated
Midilli	55	a	0.9965	0.9971
		k	0.0073	0.0056
		n	1.1298	1.1729
		b	0.0001	0.0001
	60	a	0.9892	0.9896
		k	0.0052	0.0038
		n	1.2473	1.2813
		b	0.0001	0.0001
	65	a	0.9899	0.9944
		k	0.0047	0.0049
		n	1.2792	1.2539
		b	0.0000	0.0001
	70	a	0.8375	0.9115
		k	0.0008	0.0016
		n	1.6710	1.5163
		b	0.0000	0.0000

3.2. Calculation of effective diffusivity and activation energy

Effective diffusivity values for UTR and TR samples at different drying temperatures are reported in Table 5. Effective diffusivity was calculated using equations (Eq. 4-6) described in the Materials and Methods section.

Table 5. Effective moisture diffusivity for drying of untreated and treated pumpkins.

Temperature (°C)	D_{eff} (m^2/s)	
	Untreated	Treated
55	$6.75x10^{-9}$	$7.07x10^{-9}$
60	$6.87x10^{-9}$	$7.43x10^{-9}$
65	$7.68x10^{-9}$	$8.59x10^{-9}$
70	$8.51x10^{-9}$	$9.39x10^{-9}$

The D_{eff} values of pretreated samples were higher than those of the untreated samples as shown in Table 5. These values are within the range of $6.75 \cdot 10^{-9}$- $8.51 \cdot 10^{-9}$ m^2/s and $7.073 \cdot 10^{-9}$-$9.39 \cdot 10^{-9}$ m^2/s for UTR and TR samples, respectively. This means that the pretreatment considered in this study facilitates water transport from inside to the pumpkin surface because of the preservation of pumpkin structure.
The D_{eff} values obtained for pretreated pumpkin in this study were higher than those of 0.13-4.27·10^{-9} m^2/s for pumpkins oven dried at 40-80°C reported by TUNDE-AKINTUNDE and OGUNLAKIN (2011), and 3.88-9.38 10^{-10} m^2/s obtained for pumpkin slices oven dried at 50-60°C. A plot of ln D_{eff} versus 1/T was reported in Fig. 2 and the activation energy (E_a) of UTR and TR samples dried in the range 55-70°C was obtained from Eq. (7).
The values of activation energy were found to be 28.28 and 18.63 kJ/mol for UTR and TR slices, respectively. This is an indication that less energy is required for the drying of TR samples, hence, the pretreatment aids moisture diffusion and evaporation and thus reduces energy involved in the drying process.

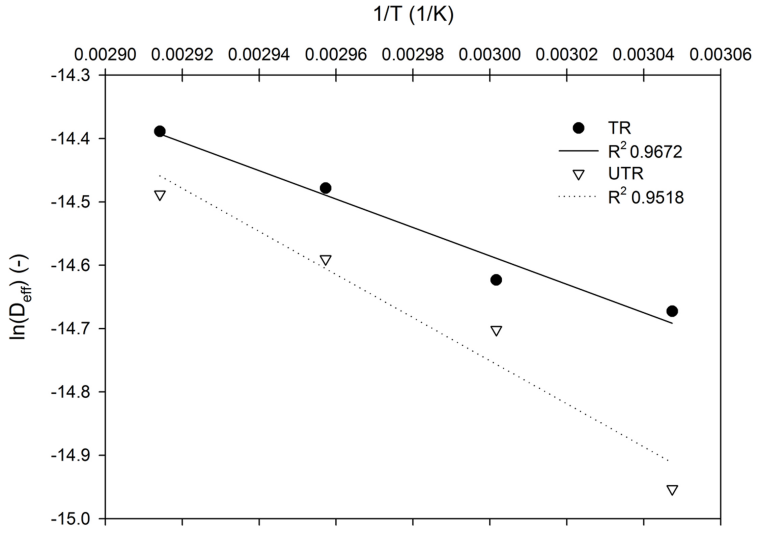
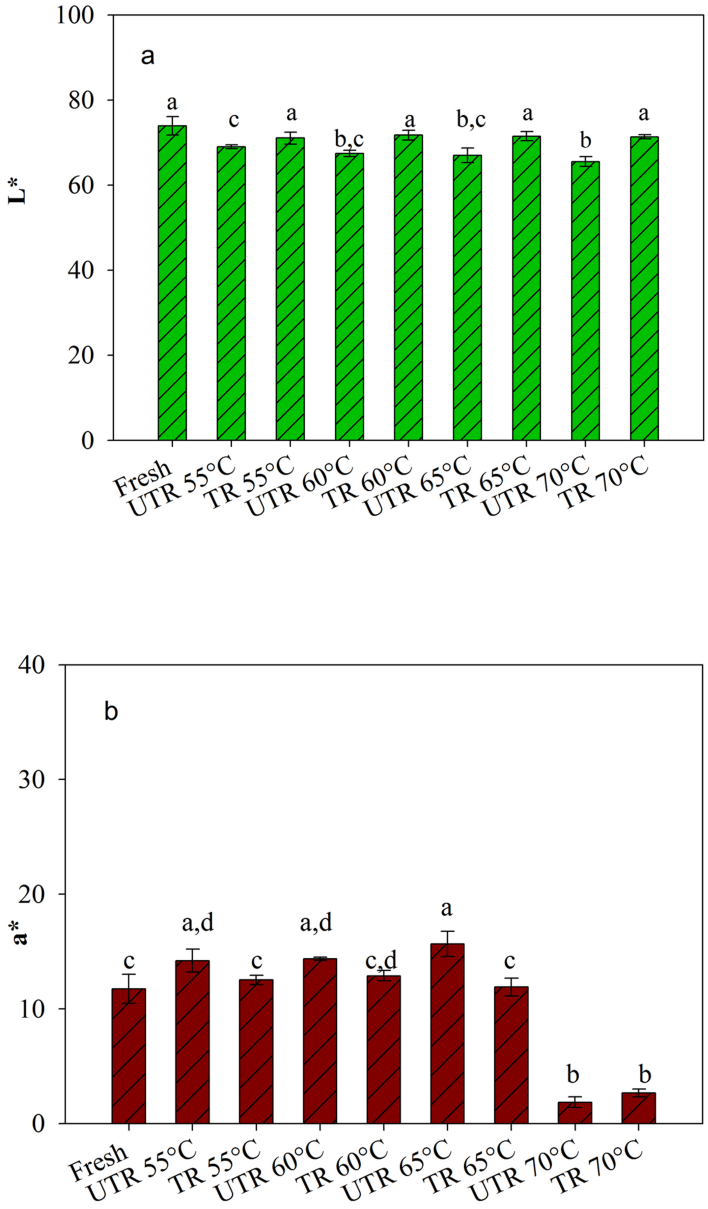


Figure 2. Relationship between diffusivity and absolute temperature for untreated (UTR) and treated (TR) samples.

3.3. Colour evaluation

The colour of dried products is an important quality factor because it reflects the sensory appeal and the quality of the foods. The average values of the colour parameters, L^* (lightness), a^* (redness), b^* (yellowness) and (ΔE) (total colour change), for fresh and dried pumpkins are presented in Fig. 3. The drying process determined a decrease of L*a*b* values for UTR samples with respect to fresh one. On the contrary, when the pumpkin was treated before drying, the colour values do not change significantly ($p<0.05$) after drying process, except for the a* value that decreases significantly at 70°C. Significant differences between UTR and TR samples dried at different temperatures were observed in Fig. 3.



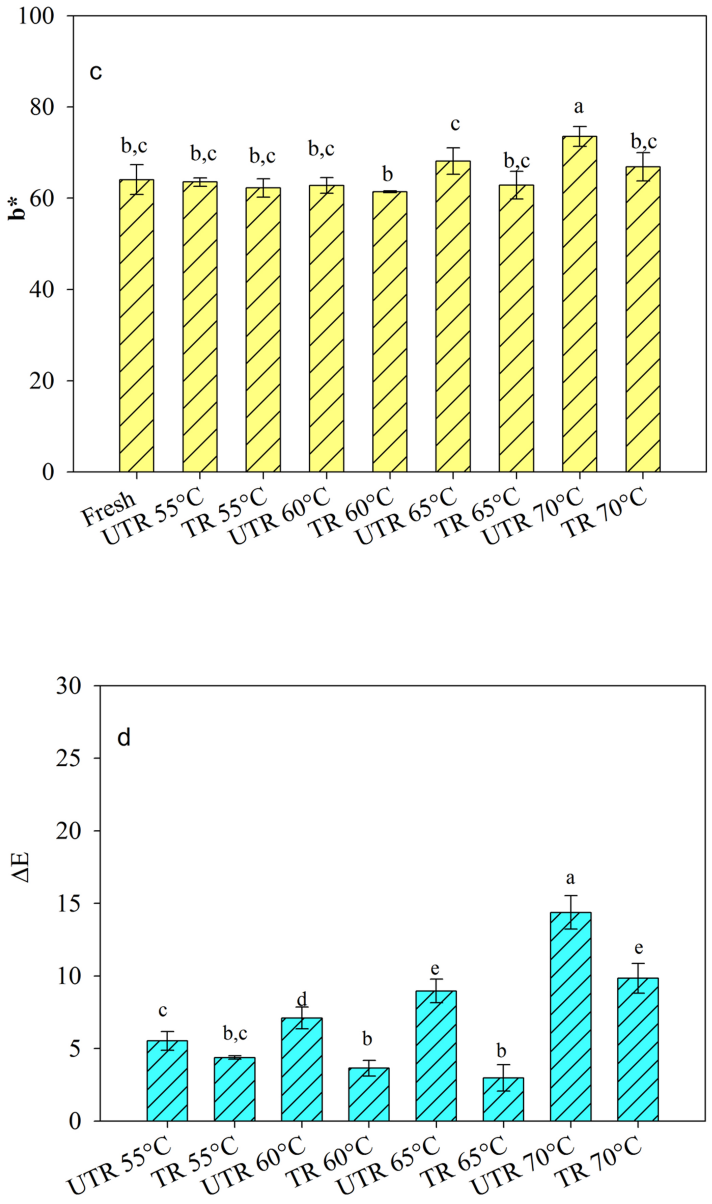


Figure 3. Colour parameters L* (a), a* (b), b* (c) and ΔE (d) for fresh, untreated (UTR) and pretreated (TR) dried samples at 55, 60, 65 and 70°C.

In particular, the lightness (L*), a property that varies from 0 (black) to 100 (white), of TR pumpkins was similar to fresh sample ($p<0.05$) and higher than that of UTR ones. Among all samples analysed, the lowest lightness was found in the UTR samples dried at 70°C. The loss of lightness may be explained by the degradation of thermo-labile pigments resulting in the formation of dark compounds that reduce luminosity, and non-enzymatic browning reaction due to heat effect, as reported by DUTTA *et al.* (2006) and GONCALVES *et al.* (2007). However, the effect of temperature on L* coordinate was smaller than that on a* and b* parameters, which turned the samples as the temperature rises.

For the greenness-redness parameter (a*), no significant differences were found between fresh and TR samples dried at 55, 60 and 65°C, while the TR one dried at 70°C presented a redness value almost ten times lower. With regard to the UTR samples, the redness values were higher than those of fresh and dried TR samples up to 65°C. At 70°C both samples became darker, and redness and vivid characteristics were lost. These colour alterations may be explained by heat carotenoid degradation; non-enzymatic browning (Maillard reaction) could also cause the degradation of colour.
A significant increase of ΔE was observed between fresh and UTR samples ($p< 0.05$) at increasing temperatures: at 70°C the maximum ΔE was obtained. Herein, both non-enzymatic browning and heat-sensitive component loss probably contributed to the changes of UTR surface colour. A significant discoloration also for TR samples was observed at 70°C. While at 55-65°C, the total colour variation (ΔE) for TR pumpkins showed the lowest values, indicating that the minimum difference in colour from the fresh sample was obtained in this temperature range.
In conclusion, the colour data showed that the pretreatment reduced the browning and preserved the L*a*b* values of pumpkin samples up to a temperature of 65°C.

3.4. Total phenolic content and antioxidant activity (EC50)

During the drying process pumpkins slices were exposed to high temperature for a long time (about 300 min), which contributed to a loss of antioxidants (ADILETTA *et al.*, 2016b). Hence, the search of the optimal conditions (pretreatment and drying temperature) necessary to preserve the original antioxidant activity is a key factor in drying process.
Results on total phenolic content and antioxidant activity were summarized in Table 6.

Table 6. Total phenolic content and antioxidant activity of fresh and dried samples.

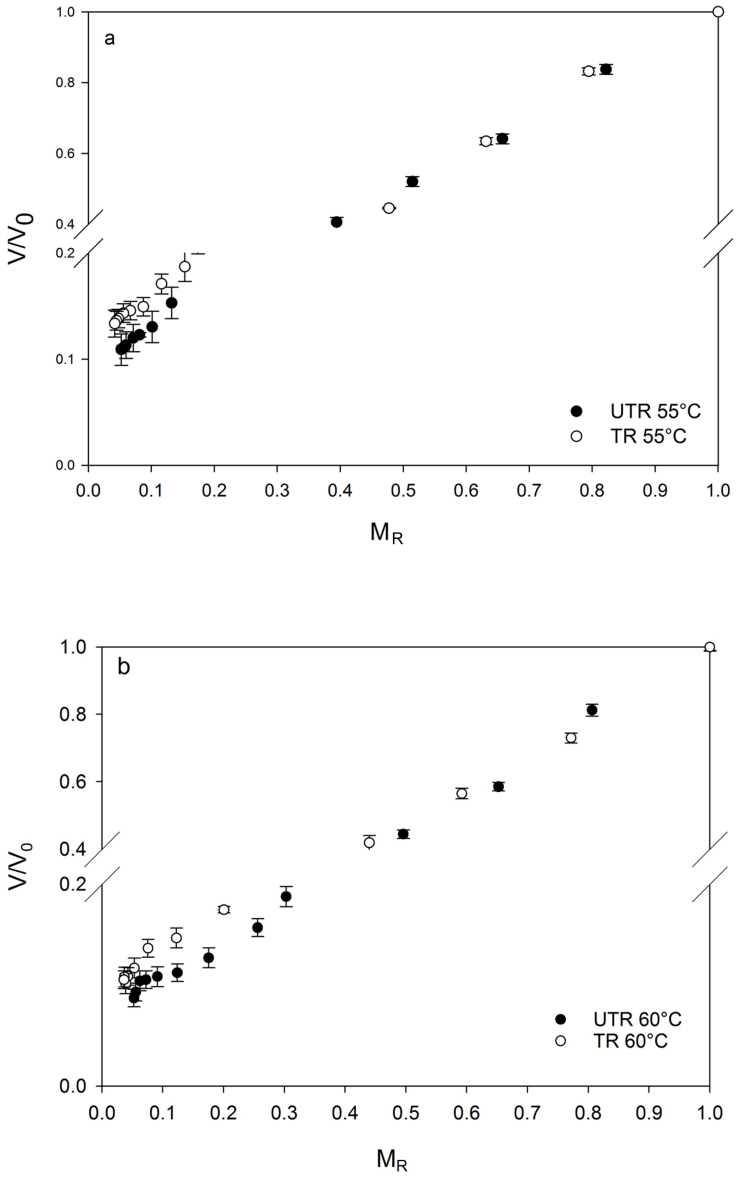
Sample	EC50 (mg/mL)	TCP (mg(GAE)/100g db)
Fresh	12.10^{h}±0.05	662.41^{a}±30.36
UTR dried at 55° C	43.22^{d}±1.08	258.01^{e}±10.21
TR dried at 55° C	19.41^{g}±0.11	486.25^{b}±11.04
UTR dried at 60°C	49.56^{c}±1.18	215.83^{f}±12.54
TR dried at 60° C	22.28^{f}±0.08	414.0^{c}±10.12
UTR dried at 65°c	64.54^{b}±2.41	170.11^{g}±8.27
TR dried at 65°C	33.84^{e}±1.04	363.26^{d}±14.55
UTR dried at 70°C	71.81^{a}±2.87	59.14^{h}±6.57
TR dried at 70°C	51.49^{c}±1.94	182.07^{g}±9.54

Fresh pumpkin had the highest total phenolics (662.41 mg GAE/100g db). The drying of pumpkins, as the temperature increased, induced a decrease in the total phenolic content for UTR samples (reduction range of 61-91% in the temperature range 55-70°C).
The pretreatment was effective in retention of total phenolic content because it reduces the drying times: in the temperature range analysed a reduction of 28, 37, 45% and 71% was obtained at 55, 60, 65 and 70°C, respectively.
The higher levels of phenolics might be responsible of the higher antioxidant activity of the different pumpkin samples as shown in Table 6. Fresh sample had the highest antioxidant activity equal to EC_{50} value of 12.10±0.05 mg/mL. The samples subjected to

pretreatment exhibited higher antioxidant activities than those of the samples without pretreatment (Table 6). Therefore, the antioxidant activity of samples dried in the range 55-65°C can be successfully preserved when the pretreatment was applied before drying.

3.5. Shrinkage effect

The removal of water during drying of biological products leads to cellular structural modifications due to reduced tension inside the cells. This phenomenon causes alterations in the shape and dimension of products including volume shrinkage (BRASIELLO *et al.*, 2017; ADILETTA *et al.*, 2015).
The Fig. 4 shows the variation of V/V_0 as a function of moisture ratio M_R during drying at the four temperatures. For both TR and UTR samples, the volume ratio V/V_0 showed a linear decrease with the moisture ratio.



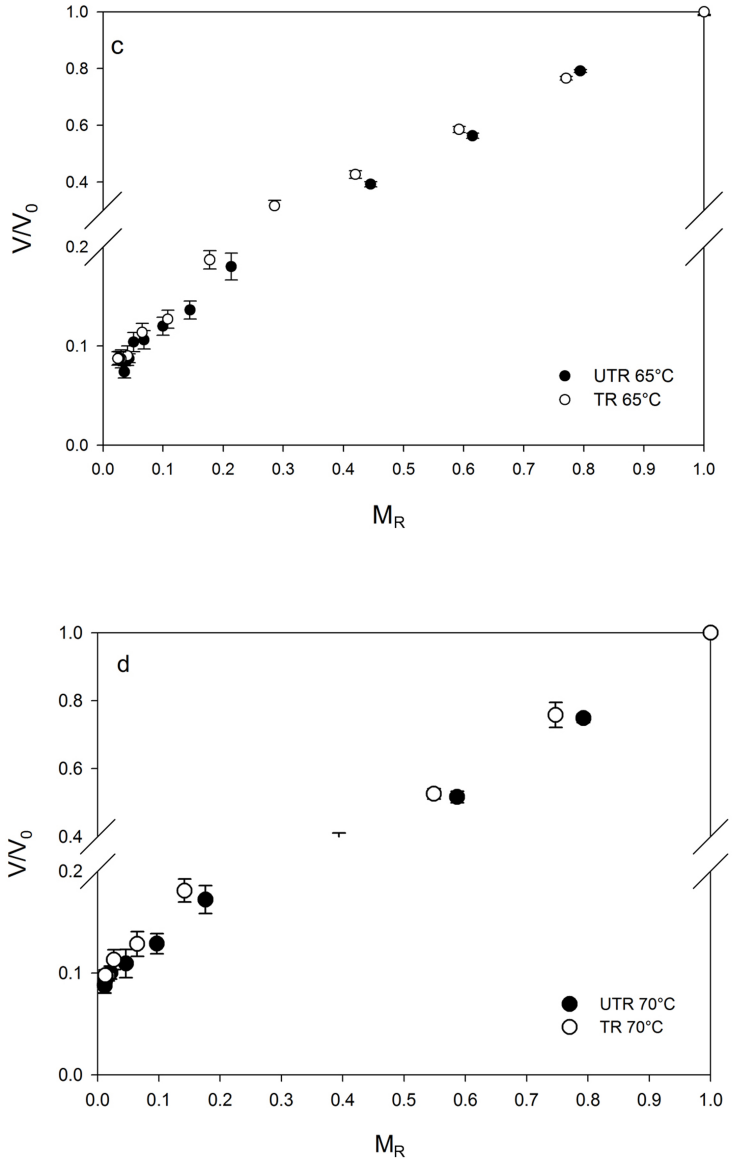


Figure 4. Volume ratio (V/V_0) vs moisture ratio (M_R) during pumpkins drying at: 55°C (a), 60°C (b), 65°C (c) and 70°C (d).

In order to quantify the effect of shrinkage, the V/V_0 ratio was fitted by using a linear relationship with the moisture ratio as (Mayor *et al.*, 2011):

$$\frac{V}{V_0} = \beta_1 + \beta_2 \frac{M_t}{M_0} \quad (11)$$

The values of the fitting parameters and of the correlation coefficient (R^2) of Eq (11) were reported in Table 7. It was found that in the case of TR samples the β_1 value, which represents the V/V_0 ratio when pumpkin is completely dried, was higher than that of the UTR samples for each temperature investigated. The higher β_1 value was found for

pumpkin treated before drying at 70°C, where probably a porous outer rigid crust that fixes the slices volume at early stages of drying process was formed.
It can be concluded that the pretreatment reduces volume shrinkage and structure modifications, assuring, as confirmed by drying kinetics and D_{eff} values, a faster transport of water from inside to the surface of the product and then evaporation.

Table 7. Fitting parameters and correlation coefficient (R^2) of the shrinkage correlation.

Temperature (°C)	Treated			Untreated		
	β_1	β_2	R^2	β_1	β_2	R^2
55	0.0686	0.9060	0.9887	0.0390	0.9431	0.9955
60	0.0515	0.8984	0.9992	0.0158	0.9497	0.9889
65	0.0459	0.9318	0.9960	0.0216	0.9356	0.9881
70	0.0689	0.8980	0.9920	0.0541	0.8782	0.9847

3.6. Rehydration capacity

Rehydration capacity is an important physical property of dried pumpkin, because it reflects the intrinsic property and molecular structure of the dried product. Rehydration is a diffusion process, during which water moves from the outside of the cells into the interior, and the rehydration capacities of samples depend on the pretreatment and the drying process that were used (ADILETTA *et al.*, 2016a). The rehydration capacity of dried pumpkin slices was quantified using the coefficient of rehydration (COR): the degree of structural disruption after the process.
The coefficient of rehydration of UTR and TR pumpkins dried at 55°C, 60°C, 65°C and 70°C are presented in Fig. 5.
The shrinkage that takes place during dehydration prevents rehydration and produces products with lower rehydration capacity. As seen in Fig. 5, the COR of all TR samples resulted in higher rehydration in comparison with UTR samples. The highest values of coefficient of rehydration were observed in the case of TR samples dried at 60 and 65°C (70 and 68%, respectively), followed by TR samples dried at 55 and 70°C (62 and 57% respectively), while it was minimum in the untreated samples.
The higher COR values might be the result of the preservation of the porous structure developed during drying, which promotes improved rehydration of the samples (BADWAIK *et al.*, 2014) as we already observed for eggplants in a recent study (ADILETTA *et al.*, 2016a).

3.7. Rehydration kinetics

Experimental rehydration curves of pumpkin slices dried at different temperatures are shown in Fig. 6. It can be observed that the drying temperature and the pretreatment influenced water absorption of pumpkins. All curves showed typical rehydration behaviour with a higher water absorption rate at the beginning of the process, then the rate decreases.
It was observed that the moisture ratio (M_i/M_d) ratio of the TR samples was higher than that of the untreated ones. These latter samples showed lesser rehydration after longer drying periods, indicating the possible presence of shrunken and closed structures that obstacle the absorption of water in agreement with shrinkage results (Fig. 4).

To examine the controlling mechanism of the rehydration processes, Peleg and Weibull models were used to test experimental data. The best rehydration kinetic curves obtained by the rehydration experiments are reported in Fig. 6 with experimental data.

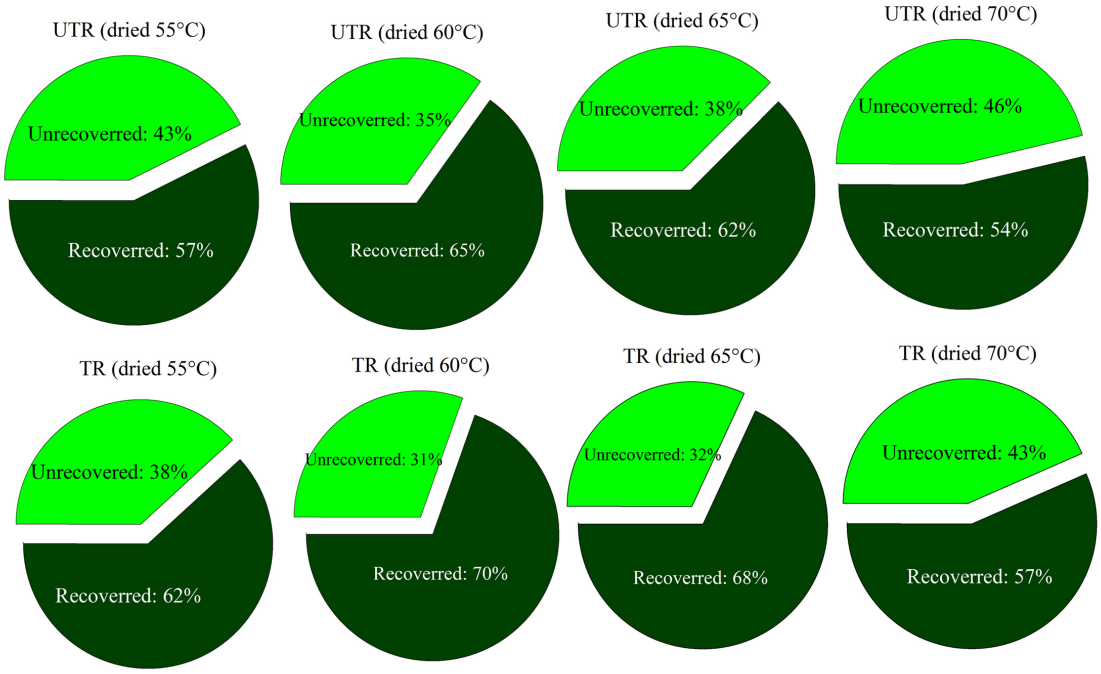


Figure 5. Coefficients of rehydration (COR) for pumpkin samples dried under different temperatures.

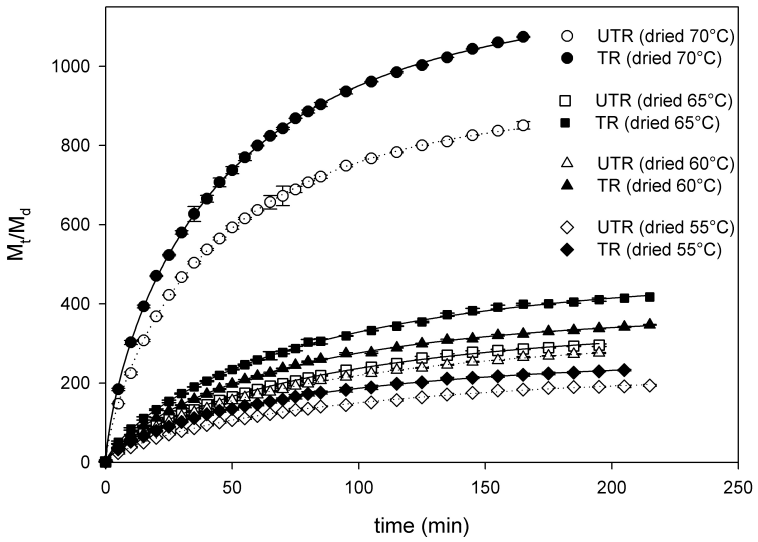


Figure 6. Experimental (symbols) and predicted (lines) rehydration curves of untreated (UTR) and pretreated (TR) samples dried at (a) 55°C, (b) 60°C, (c) 65°C and (d) 70°C.

The values of R^2, MRE, χ^2, RMSE were summarized in Table 8. Nonlinear regression was used to obtain each parameter value of every model. The best model describing the

rehydration characteristics of pumpkins slices was chosen as the one with the highest R^2, MRE below 10% (an indicator of a reasonably good fit) and the lowest χ^2 and RMSE. The values of parameters for each model were reported in Table 9.
The results in Table 8 show that all R^2 values were higher than 0.997, indicating a good fitting.
From the analysis of the results given in Table 8, the Weibull model was the best model for describing the water uptake in pumpkins during rehydration with the lowest values of MRE (<0.8813), RMSE (<6.0241) and χ^2 (<2.6533).

Table 8. Correlation coefficients (R^2, MRE, RMSE, $\chi 2$) of the rehydration models.

Model name	T (°C)	Treated				Untreated			
		R^2	MRE	RMSE	χ^2	R^2	MRE	RMSE	χ^2
Peleg	55	0.9978	2.4374	2.9222	9.1495	0.9976	2.7975	2.6084	7.2730
	60	0.9992	1.7421	2.7332	7.9858	0.9989	1.8624	2.5450	6.9567
	65	0.9982	2.4407	5.9473	25.0370	0.9985	2.4173	3.1755	10.8309
	70	0.9986	0.7982	7.9582	39.3140	0.9994	1.0954	5.5797	33.7280
Weibull	55	**0.9996**	0.8813	1.3121	1.9128	**0.9993**	0.6881	1.4037	2.1816
	60	**0.9996**	0.6365	2.4117	1.4759	**0.9998**	0.6294	1.0850	1.3132
	65	**0.9994**	0.5806	4.8396	2.3177	**0.9996**	0.2151	3.3250	1.7266
	70	**0.9999**	0.3957	6.0241	2.6533	**0.9997**	0.3007	4.2487	1.9387

Table 9 shows the Weibull parameters: A increased with drying temperature, a ranged between 0.7679 and 0.8447 and b varies between 46.6227 and 94.3970.

Table 9. Parameters of the Weibull model for rehydration kinetics.

Model name	Temperature (°C)	Parameter	Samples	
			Treated	Untreated
Weibull	55	A	265.4958	231.4673
		β	77.6382	94.3970
		α	0.7679	0.7733
	60	A	376.1352	322.1087
		β	71.3801	84.6131
		α	0.8263	0.7992
	65	A	478.9671	349.3444
		β	83.0645	85.8291
		α	0.7909	0.8142
	70	A	1172.4673	906.4519
		β	50.4266	46.6227
		α	0.8447	0.7763

4. CONCLUSIONS

The effect of an alternative chemical pretreatment on drying of Italian ecotype pumpkin slices at four temperatures was investigated in this study. The increase in drying air temperature from 55 to 70° C decreased significantly the drying time of samples, especially when the samples were pretreated before drying. The drying kinetics were well described by the Midilli model for both untreated and treated pumpkin slices. The water effective diffusion coefficients resulted to be higher in the treated samples with respect to the non-treated ones indicating the faster water transfer during drying: D_{eff} was in the range $6.75 \cdot 10^{-9}$ - $8.51 \cdot 10^{-9}$ m^2/s for UTR samples and of $7.073 \cdot 10^{-9}$ - $9.39 \cdot 10^{-9}$ m^2/s for TR ones. While the activation energy for moisture diffusion was found equal to 28.28 and 18.63 kJ/mol for UTR and TR slices, respectively, indicating that less energy is required for the drying of TR samples.

Moreover, the pretreatment effectively protected the quality of dried pumpkin slices. Compared with fresh samples, TR pumpkin slices dried in the temperature range 55-65°C, had lower changes of colour, shrinkage, phenolic content, antioxidant activity and rehydration capacity then UTR ones.

The higher rehydration capacity and the lower shrinkage, was probably due to the preservation of the porous structure developed during drying assuring, according to the D_{eff} values, a faster transport of water from inside to the surface of the product and then evaporation. In fact, the use of disaccharides, such as trehalose or sucrose, in the pretreatment solution has the main effect of maintaining general protein structure in the dry state. In this way, the cell membrane is protected and upon rehydration its functionality is restored.

At temperature of 70°C structure changes and loss of colour and of phenolic compounds are more evident.

Regarding to the rehydration kinetics, it was found that the Weibull model was the best model for describing the water uptake in pumpkins during rehydration.

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PAPER

COMPARISON OF THE FATTY ACID PROFILE IN THE MEAT OF PIGS AND WILD BOARS

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ABSTRACT

The aim of the study was to compare the fatty acid profile of the *longissimus lumborum muscle* from organically raised pigs: 20 samples from Złotnicka Spotted pigs, 20 samples from F_1 crossbred pigs (Polish Large White x Polish Landrace) and 16 samples from wild boar. The content of saturated fatty acids in the meat of animals from all the groups was similar. Statistically significant differences were calculated for monounsaturated fatty acids and polyunsaturated fatty acids. The meat of wild boar had the highest content of arachidonic acid but the lowest content of palmitoleic acid, oleic acid and α-linolenic acid.

Keywords: fatty acid profile, meat, pigs, wild boar

1. INTRODUCTION

Due to its content of many nutrients, meat plays an important role in the human diet. Meat provides our bodies with high value protein, essential amino acids, as well as trace elements, B-group and antioxidant vitamins and fatty acids (FA). The composition of fatty acids, especially the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA), is significant for human health (STRAZDINA *et al*., 2013). According to SERRANO *et al*. (2007), saturated fatty acids are regarded as the cause of cardiovascular diseases, as they increase blood pressure and the concentration of the LDL fraction of cholesterol, while monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) decrease the concentration of bad cholesterol (LDL) and increase the concentration of the good cholesterol (HDL), which results in reducing the risk of heart diseases and atherosclerosis (GARCIA REBOLLO *et al*., 1998; GERHARD *et al*. ,2004).
Compared to beef, pork is characterised by a favourable fatty acid profile, i.e. lower SFA content and higher PUFA content. In comparison with poultry meat, pork (despite a lower total PUFA content) shows a much more beneficial n-6 to n-3 fatty acids ratio (BLICHARSKI *et al*., 2013; IVANOVIĆ *et al*., 2013).
Recently, more and more consumers have been paying attention not only to the quality and safety of food, but also to the environmental aspects of its production. The main regulations regarding organic food production are included in the following legal acts: Council Regulation (EC) No. 834/2007 and Regulation (EC) No. 889/2008. According to these regulations, livestock should be fed with plant feeds and feed produced in accordance with the principles of organic farming. At the same time, the laws take into account the possibility of using additives containing some trace elements, vitamins and minerals (SUNDRUM *et al*., 2000). In organic farms, plant protection products or veterinary medicines should not be used. Organically raised animals are fed diets without synthetic additives and GMO preparations. These requirements undoubtedly affect the quality of the product obtained. The meat quality of domestic animals, which were bred in natural conditions means greater food safety for the consumer (SKOBRÁK *et al*., 2011). According to GRELA and KOWALCZUK (2009), organic meat products derived from fattening pigs are characterised by a higher content of nutrients. Pork obtained from pigs reared in the organic system contains higher amounts of intramuscular fat and more unsaturated fatty acids (ANGOOD *et al*., 2008; SUNDRUM *et al*., 2000; HANSEN *et al*., 2006).
In turn, the main source of feed for wild boar living in their natural habitats is plants (grasses, leaves, roots, shrubs, seeds, forest fruits) and, less frequently, avian eggs, snails, insects, earthworms, larvae and beetles (SKOBRÁK *et al*., 2011; ROŻMAITE *et al*., 2012). Due to the natural environment in which wild animals live, venison is often defined as an organic food.
The aim of the study was to compare the fatty acid profile of the *longissimus lumborum muscle* from organically raised Złotnicka Spotted and F_1 crossbred pigs (Polish Large White × Polish Landrace) and from wild boar.

2. MATERIALS AND METHODS

2.1. Animals

The study used 20 Złotnicka Spotted pigs, 20 F_1 crossbred pigs (Polish Large White × Polish Landrace) and 16 wild boar (hogs and gilts in equal amounts). In each group, the gender ratio of the tested animals was close to 1:1. The pigs originated from an organic

farm in the Kujawsko-Pomorskie province, where they were fed a diet containing 12.6 MJ metabolisable energy and 156 g total protein. The feed was comprised of 25% triticale, 20% rye, 10% barley, 10% wheat, 10% oats, 10% lupin, 5% pea, 5% rapeseed and 5% vitamin-mineral mixture. The piggery m*et al*l the conditions of welfare defined by Polish law (Regulation of the Minister of Agriculture and Rural Development, 2010). At the end of fattening, when Złotnicka Spotted pigs reached 106.2±3.66 kg of body weight (aged 5.5-6 month) and F_1 crosses reached 114.25±2.59 kg (aged 6.5-7 month), the animals were slaughtered under uniform standard production conditions in accordance with Polish law and standards in place. From the right halves of the carcasses, samples of the *longissimus lumborum muscle* were collected (between the 1st and the 4th lumbar vertebra).
In turn, two-year-old wild boar (weighing 38-60 kg on average) were shot in the Podkarpackie province during the hunting season by different hunters, in accordance with Regulation No. 45 and No. 48 of the Minister of the Environment. The samples were removed from the *longissimus lumborum* approximately at the level of the 1-2 lumbar vertebra of the carcasses. Local hunters provided all samples within 24 hours after shooting.

2.2. Samples and fatty acid analysis

For further analyses, samples were placed in sterile, tightly sealed bags, chilled to 4°C and transported to a laboratory. After freeze-drying (Lyovac GT2, Finn-Aqua), the samples were analysed for fatty acids through extraction with a chloroform and methanol solution in accordance with the method described by FOLCH *et al*. (1956). The fatty acid profile of methyl esters was determined by gas chromatography (Varian 3800 GC, USA) with a flame ionisation detector, using a Supelcowax 10 column (30 m × 0.32 mm × 0.25 μm). The temperature of the injector was 230°C, and that of the detector was 250°C. The volume of the injected sample was 1μl (split 1:50). The carrier gas was helium at a flow rate of 1.5 $cm^3 min^{-1}$. The analyses were performed at a temperature range of 90 to 225°C (11°C min^{-1}), 225°C for 6 min, and then an increase from 225 to 240°C (6°C min^{-1}) and 240°C for 19 min. The fatty acid methyl esters were identified with Supelco PUFA-2 Animal Source and Supelco 37 Component Fame Mix standards (Supelco, USA). The composition of fatty acids was expressed as a percentage of total fatty acids.
In addition, the indices of fatty acid metabolism were specified. The elongase index was calculated as the ratio of C18:0 to C16:0. The thioesterase index was calculated as the ratio of C16:0 to myristic acid (C14:0). The 9-desaturase index was calculated as 100 times the ratio of the palmitoleic acid (C16:1) percentage to the sum of acids: C16:1 and C16:0. The 9-desaturase index was calculated as 100 times the ratio of oleic acid (C18:1) to the sum of acids: C18:1 and C18:0 (ZHANG *et al*., 2007).

2.3. Statistical analysis

The results were statistically analysed with Statistica 8.0, and the means and standard deviations are provided in the table. One-way analysis of variance (ANOVA) and the post-hoc Scheffe test were used to compare the proportion of different fatty acids in the *longissimus lumborum muscle* of the animals. The normality of data distribution and the homogeneity of variance were tested with the Shapiro-Wilk and Levene tests, respectively. The correlations between the analysed fatty acids were determined based on the coefficients of Pearson's correlation. Differences were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

The percentage of different fatty acids in the *longissimus lumborum muscle* of the animals, depending on genetic type and species, is presented in Table 1.

Table 1. Fatty acid profile (% of total FA) of fat from the *m. longissimus lumborum* of the studied animals.

	ZS n=20	PLW x PL n=20	WILD BOAR n=16
C14:0	1.02±0.21[a]	1.26±0.27[a]	1.80±0.39[b]
C16:0	26.88±1.48[a,b]	28.22±0.91[a]	25.14±4.21[b]
C18:0	14.91±2.82[a]	13.29±1.60[a]	15.36±0.85[a]
C16:1 n7	3.05±0.45[a]	3.64±0.74[c]	1.30±0.68[b]
C18:1 n9	41.65±2.58[a]	40.76±3.38[a]	31.59±6.17[b]
C18:3 n3	2.09±0.66[a]	2.03±0.59[a]	0.79±0.16[b]
C20:4 n6	10.40±1.87[a]	10.80±2.99[a]	24.01±3.15[b]
SFA	42.81±3.50[a]	42.78±0.95[a]	42.31±3.93[a]
MUFA	44.70±2.81[a]	44.40±3.90[a]	32.90±5.77[b]
PUFA	12.48±2.34[a]	12.83±3.36[a]	24.80±3.10[b]
n3/n6	0.20±0.05[a]	0.19±0.05[a]	0.03±0.01[b]
n6/n3	5.34±1.46[a]	5.62±1.87[a]	32.03±9.89[b]
thioesterase index[1]	27.07±4.35[a]	23.23±4.63[a]	14.88±5.24[b]
elongase index[2]	0.55±0.10[a,b]	0.47±0.07[a]	0.63±0.12[a]
Δ^9 desaturase (C16) index[3]	10.10±1.35[a]	11.35±1.88[a]	4.74±1.95[b]
Δ^9 desaturase (C18) index[4]	73.69±4.48[a]	75.32±3.52[a]	66.79±4.27[b]

Results were expressed as means±SD. Values marked in the same row with different letters (a,b) are statistical significantly different at $P < 0.05$. SFA, MUFA, PUFA, n6, n3 = sum of all saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), n6 and n3 fatty acids, ZS - Złotnicka Spotted pigs, PLW x PL - crossbred pigs (Polish Large White × Polish Landrace), [1]Calculated as 16:0/14:0, [2]Calculated as 18:0/16:0, [3]Calculated as 100 × [16:1n-9/(16:1n-9 + 16:0)], [4]Calculated as 100 × [18:1n-9/(18:1n-9 + 18:0)]

Among saturated fatty acids (SFA), the presence of myristic (C14:0), palmitic (C16:0) and stearic acids (C18:0) was found. Compared to pig muscle, the wild boar muscle had a significantly highest content of C14:0 (approx. 1.8%). In turn, the muscle of PLW x PL crossbreeds contained statistically more C16:0 acid than wild boar muscle (approx. 3.08%). Unlike JANKOWIAK *et al*. (2010), the present statistical analysis showed no significant differences in the content of palmitic acid between the meat of Złotnicka Spotted pigs and F_1 crossbreeds (PLW × PL). Despite the differences in the content of individual fatty acids, the total SFA in the meat of the animals in each group did not exceed 43%. The obtained result was confirmed in the studies of other authors (PETROVIĆ *et al*., 2014; GRELA and KOWALCZUK, 2009).

While SFA content remained at the same level in all the groups, considerable differences occurred in the group of monounsaturated fatty acids (MUFA). The lowest concentration of MUFA was observed in wild boar muscle, in which the content of palmitoleic (C16:1 n7) and oleic acids (C18:1 n9) differed significantly from that determined for the pig muscle from both study groups. Compared to other authors, the content of acids (C16:1 n7 and

C18:1 n9) determined in the present study was very similar, although the content of MUFA was lower by 4.81% for organically raised pigs (GRELA and KOWALCZUK, 2009) and by 3.9% for wild boar (IVANOVIĆ *et al*., 2013). This difference is caused by the presence of additional acids (C18:1 n7).

The highest total PUFA (24.8% of all fatty acids) was determined in wild boar muscle. This value was confirmed in the research of other authors (SALES and KOTRBA, 2008). The high level of these acids translates into an appropriate PUFA/SFA ratio, which, according to WOOD *et al*. (2003), should exceed 0.4. For wild boar muscle, this ratio is 0.5861. The present level was slightly lower than published by DANNENBERGER *et al*. (2013) for wild boar living in the northern part of Germany (0.65÷1.05). In our study, the content of PUFA in wild boar meat was twice as high as the values obtained for the muscles of the pigs from both study groups. Their PUFA level was similar to the values published by CEBULSKA *et al*. (2018), for Złotnicka Spotted pigs, and by GRZEŚKOWIAK *et al*. (2010), who determined the fatty acid profile of the muscle of Polish Landrace × Polish Large White pigs. In the present study, arachidonic acid (C20:4 n6) showed the highest percentage among PUFA. Likely, the high content of C20:4 n6 is due to the absence of C18: 2 n6. This was significantly higher in wild boar muscle compared to pig muscle. Statistical analysis did not reveal significant differences in the content of this acid between the meat from Złotnicka Spotted and PLW × PL pigs. The second determined PUFA acid was α-linolenic acid (C18:3 n3; ALA). The muscles of pigs from both study groups contained similar amounts of this acid, which formed approx. 2% of total fatty acids. In contrast, the wild boar meat was less abundant in this acid (only 0.79%). In comparison with other authors, the ALA acid content determined in our own research was higher. According to PEDRAZZOLI *et al*. (2017), meat of wild boars up to 2 years should contain on average 1.47% of this acid (0.62% more than in the muscle of pigs), while the meat of older wild boars only 0.99% (0.2% more than in the present study).

The ALA (α-linolenic acid, C18: 3 n3) and LA (linoleic; C18: 2 n6) acids supplied with food may undergo enzymatic transformation. Elongase enzymes lengthen carbon chains, and desaturases produce additional double bonds, resulting in the formation of polyunsaturated fatty acids with lengths of at least 20 C atoms (ACHREMOWICZ and SZARY-SWORST, 2005). In fatty acid synthesis, thioesterase is responsible for terminating the reaction and releasing the newly synthesised fatty acid. The thioesterase index (C16:0/C14:0), which indicates a catalysis of palmitic acid synthesis from miristic, was higher ($P < 0.05$) in pig muscle than in wild boar, while the elongase index, as an indicator of C18:0 synthesis from C16:0 (C18:0/C16:0), remained at the same level (0.47-0.63) in all the groups. Δ^9-desaturase catalyses the conversion of C16:0 and C18:0 to C16:1 and C18:1, the 2 major MUFA of pork lipids. Greater index values mean greater desaturase activity. The highest Δ^9-desaturase (C16) and (C18) indexes were found in pig muscle. These results agree with those obtained by DAZA et.al. (2017) and ZHANG *et al*. (2007).

Between the analysed fatty acids, 11 correlations were found for the meat of ZS pigs and 16 correlations for the meat of PLW × PL pigs. Statistically significant relationships recurred between myristic acid and palmitic, palmitoleic and arachidonic acids, between palmitoleic acid and stearic and oleic acids, and between stearic acid and oleic and arachidonic acids (Tables 2-3).

Table 2. Coefficients of correlation (r_{xy}) between fatty acids determined in the *m. longissimus lumborum* of ZS pigs.

C16:0	0.81*					
C16:1	0.56*	0.18				
C18:0	–0.22	0.21	–0.57*			
C18:1	–0.10	–0.45*	0.43*	–0.68*		
C18:3	0.05	–0.16	–0.02	–0.40*	–0.19	
C20:4	–0.44*	–0.56*	–0.19	–0.43*	–0.02	0.64*
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:3

* significant at $P < 0.05$

Table 3. Coefficients of correlation (r_{xy}) between fatty acids determined in the *m. longissimus lumborum* of F_1 crossbred pigs (PLW × PL).

C16:0	0.77*					
C16:1	0.82*	0.66*				
C18:0	–0.81*	–0.77*	–0.95*			
C18:1	0.63*	0.38	0.65*	–0.67*		
C18:3	–0.87*	–0.50	–0.58*	0.57	–0.61*	
C20:4	–0.63*	–0.45	–0.63*	0.64*	–0.98*	0.56
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:3

* significant at $P < 0.05$

Contrary to pig meat, only 5 correlations were found for wild boar meat (Table 4). The only correlation shared by the analysed fatty acids for all study groups was a negative correlation between palmitoleic acid and stearic acid (r= -0.57 for ZS; r= -0.95 for PLW × PL; r= -0.76 for wild boar; $P < 0.05$).

Table 4. Coefficients of correlation (r_{xy}) between fatty acids determined in the *m. longissimus lumborum* of wild boar.

C16:0	–0.15					
C16:1	0.10	0.73*				
C18:0	–0.27	–0.34	–0.76*			
C18:1	0.23	–0.90*	–0.63*	0.29		
C18:3	0.05	0.02	–0.01	0.25	0.10	
C20:4	–0.33	0.38	0.24	–0.20	–0.74*	–0.35
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:3

* significant at $P < 0.05$

To ensure normal function of the human body, it is particularly important to maintain the proper PUFA n6 to PUFA n3 ratio, which should range between 1:1 and 4:1 (SIMOPOULOS, 2002). In both pig groups under study, this ratio was slightly higher (5.3:1

and 5.6:1), while in the case of wild boar muscle, it was by the highest (32.03:1). According to MARCINIAK-ŁUKASIK (2011), excess n6 fatty acids in the diet inhibits the metabolism of n3 fatty acids, which disrupts the physiological balance of the biologically active compounds obtained from them.

4. CONCLUSIONS

Wild boar meat does not differ significantly in SFA content from the meat of organically raised pigs of the ZS breed and F_1 crossbreds (PLW × PL). Statistically significant differences were noted in the MUFA and PUFA content. Wild boar muscle proved the richest in polyunsaturated fatty acids.
The appropriate amounts of individual fatty acids determined in the pig muscles translate into a more health-promoting ratio of n6 to n3 acids. The n6 to n3 fatty acids ratio determined in wild boar muscle was the highest, but the least desirable.

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PAPER

EFFECT OF HEATING ON CHEMICAL PARAMETERS OF EXTRA VIRGIN OLIVE OIL, POMACE OLIVE OIL, SOYBEAN OIL AND PALM OIL

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ABSTRACT

This work studied the oxidative stress on the chemical properties of extra virgin olive oil, pomace olive oil, soybean oil and palm oil during heating. The highest relative increase in free acidity was found in pomace olive oil. Peroxide value as an absolute value (meq O_2/kg) was lowest in palm oil 1.4 (unheated), 4.0 (180°C - 120 min), 6.4 (220°C - 120 min). Extra virgin olive oil had lower spectrophotometric indices (K232, K270 and ΔK) compared to the solvent extracted oils. Total phenols were highest in the extra virgin olive oil (196.8 mg/kg) and decreased to 59.8 and to 66.8 mg/kg after 120 min of heating at 180°C and 220°C respectively. A decreasing trend was also found in the tocopherol content with the highest % reduction (-79.5%) in EVOO heated at 220°C for 120 min. This was in agreement with the antioxidant activity trend measured with the ABTS assay (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (155.9 μM TE/100 g) and with the oxygen radical absorbance capacity (ORAC) assay (316.1 μM TE/100 g) in the unheated extra virgin olive oil.

Keywords: ABTS, DPPH·, EVOO, heating, ORAC, oxidation

1. INTRODUCTION

Lipids play a key role in human health. In the human diet, they are an important and essential part along with carbohydrates and proteins, representing not only a source of energy and protection for the organs and the body thanks to their functional properties, but also taking part in the metabolic processes, being components of bio-membranes and serving as carriers of biologically active substances (VASKOVA and BUCKOVA, 2015).
Edible oils are chemically unstable and susceptible to oxidation processes, especially if they are in the presence of oxygen, light, moisture, heat (CALVO *et al*., 2012), enzymes and traces of metals (VASKOVA and BUCKOVA, 2015).
Oxidation is the main cause of deterioration of oils and fats, which besides reducing shelf-life, sensorial characteristics and nutritional value, also produces toxic compounds (POYATO *et al*., 2014). More than 400 chemical components have been detected in deteriorated fried edible vegetable oils (PAUL and MITTAL, 1996).
Introducing lipid oxidation products into the diet may lead to an increased risk of cardiovascular disorders, such as the formation of atherosclerotic plaques (HALLIWELL and CHIRICO, 1993). Oxidative stress seems to be linked to many multi-factorial diseases, especially cancers, cardiovascular diseases and inflammatory disorders. Oxidation alters physiologically important molecules, including proteins, lipids, carbohydrates and nucleic acids, together with the modulation of gene expression and the inflammatory response (LAGUERRE *et al*., 2007). The endogenous antioxidants of vegetable oils provide a natural resistance to oxidative stress.
Among all categories of olive oil, extra virgin olive oil has gained significant importance from the gastronomical, nutritional, therapeutic and economic point of view. It is considered the best olive oil for its organoleptic characteristics, stability and chemical composition (CALVO *et al*., 2012).
The biological activities associated with the consumption of extra virgin olive oil (antioxidant, anti-inflammatory, chemo-preventive and anti-cancer) have promoted the use of this oil, not only as food but also as an ingredient in a wide range of industrial food products (CALVO *et al*., 2012). Virgin olive oil is fundamental in the Mediterranean diet and contributes to its health benefits (BOSKOU, 2015) and it is widely used in the Countries of the Mediterranean basin, such as Italy (PROTO and ZIMBALATTI, 2015). Pomace olive oil is a secondary product in the olive oil industry, but it is important because otherwise olive pomace would be considered a waste. Although, palm oil is the most widely consumed edible vegetable oil in the world, with a production of 60.96 million metric tons in 2015-2016 (statista.com, 2017), controversial results have been found in relation to its use and human health (MANCINI *et al*., 2015). Soybean oil is the second most widely consumed edible vegetable oil in the world, with a production of 51.45 million metric tons in 2015-2016 (statista.com, 2017). After oil extraction, the residue is widely used as animal feed. This work has been based on these premises and aims to study the chemical property variations during heating of the three most popular edible vegetable oils (extra virgin olive oil, soybean oil and palm oil), together with pomace olive oil, which could become more important in the edible vegetable oil market.
The aim of this work was to study the variation in the chemical properties of the three most popular edible vegetable oils during heating at 180 and 220°C and for 30, 60 and 120 min.

2. MATERIALS AND METHODS

2.1. Vegetable oils

Four vegetable oils were used in this experiment: extra virgin olive oil (EVOO) and pomace olive oil (PO) were produced in the harvest year 2016-2017 in the Region of Calabria (South Italy) and bought directly from the producer, Palm oil (P) and Soybean oil (SO) were purchased in a supermarket. The oils were analysed before heating and after heating at two different temperatures (180°C and 220°C) for 30, 60 and 120 minutes for each temperature. A 100 g aliquot of each oil was placed in a glass pyrex container which was heated in an oven. Nine glass pyrex containers for each oil and for each temperature (180 and 220°C) were prepared, three of them for each temperature were taken out of the oven after each time established by the experimental design (30, 60 and 120 min) to conduct analyses in triplicate.

2.2. Reagents

Diethyl ether, ethyl alcohol, sodium hydroxide, phenolphtalein, chloroform, acetic acid, potassium iodide, soluble starch, cyclohexane, *p*-anisidine, methanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·), 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH), Trolox, 2,2-azinobis-(3-ethylbensothiazoline)-6-sulfonic acid (ABTS), potassium peroxodisulphate, ethanol were from Sigma-Aldrich (St. Louis, MO, USA), fluorescein, sodium thiosulphate, buffer phosphate were from Carlo Erba, (Milan, Italy).

2.3. Free Acidity (FA)

FA analysis was conducted according to Annex II of the Consleg (2015) for olive oil analyses. A 5 g aliquot of each oil was dissolved in 25 mL solution of diethyl ether/ethylic alcohol (1:1, v/v) and titrated with a 0.1 N NaOH aqueous solution using 1% phenolphthalein in ethanol as an indicator. Results are expressed as g oleic acid/100 g.

2.4. Peroxide Value (PV)

Determination of PV was performed according to Annex III of the Consleg (2015) for olive oil analyses. A 2 g aliquot of each oil was dissolved in a 25 mL solution of acetic acid/chloroform (3:2, v/v) and 1 mL of a saturated aqueous solution of potassium iodide was added. The mixture was shaken for 1 minute before being placed in the dark for five minutes. After this time, it was titrated with a 0.01 N sodium thiosulphate solution using a 1% starch soluble solution as an indicator. Results are expressed as meq O_2/kg.

2.5. *p*-Anisidine Value (*p*-AnV)

The *p*-AnV analysis was conducted as described by the Norme Grassi e Derivati method NGD C 36-79 (NGD 1979). Each sample was diluted 1:100 (m/v), with isooctane (for spectrophotometry type), after which it was allowed to react with *p*-anisidine. The optical density of the solution was measured at 350 nm in a UV/Vis Spectrometer model Lambda 2, Perkin Elmer, Waltham, Massachusetts USA.

2.6. TOTOX

This index is given as the sum of 2PV and *p*-AnV.

2.7. Spectrophotometric indices

Spectrophotometric indices were determined as described in Annex IX of the European Regulation (Consleg, 2015). Each sample was diluted 1:100 (m/v) with cyclohexane and the specific extinctions were measured at 232, 266, 270 and 274 nm against a blank (only cyclohexane). An UV/Vis Spectrometer model Lambda 2, Perkin Elmer (Waltham, MA, USA), was used.

2.8. Antioxidant Extract (AE)

AE was obtained with the method proposed by GOLDSMITH *et al.* (2014) modified as follows: 5 g of each sample was mixed for extraction with a 5 mL of methanol/water solution (80:20, v/v). The mixture was vigorously shaken with a Vortex for 1 min and then centrifuged at 5000 rpm for 7 min. The supernatant containing the antioxidants was kept. The operation was repeated one more time and the two extracts were mixed together to obtain the first AE. After this, two more AE were prepared to obtain three different AE from the same oil and to analyse each oil in triplicate.

2.9. Total phenolic content

Total phenolic content of the AE was determined using the Folin-Ciocalteu assay (SINGLETON *et al*., 1999; GIUFFRÈ *et al*., 2017a). Two mL of AE, 10 mL of bi-deionised water, 2.5 mL of Folin-Ciocalteu reagent and 10 mL of a 7% sodium carbonate in bi-deionised water solution were placed in a 50 mL glass flask. At this point the volume was made up to 50 mL with bi-deionised water. A blank was prepared substituting AE with bi-deionised water. After 1 hour in the dark, the absorbance was measured at 765 nm in an Agilent 8453 spectrophotometer (Santa Clara, CA, USA). The total phenolic content was calculated on the basis of a calibration curve. Data were expressed as mg gallic acid/kg.

2.10. Antioxidant Activity (DPPH assay) hydrophilic

The DPPH assay measures the radical scavenging activity of a vegetable extract, in this case from a vegetable oil. The DPPH assay method was developed by BRAND-WILLIAMS *et al*. (1995) and it was adapted to an olive oil application (KALANTZAKIS *et al*., 2006). It is spectrophotometrically determined by measuring the disappearance of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) at 515 nm. A 0.10 mL aliquot of AE was added to 2.40 mL of a 60 μM DPPH methanolic solution. The mixture was shaken for five minutes in the dark. After this, the decrease in absorbance was measured at 515 nm in an Agilent 8453 spectrophotometer (Santa Clara, CA, USA). Results were expressed as % inhibition (mean ± S.D.) using the following formula: % inhibition = [(T0 – T5)/T0] x 100.

2.11. Antioxidant Activity (DPPH assay) oil

The DPPH assay measures the radical scavenging activity of a vegetable oil. It was conducted in an UV/Vis Spectrometer model Lambda 2, Perkin Elmer (Waltham, MA, USA), using the method proposed by KALANTZAKIS *et al*. (2006), modified as follows. Firstly, the oil was diluted with ethyl acetate (1:10, v/v). Secondly, 500 μL of diluted oil

were added to 2 mL of a 10^{-4} M DPPH· solution, previously prepared with ethyl acetate and, thirdly, the absorbance of the mixture was measured immediately at 515 nm (t0) and after 30 minutes of incubation (t30). The results were calculated with the following formula: % inhibition = [(T0 – T30)/T0] x 100 and they were expressed as % inhibition.

2.12. Antioxidant Activity (ABTS assay)

The ABTS assay determines the radical scavenging activity of an extract using an ethanol solution of 2,2-azinobis-(3-ethylbensothiazoline)-6-sulfonic acid (ABTS) and potassium peroxydisulphate. For its determination the method proposed by RE *et al*. (1999) was applied, with the following modifications.
A 0.050 mL aliquot of AE was added to 2.450 mL of a 7mM ABTS ethanolic solution and was vigorously shaken in the dark for 6 minutes. After this, the decrease in absorbance was measured at 734 nm in an Agilent spectrophotometer, model 8453 (Santa Clara, CA, USA). Results were expressed as % inhibition using the following formula: % inhibition = [(T0 – T6)/T0] x 100.

2.13. Antioxidant Activity (ORAC assay) AE

The ORAC assay was proposed by CAO *et al*. (1993). The ORAC assay measures the antioxidant activity of an oil extract and is determined on the basis of the oxidative damage to the fluorescent protein (NINFALI *et al*., 2001). AAPH was used as the generating species of peroxyl radicals, and Trolox as an antioxidant standard. A 150 μL aliquot of fluorescein solution (96 nM in a 7.4 pH buffer phosphate solution) and 30 μL of AAPH (133 mM in a 7.4 pH buffer phosphate solution) were added to 20 μL of AE previously diluted 1:30 (v/v) in a 7.4 pH buffer phosphate solution. The fluorescence decrease was measured using a Perkin Elmer Victor X2 (Waltham, MA, U.S.A.). The final reaction tested and the concentrations of the different reagents were determined following FERNÁNDEZ-PACHON *et al*. (2005). Results were expressed as μmol Trolox/100 g.

2.14. Statistical analysis

Analyses of samples were conducted in triplicate and mean and standard deviation were calculated by the Excel 2010 version software. Analysis of variance (one-way ANOVA) was performed by SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.), using the Tukey test and the significance level was set at $p < 0.05$. The effect of temperature and heating duration were analysed by a two-way ANOVA by SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.).

3. RESULTS AND DISCUSSION

3.1. Free acidity

A vegetable oil is mainly composed of tryglycerides. Each vegetable oil has a specific triglyceride composition: olive oil contains mainly triolein (GIUFFRÈ, 2013; 2014); palm oil contains mainly dioleylpalmitoylglycerol (21-25%) and dipalmitoyloleylglycerol (30-34%) (ENDO *et al*., 2011); soybean oil contains mainly trilinolein (21-22%) and dilinoleolein (15-16%) (SUDAR *et al*., 2003).
The hydrolysis of tryglycerides produces free fatty acids as the main degradation products. When the oil temperature reaches 150°C a part of the glycerol evaporates and

the remaining glycerol promotes the production of free fatty acids by hydrolysis (NAZ *et al*., 2005). This process is accelerated when a food containing water is added to the oil. The higher the water quantity, the higher the oil degradation. In our work, EVOO had the highest initial FA but it showed the lowest percentage increase with temperature and with time. When the oils were heated at 180°C for 30, 60 and 120 min, the highest percentage increase in FA was in P: 30, 40 and 50% respectively. When the oils were heated at 220°C for 120 min, the highest percentage increase in FA was in PO: 146.7% (Table 1). If the absolute FA data are considered, the lowest values were found in P and in SO oils in which 0.18 g/100 g and 0.17 g/100 g (as oleic acid) were found after the most drastic treatment (220°C for 120 min). AZIMAH *et al.* (2017) used palm oil to fry potatoes at 175°C for 0, 10 and 20 times and found an initial increase in free acidity from 0.18 % (0 times, i.e. no fried oil) to 0.27% (10 times fried) whereas no increase was found from 10 to 20 times of frying. BULUT and YILMAZ (2010) used a refined pomace olive oil to fry 35 g patties whose dough contained flour (56%), water (42%) dry yeast (0.5%), baking soda (0.5%) and salt (0.5%) and found an increase from 0.27% to 0.28%, 0.34%, 0.43%, 0.52% and 0.59% in fried oil after respectively 0, 1, 2, 3, 4 and 5 days.

3.2. Peroxide value

The PV analysis is based on the quantification of the primary oxidation products, mainly hydroperoxides (SAAD *et al*. 2007). During peroxidation, unsaturated fatty acids are oxidized by O_2. This is an auto-catalytic reaction which induces the formation of free radicals from fatty acids and starts the oxidation of the remaining non-oxidized fatty acids. In EVOO, the initial PV was 8.1 meq O_2/kg, i.e. well below the maximum (20 meq O_2/kg) stated by both the Consleg (2015) and the IOC (2015). After 120 min heating, PV increased in EVOO up to 19.4 meq O_2/kg (+139.5%) at 180°C and 20.9 meq O_2/kg (+158.0%) at 220°C. Under the worst conditions (220°C and 120 min heating), PO and SO were 11.8 and 9.5 meq O_2/kg respectively and P showed at the same time both the lowest absolute PV and the highest percentage increase (357.1%), this was due to the very low initial PV (1.4 meq O_2/kg) which is well below the 10 meq O_2/kg required by the Codex Stan (2013) for a refined edible vegetable oil. After 120 min of P heating, PV increased to 4.0 meq O_2/kg (+185.7%) at 180°C and to 6.4 meq O_2/kg (+357.1%) at 220°C. After 60 min heating at 180°C, the PV was 9.9, 4.92, 5.7 and 3.9 meq O_2/kg for EVOO, PO, SO and P respectively (Table 2), i.e. always below the maximum of 10 meq O_2/kg, stated by the Codex Stan (2013). P showed the lowest increase in absolute value: 6.4 meq O_2/kg after 120 min at 220°C. GHARBY *et al*. (2016) heated extra virgin olive oil and refined olive oil from Morocco (cv Picholine) at 100°C for 120 h and found a variation from 2.30 to 32.43 meq O_2/kg oil in the former and a variation from 0.60 to 375.10 meq O_2/kg oil in the latter. JAARIN and KAMISAH (2002) fried sweet potatoes in palm oil and soy oil for 10 min at 180°C and used the same oil five times with an interval of at least five hours between each heating and found an increase from 2 (fresh oil) to more than 9 meq O_2/kg oil in the palm oil and an increase from 5 (fresh oil) to 11 meq O_2/kg oil in the soy oil.

3.3. *p*-Anisidine value

p-AnV is an appropriate method for evaluating the secondary products of lipid oxidation (QING *et al*., 2016) and it is related to the formation of non-volatile aldehydes (2-alkenals) and ketones which are responsible of the rancid odour and taste in a fat.

Table 1. Free acidity (g oleic acid/100 g). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (**, $p < 0.01$; ***, $p < 0.001$).
At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		**		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	0.50±0.02 b	--	0.15±0.01 c	--	0.12±0.01 c	--	0.10±0.01 c	--
180°C/30min	0.51±0.01 b	2.0	0.17±0.01 c	13.3	0.14±0 bc	16.7	0.13±0.01 bc	30.0
180°C/60min	0.53±0.02 b	6.0	0.18±0.01 bc	20.0	0.14±0.01 abc	16.7	0.14±0 b	40.0
180°C/120min	0.53±0.01 b	6.0	0.19±0.01 bc	26.7	0.16±0.02 ab	33.3	0.15±0.01 ab	50.0
220°C/30min	0.51±0.01 b	2.0	0.17±0.01 c	13.3	0.14±0.01 abc	16.7	0.14±0 b	40.0
220°C/60min	0.52±0.01 b	4.0	0.21±0.01 b	40.0	0.16±0.01 ab	33.3	0.16±0.01 ab	60.0
220°C/120min	0.61±0.02 a	22.0	0.37±0 a	146.7	0.17±0.01 a	41.7	0.18±0.01 a	80.0
Temperature	n.s.		n.s.		n.s.		n.s.	
time	n.s.		**		*		n.s.	
Temperature x time	***		n.s.		n.s.		n.s.	

Table 2. Peroxide Value (meq O_2/kg). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$).
At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	8.1±0.3 f	--	3.9±0.01 f	--	2.4±0.03 g	--	1.4±0.05 f	--
180°C/30min	9.4±0.1 e	16.1	4.4±0.04 e	12.8	4.3±0.10 f	79.2	3.7±0.06 e	164.3
180°C/60min	9.9±0.2 e	22.2	4.9±0.07 c	25.6	5.7±0.01 d	137.5	3.9±0.04 d	178.6
180°C/120min	19.4±0.5 b	139.5	5.9±0.11 b	51.3	7.3±0.04 b	204.2	4.0±0.05 d	185.7
220°C/30min	11.6±0.6 d	43.2	4.7±0.01 d	20.5	4.6±0.05 e	91.7	4.2±0.04 c	200.0
220°C/60min	14.8±0.2 c	82.7	5.9±0.08 b	51.3	7.1±0.06 c	195.8	5.5±0 b	292.9
220°C/120min	20.9±0.2 a	158.0	11.8±0.03 a	202.6	9.5±0.04 a	295.8	6.4±0.04 a	357.1
Temperature	n.s.		n.s.		n.s.		n.s.	
Time	*		n.s.		*		n.s.	
Temperature x time	***		***		***		***	

The importance of *p*-AnV, mainly in a rectified oil, is due to the scarce effect on removing the secondary oxidisation products by deodorisation which, instead, diminishes the PV. For this reason, if before its rectification, an edible vegetable oil has suffered a heavy and continuous oxidative damage, this can be revealed by the *p*-AnV analysis. After 120 min heating at 220°C, P showed the lowest initial *p*-AnV (31.4) of the studied oils, whereas SO showed the highest *p*-AnV (94.4) and the highest percentage increase (1715.4%) compared to the unheated oils (Table 3).
Considering values after heating at 180°C for 120 min, the *p*-AnV was 2.6 (EVOO), 3.1 (PO), 2.8 (SO) and 2.0 (P) times lower than *p*-AnV found at 220°C heating for 120 min.
If values at 220°C are considered, it is worthy of note that *p*-AnV after 120 min heating is almost double in PO and P and more than double in EVOO compared to *p*-AnV found after 60 min heating, whereas a very little increase was observed at 180°C between 60 and 120 min heated oils.
XU *et al*. (2015) studied the *p*-AnV variation in palm oil used to fry potatoes at 170°C for 75 frying batches conducted over 3 days and found a constant increase with 85 as the final value. AHMAD TARMIZI and ISMAIL (2014) used refined, bleached, and deodorized palm olein to fry potatoes at 180°C for a 56 h period and found an initial *p*-AnV increase from 0.8 (0 h – fresh palm olein) to 37.0 (32 h heating), thereafter they measured a constant decrease until 31.4 (48 h) and a final increase 35.6 (56 h). They also mixed palm olein with sunflower oil, canola oil and cotton seed oil and in all cases the *p*-AnV during heating was higher than in palm olein.

3.4. TOTOX

This is an indicator of the overall oxidation state and quality of the oil (SAAD *et al*., 2007). The higher the TOTOX the higher the oil's oxidisation. TOTOX values are listed in Table 4. The initial lowest TOTOX value was found in P (4.5) followed by SO (10.1), PO (16.0) and EVOO (20.6). If TOTOX is considered after 120 min heating at 180°C, the initial classification varies from P (23.6), PO (35.7), SO (48.3) to EVOO (59.9). If TOTOX is considered after 120 min heating at 220°C, the initial value increases to 44.3 in P, 96.5 in EVOO, 97.6 in PO and to 113.4 in SO. This was due to the different antioxidant content and to the different fatty acid composition of each oil. EVOO had the highest total phenolic content (Table 8) and P had the highest saturated fatty acid content (data not published). As a consequence, even if EVOO had the highest initial TOTOX, the heating treatment caused the lowest percentage increase after 120 min at 220°C (368.4%): this was due to the highest phenolic content (Table 8). Another low increase in terms of TOTOX was found in P (44.3 after 120 min at 220°C), because of the lowest unsaturated fatty acid content (less than 60%) and the lowest polyunsaturated fatty acid content (less than 13%) of this oil (data not published).
XU *et al*. (2015) studied palm oil during the frying of potatoes at 170°C and found a constant increase from 10 (fresh oil) to 115 after 75 frying cycles (5 min each one). SRIVASTAVA and SEMWAL (2015), in coconut oil heated at 180°C for 8 h found a continuous and significant increase in TOTOX value from 8.91 (fresh oil/0 h) to 33.95 after 8 h heating.

3.5. K232, K270 and ΔK

Thermal oxidation causes the isomerisation of double bonds contained in the unsaturated fatty acids and the formation of trans isomers (MARINOVA *et al*., 2012). The absorbance at 232 nm gives information about the presence of diene conjugates, which are formed during the oil rectification. Also, the oxidation products present in an edible vegetable oil vary the spectrum of UV absorption and increase values read on the spectrophotometer. The lower this value, the better the oil quality.

Table 3. *p*-Anisidine Value. At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	4.4±0.1 f	--	8.3±0.07 f	--	5.2±0.12 f	--	1.8±0.10 e	--
180°C/30min	7.4±0.6 e	68.2	9.6±0.08 e	15.7	5.6±0.04 f	7.7	2.0±0.07 e	11.1
180°C/60min	17.4±0.5 c	295.5	21.2±0.13 d	155.4	20.2±0.69 e	288.5	11.9±0.04 d	561.1
180°C/120min	21.0±0.1 b	377.3	24.0±0.39 c	189.2	33.6±0.83 c	546.2	15.5±0.33 c	761.1
220°C/30min	13.4±0.6 d	204.5	21.4±0.11 d	157.8	27.2±0.64 d	423.1	2.4±0.15 e	33.3
220°C/60min	20.8±0.1 b	372.8	40.5±0.08 b	388.0	47.6±1.06 b	815.4	17.5±0.36 b	872.2
220°C/120min	54.6±1.2 a	1140.9	73.9±0.01 a	790.4	94.4±1.13 a	1715.4	31.4±0.33 a	1644.4
Temperature	n.s.		n.s.		n.s.		n.s.	
Time	n.s.		n.s.		n.s.		n.s.	
Temperature x time	***		***		***		***	

Table 4. TOTOX. At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	20.6±0.7 f	--	16.0±0.1 f	--	10.1±0.2 g	--	4.5±0.2 g	--
180°C/30min	26.1±0.8 d	26.7	18.5±0.01 e	15.6	14.2±0.2 f	40.6	9.5±0.1 f	111.1
180°C/60min	37.3±0.3 d	81.1	31.1±0.3 d	94.4	31.6±0.7 e	212.9	19.8±0.1 d	340.0
180°C/120min	59.9±1.0 b	190.8	35.7±0.6 c	123.1	48.3±0.8 c	378.2	23.6±0.4 c	424.4
220°C/30min	36.6±0.6 d	77.7	30.8±0.1 d	92.5	36.4±0.6 d	260.4	10.9±0.2 e	142.2
220°C/60min	50.4±0.5 c	144.7	52.3±0.2 b	226.9	61.7±1.2 b	510.9	28.5±0.4 b	533.3
220°C/120min	96.5±1.2 a	368.4	97.6±0.1 a	510.0	113.4±1.2 a	1022.8	44.3±0.4 a	884.4
Temperature	n.s.		n.s.		n.s.		n.s.	
time	n.s.		n.s.		n.s.		n.s.	
Temperature x time	***		***		***		***	

The best findings were revealed in EVOO (1.686 for unheated EVOO and 2.663 for H-EVOO at 220°C for 120 min). P showed the second lowest values at 180°C and at 220°C after 30 min heating. In P a slight reduction in K232 was found at both 180 and 220°C from 60 and 120 min heating (Table 5). PO showed the second lowest findings at 220°C and 60-120 min heating.
The absorbance at 270 nm gives information about the presence of triene conjugates. Linoleate oxidation products or degradation of hydroxylinoleate produce conjugated trienes absorbing at 270 nm (MARINOVA *et al*., 2012). Findings are listed in Table 6. SO showed the initial absolute highest value and this negative condition was found after all the studied treatments, whereas it showed the lowest relative increase during heating (68.9% after 120 min at 220°C). EVOO always showed both the absolute lowest value and the highest relative increase with time and with temperature. The lowest EVOO values were due to its being the only non-rectified vegetable oil in this study. P showed the second lowest findings after EVOO.
ΔK is a spectrophotometric index indicating the maximum absorbance at 270 nm. In the case of a rectified oil, and mainly in the case of PO, the absorbance value in this zone increases and the spectrophotometric profile has a characteristic trend with three maximum levels due to the presence of trienes. Of the three peaks, the most pronounced is the central one at 270 nm. To judge an edible vegetable oil it is also important to take into account the absorbance of the two lateral peaks at 266 nm and 274 nm. EVOO showed the lowest ΔK before and after heating, this was because EVOO is not rectified and because of the low ΔK value before heating (0.001), which was reversed in the subsequent steps during heating (Table 7). It is noteworthy that the ΔK value observed in EVOO after 120 min of heating at 220°C was lower than the ΔK value observed in the three other oils before heating; this demonstrates, if necessary, the better food properties of EVOO if compared to other edible vegetable oils. SO always presented the highest values and from the ΔK point of view this is the worst oil.
GHARBY *et al*. (2017) treated edible oils at 100°C for 120 h and found an increase from 1.57 (fresh oil) to 2.59 (after 120 h heating) in an EVOO and a progress from 1.78 to 2.79 in a refined olive oil under the same thermal and time conditions. In the same study K270 was found to increase from 0.13 to 0.33 in EVOO and from 0.55 to 1.99 in a refined olive oil. AZIMAH *et al*. (2017) measured the presence of conjugated dienes at 234 nm in palm oil used to fry potatoes at 175°C, they read 5.36 as a specific extinction in the fresh oil and 5.40 and 5.21 after 10 and 20 frying cycles respectively.

3.6. Total phenolic content

Vegetable oils contain many biologically active components, which exert antioxidant activity, EVOO is consumed unrefined, differently from other edible vegetable oils. This implies that EVOO contains many minor bioactive compounds such as phenols whose content was found to decrease in EVOO during olive fruit ripening (SICARI *et al*., 2009; SICARI *et al*., 2010).
Table 8 describes the total phenolic content evolution of the four studied oils during heating treatments. The highest total phenolic content was found in EVOO (196.8 mg/kg) whereas the lowest content was found in SO (15.0 mg gallic acid/kg).
Heating lowered the total phenolic content and a continuous decrease was measured with heating and with time in all the studied oils. The lowest total phenolic content was found in the samples heated for 120 min at 220°C. The highest loss in total phenolic content (as a percentage) was in EVOO because it had (ab origine) the highest total phenolic content, thus, the highest total phenolic content to be lost during heating.
SANTOS *et al*. (2018) studied EVOO during the frying of white potatoes at 175°C and found a loss in total phenolic content from fresh oil (564 mg/kg) to the 28 hrs fried oil (171 mg/kg), with the minimum content (114 mg/kg) after 16 hrs frying.

Table 5. K 232. At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (*, $p < 0.05$; ***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		*		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Differenc %**	**Palm**	**Difference %**
Unheated oil	1.686±0.01	--	2.858±0.02	--	2.967±0.04	--	2.287±0.13	--
180°C/30min	1.802±0.02	6.9	2.898±0.07	1.4	3.012±0.08	1.5	2.568±0.06	12.3
180°C/60min	1.931±0.02	14.5	2.927±0.02	2.4	3.099±0.06	4.4	2.742±0.01	19.9
180°C/120min	2.297±0.01	36.2	2.987±0.12	4.5	3.113±0.01	4.9	2.722±0.01	19.0
220°C/30min	1.820±0.02	7.9	2.922±0	2.2	3.053±0.06	2.9	2.827±0.03	23.6
220°C/60min	2.594±0.01	53.9	2.934±0.01	2.7	3.104±0	4.6	3.518±0.05	53.8
220°C/120min	2.663±0.03	57.9	3.039±0.05	6.3	3.143±0.01	5.9	3.212±0.06	40.4
Temperature	n.s.		n.s.		n.s.		n.s.	
time	n.s.		*		**		n.s.	
Temperature x time	***		n.s.		n.s.		***	

Table 6. K270. At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	0.113±0.01 g	--	1.203±0.01 g	--	2.174±0.05 e	--	0.971±0.03 f	--
180°C/30min	0.166±0.01 f	46.9	1.466±0.01 f	21.9	2.308±0.10 d	6.2	1.054±0 e	8.5
180°C/60min	0.407±0.01 d	260.2	1.713±0.02 d	42.4	2.550±0.09 d	17.3	1.211±0.02 d	24.7
180°C/120min	0.545±0 b	382.3	1.871±0.02 c	55.5	3.237±0.18 b	48.9	1.555±0 c	60.1
220°C/30min	0.350±0 e	209.7	1.639±0.01 e	36.2	2.841±0.01 c	30.7	1.227±0.02 d	26.4
220°C/60min	0.511±0 c	352.2	2.279±0.01 b	89.4	3.664±0.03 a	68.5	1.639±0.04 b	68.8
220°C/120min	0.890±0.01 a	687.6	2.729±0.03 a	126.8	3.672±0.04 a	68.9	2.364±0.01 a	143.5
Temperature	n.s.		n.s.		n.s.		n.s.	
time	n.s.		n.s.		n.s.		n.s.	
Temperature x time	***		***		***		***	

Table 7. ΔK. At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	Difference %	Pomace	Difference %	Soybean	Difference %	Palm	Difference %
Unheated oil	0.001±0 g	--	0.071±0 c	--	0.194±0.03 d	--	0.082±0 e	--
180°C/30min	0.005±0 f	400.0	0.077±0 c	8.5	0.234±0.04 d	20.6	0.086±0 e	4.9
180°C/60min	0.025±0 d	2400.0	0.104±0 bc	46.5	0.249±0.03 cd	28.4	0.105±0 d	28.0
180°C/120min	0.048±0.02 b	4700.0	0.106±0 bc	49.3	0.599±0.19 ab	208.8	0.152±0 b	85.4
220°C/30min	0.019±0 e	1800.0	0.109±0.01 bc	52.5	0.460±0.02 bc	137.1	0.116±0 c	41.5
220°C/60min	0.031±0 c	3000.0	0.136±0.01 d	91.5	0.650±0.04 ab	235.0	0.155±0.01 b	89.0
220°C/120min	0.061±0 a	6000.0	0.172±0.03 a	142.3	0.684±0.01 a	252.6	0.447±0 a	445.1
Temperature	*		n.s.		n.s.		n.s.	
time	*		n.s.		n.s.		n.s.	
Temperature x time	n.s.		n.s.		*		***	

Table 8. Total phenolic content (mg gallic acid/kg). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	Difference %	Pomace	Difference %	Soybean	Difference %	Palm	Difference %
Unheated oil	196.8±5.1 a	--	40.0±0.2 a	--	15.0±0.6 e	--	43.0±0.6 a	--
180°C/30min	155.5±3.0 b	-21.0	35.5±3.0 b	-11.3	12.2±0.3 b	-18.7	39.5±1.6 b	-8.1
180°C/60min	95.1±4.0 c	-51.7	29.6±0.3 c	-26.0	10.5±0.4 c	-30.0	33.3±0.9 c	-22.6
180°C/120min	59.8±2.2 d	-69.6	27.6±1.1 cd	-31.0	10.1±0.3 c	-32.6	26.5±2.0 d	-38.4
220°C/30min	91.5±2.9 c	-53.5	30.0±0.7 c	-25.0	9.6±0.5 c	-36.0	33.1±0.6 c	-23.0
220°C/60min	63.8±1.8 d	-67.6	24.3±0.2 de	-39.3	7.1±0.5 d	-52.7	27.2±0.9 d	-36.7
220°C/120min	66.8±1.2 d	-66.1	22.5±1.3 e	-43.8	5.2±0.4 e	-65.3	21.8±0.6 e	-49.3
Temperature	n.s.		***		*		**	
time	n.s.		***		n.s.		**	
Temperature x time	***		n.s.		***		n.s.	

3.7. Total tocopherol content

Tocopherols are important components of Vitamin E which was found to prevent the risk of prostate cancer (COC, 2015), to maintain the integrity of long-chain PUFAs in the membranes of cells and thus maintain their bioactivity (TRABER and ATKINSON, 2007), to have beneficial effects as an antioxidant against reproductive disorders, thus it is recommended for women of reproductive age (MUTALIP *et al*., 2018), and to exert an antioxidant activity during a vegetable oil's shelf-life (EVANS *et al*., 2002).
The initial tocopherol content depends on many factors such as cultivar and extraction procedure, for this reason different tocopherol contents are present in the literature in the unheated oils. The following contents have been reported: for EVOO 125-214 mg/kg (NINFALI *et al*., 2002); for PO: 300 mg/kg (PIGNITTER *et al*., 2016); for S: 1030 mg/kg (EVANS *et al*., 2002), 340 mg/kg (GRILO *et al*., 2014); for P: 201 mg/kg (XU *et al*., 2015), 500 mg/kg (KOUSKI *et al*., 2015). In the oils studied in our work, P contained the highest initial total tocopherol content (249 α-tocopherol mg/kg) and showed the lowest percentage decreasing trend at 180°C and at 220°C. SO was found to have the second highest tocopherol content (199.6 mg/kg), whereas PO had the lowest content i.e. 68.6 mg/kg in the unheated PO and 37.3 mg/kg after 120 min heating at 180°C and 18.8 mg/kg after 120 min at 220°C heating (Table 9). Our results are confirmed by other Authors who always found a decreasing trend in tocopherol content during heating even if with a different rate depending on the type of oil, cooking system and applied temperature (HASSANEIN *et al*., 2003; HAMID *et al*., 2014; JAVIDIPOUR *et al*., 2017).

3.8. Antioxidant Activity (ABTS assay)

The AA is partially a consequence of total phenolic content and, more generally, it is a consequence of the physico-chemical properties of each oil studied in this work. EVOO showed the highest ABTS-AA at the start of the experiment (155.9 μM TE/100 g) and in all the six applied treatments. Of the refined oils, P and SO showed the lowest percentage difference in all the six treatments, i.e. the lowest decrease in terms of percentage (Table 10). AYDENIZ and YILMAZ (2016) studied a refined winterized peanut oil and found a decreasing trend in Antioxidant Activity during frying of patties at 180°C for four consecutive days (5-5.5 hrs per day): the oil showed 3.1 mM TEAC/100 g oil at 0 day and 1.8 (day 1), 1.2 (day 2), 0.8 (day 3), 0.5 (day 4); in this experiment the Antioxidant Activity was lower than in our experiment but the peanut oil had a very low initial total phenolic content (0.013 g/kg oil).

3.9. Antioxidant Activity (DPPH hydrophilic assay)

P showed the highest AA-DPPH-hydro in the hydrophilic extract after each treatment (51.8-38.9 μM TE/100 g), whereas SO always showed the lowest AA-DPPH-hydro (6.6-3.7 μM TE/100 g). EVOO showed the second highest AA-DPPH-hydro. This was probably due to the high phenolic content in EVOO (Table 8) and to the high tocopherol content in P, in accordance with findings of antioxidant content reported by other authors (HAMID *et al*., 2014). In all our studied oils a constant decrease in terms of AA was revealed (Table 11).

3.10. Antioxidant Activity (DPPH oil assay)

The highest AA-DPPH-oil was found in unheated SO (117.0 μM TE/100 g) and P (115.7 μM TE/100 g), whereas in unheated EVOO the Antioxidant Activity was 73.5 μmol TE/100 g (Table 12). According to KALANTZAKIS *et al*. (2006) who compared virgin olive oil with SO during 10 hours heating and found SO to have a higher AA-DPPH-oil before and after heating, this was probably due to the higher presence of tocopherol content in SO.

Table 9. Total tocopherol content (mg α-tocopherol/kg). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	169.2±2.7 a	--	68.6±0.3 a	--	199.6±0.7 a	--	249.1±1.2 a	--
180°C/30min	105.8±2.1 b	-37.5	51.3±0.8 b	-25.2	169.5±0.9 b	-15.1	213.0±0.4 b	-14.5
180°C/60min	77.2±1.5 c	-54.4	46.0±0.8 c	-32.9	138.0±0.3 d	-30.8	179.1±0.7 d	-28.1
180°C/120min	44.4±1.4 e	-73.8	37.3±0.6 d	-45.6	86.4±0.3 f	-56.7	126.5±0.3 f	-49.2
220°C/30min	59.4±3.9 d	-64.9	44.8±0.5 c	-34.7	158.1±0.6 c	-20.8	201.5±0.3 c	-19.1
220°C/60min	48.4±0.7 e	-71.4	25.7±0.6 e	-62.5	116.6±0.4 e	-41.6	156.3±0.4 e	-37.2
220°C/120min	34.7±1.6 f	-79.5	18.8±0.4 f	-72.5	53.7±0.5 g	-73.1	91.2±0.8 g	-63.4
Temperature	n.s.		n.s.		n.s.		n.s.	
time	n.s.		n.s.		*		*	
Temperature x time	***		***		***		***	

Table 10. Antioxidant Activity, ABTS assay (μM TE/100g). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	155.9±3.83 a	--	62.5±2.47 a	--	51.8±0.22 a	--	51.8±0.36 a	--
180°C/30min	145.6±1.28 b	-6.6	56.3±1.32 ab	-9.9	37.9±0.22 b	-26.9	49.1±2.12 a	-5.3
180°C/60min	131.3±1.71 c	-15.8	52.8±4.56 bc	-15.5	36.4±0.27 bc	-29.9	44.8±1.20 b	-13.5
180°C/120min	112.3±0.31 d	-27.9	45.5±1.55 cd	-27.1	30.7±0.79 de	-40.8	43.1±0.21 b	-16.9
220°C/30min	124.6±1.17 c	-20.1	44.9±5.51 cd	-28.0	32.4±3.27 cd	-37.6	44.5±2.14 b	-14.2
220°C/60min	111.1±3.69 d	-28.7	42.9±3.80 d	-31.3	33.8±1.24 bcd	-34.8	39.1±0.25 c	-24.6
220°C/120min	100.5±3.29 e	-35.5	29.1±2.01 e	-53.4	27.2±1.58 e	-47.5	38.9±0.44 c	-24.9
Temperature	*		*		*		*	
time	*		n.s.		*		*	
Temperature x time	**		n.s.		n.s.		n.s.	

In all the oils studied in our work, the AA-DPPH-oil assay showed higher values if compared to AA-DPPH-hydro (Tables 11-12). This was in accordance with the findings of ESPÍN *et al*. (2000) who analysed the AA-DPPH of untreated edible vegetable oils (total fats or FT), of its methanolic extracts (MF) and of the oil after methanolic extraction (LF) and found the sum MF + LF always quantitatively comparable with TF value. The decreasing trend of the AA-DPPH-oil was demonstrated by other Authors in different heating conditions. GOMEZ-ALONSO *et al*. (2003) studied EVOO used for French fries at 180°C and found a decrease in the AA of the oil from 740 μmol TE/kg (fresh oil) to less than 250 μmol TE/kg during a total of 2 h frying over 6 days. A reduction in radical scavenging activity (DPPH assay) was also found during deep-frying in palm oil and rice bran oil (HAMID *et al*., 2014). KOBYLIŃSKI *et al* (2016) studied the effect of specific oil surface in rapeseed oil during heating at 180°C and found 459.5 μmol TE/100g as an AA-DPPH value in the fresh oil and an AA-DPPH value ranging from 3.3 μmol TE/100g to 72.65 μmol TE/100g when the level of oil in pan-five different oil layer heights was changed from 0.5 cm to 2.5 cm; this was expected considering that during heating of a thin oil layer there is a greater oxygen absorption per unit oil than during heating in a larger amount of oil.

3.11. Antioxidant Activity (ORAC assay)

The ORAC assay confirmed results obtained by ABTS assay with EVOO showing the highest ORAC-AA. Before heating EVOO had the highest ORAC-AA value (316.1 μM TE/100 g), after 30 min heating it decreased to 220.9 μM TE/100 g (-30.1%) and to 142.6 μM TE/100 g (-54.9%) at 180°C and 220°C respectively (Table 13). P showed the second highest ORAC-AA (65.4 μM TE/100 g) and it decreased to 40.7 μM TE/100 g (after 30 min heating) and to 32.5 μM TE/100 g (after 120 min heating) at 180°C. When P was heated at 220°C, the ORAC-AA was 27.1 μM TE/100 g (-58.6%) and 15.9 (-75.7%) respectively after 30 and 120 min heating. It is worthy of note that oils from different cultivars could have a different ORAC even if they have the same total phenolic content, in fact ZULLO and CIAFARDINI (2008) studied some single components of the phenolic fraction in an EVOO and found gallic acid to have a greater influence on ORAC than caffeic acid and oleuropein.

3.12. ONE-WAY ANOVA, TWO-WAY ANOVA, CORRELATION MATRIX

3.12.1 Free acidity

One-way ANOVA showed high significant differences in SO and showed very high significant differences in every other oil ($p < 0.001$), (Table 1). Two-way ANOVA analysis showed that temperature had no significant effect on the FA variation in the four oils analysed in this study (Table 1). The same was for heating duration on EVOO and P, whereas the heating duration influenced significantly the FA variation in SO ($p < 0.05$) and highly significantly in PO ($p < 0.01$). FA showed a very good positive correlation with *p*-AnV especially in EVOO (0.9175), in PO (0.9567) and in P (0.8193), a good correlation was found in SO (0.7634), (Tables 14-17). FA was negatively very well correlated with AA-DPPH-oil in P (-0.8569), in SO (-0.8372), in EVOO (-0.8067) and a good correlation was found in PO (-0.7820), (Tables 14-17). Similar studies have been conducted on other edible vegetable oils. GIUFFRÈ *et al*. (2017b), studied the influence of high temperature and time of heating on sunflower seed oil at 180-210-240°C for 15-30-60-120 min and found a constant slight increase in FA which was influenced by time of heating ($p < 0.01$) and by the interaction between temperature and time of heating ($p < 0.001$).

Table 11. Antioxidant Activity, DPPH hydrophilic assay (μM TE/100g). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (**, $p < 0.01$; ***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		**		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	47.0±3.37 a	--	10.7±0.44 a	--	6.6±0.30 a	--	51.8±0.36 a	--
180°C/30min	43.6±0.88 a	-7.3	8.3±2.82 b	-22.9	6.4±0.14 a	-3.9	49.1±2.12 b	-13.4
180°C/60min	29.6±1.71 b	-37.1	7.8±0.41 b	-27.4	6.3±0.17 a	-5.5	44.8±1.20 bc	-21.5
180°C/120min	19.9±0.35 c	-57.7	6.8±1.05 b	-37.0	3.8±0.45 b	-42.9	43.1±0.21 bc	-24.9
220°C/30min	28.8±2.05 b	-38.7	7.5±0.06 b	-29.8	4.5±0.25 b	-32.7	44.5±2.14 b	-14.9
220°C/60min	19.6±0.46 c	-58.3	6.6±0.11 b	-38.7	3.8±0.45 b	-42.6	39.1±0.25 cd	-32.1
220°C/120min	12.7±2.87 d	-73.0	5.0±1.78 b	-53.6	3.7±0.24 b	-43.7	38.9±0.44 d	-43.3
Temperature	*		n.s.		n.s.		n.s.	
Time	*		n.s.		n.s.		n.s.	
Temperature x time	**		n.s.		***		*	

Table 12. Antioxidant Activity, DPPH oil assay (μM TE/100g). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	73.5±2.02 a	--	69.8±0.14 a	--	117.0±0.21 a	--	115.7±1.97 a	--
180°C/30min	71.7±1.84 a	-2.3	68.0±0.19 b	-2.6	107.4±0.46 b	-8.2	109.9±1.14 ab	-5.0
180°C/60min	56.7±1.59 c	-22.8	66.2±0.19 c	-5.2	105.6±1.06 c	-9.8	106.8±0.46 bc	-7.7
180°C/120min	43.4±1.82 e	-40.9	61.4±0.55 d	-12.0	101.2±0.49 e	-13.5	101.2±0.34 cd	-12.6
220°C/30min	64.5±1.14 b	-12.1	49.9±0.19 e	-28.5	105.2±0.60 c	-10.1	107.4±4.24 bc	-7.1
220°C/60min	50.3±1.97 d	-31.5	48.5±0.25 f	-30.6	103.3±0.42 d	-11.7	95.9±2.44 d	-17.1
220°C/120min	31.3±0.92 f	-57.3	39.0±0.56 g	-44.2	96.5±0.28 f	-17.5	76.9±3.59 e	-33.5
Temperature	*		**		n.s.		n.s.	
time	*		n.s.		*		n.s.	
Temperature x time	*		***		**		***	

Table 13. Antioxidant Activity, ORAC assay (μM TE/100 g). At the top of the table, one-way ANOVA experiment where unheated and heated oils are considered: means followed by different letters in the same column are significantly different according to Tukey's test (***, $p < 0.001$). At the bottom of the table, two-way ANOVA experiment where only heated oils are considered: temperature, time, temperature x time (n.s., $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$). Difference (%) is calculated on the unheated oil.

Sign.	***		***		***		***	
	EVOO	**Difference %**	**Pomace**	**Difference %**	**Soybean**	**Difference %**	**Palm**	**Difference %**
Unheated oil	316.1±44. 9 a	--	18.5±0.4 a	--	9.1±0.2 a	--	65.4±0.5 a	--
180°C/30min	220.9±9.1 b	-30.1	17.5±0.2 b	-5.4	6.0±0.3 b	-34.1	40.7±0.5 b	-37.8
180°C/60min	164.8±15.5 c	-47.9	14.9±0.5 d	-19.5	5.4±0.3 b	-40.7	33.0±1.2 c	-49.5
180°C/120min	117.2±3.6 de	-62.9	10.4±0.5 f	-43.8	1.9±0.4 e	-79.1	32.5±1.7 c	-50.3
220°C/30min	142.6±4.8 cd	-54.9	15.8±0.1 c	-14.6	5.6±0.3 b	-38.5	27.1±1.7 d	-58.6
220°C/60min	114.6±6.2 de	-63.8	11.4±0.2 e	-38.4	3.7±0.4 c	-59.3	25.7±1.6 d	-60.7
220°C/120min	93.2±3.5 e	-70.5	8.3±0.6 g	-55.1	2.0±0.4 e	-78.0	15.9±0.3 e	-75.7
Temperature	n.s.		*		n.s.		*	
time	n.s.		*		n.s.		n.s.	
Temperature x time	***		***		***		***	

Table 14. Correlation matrix between chemical properties of Extra Virgin Olive Oil before and after heating.

	Free Acidity	**Peroxide Value**	***p*-Anisidine Value**	**TOTOX**	**K232**	**K270**	**ΔK**	**Total Phenolic Content**	**Total Tocopherol Content**	**ABTS assay**	**DPPH oil assay**	**DPPH hydro assay**	**ORAC assay**
Free Acidity	1												
Peroxide Value	0.7212	1											
p-Anisidine Value	0.9175	0.8327	1										
TOTOX	0.8782	0.9339	0.9756	1									
K232	0.6966	0.8660	0.8148	0.8692	1								
K270	0.8467	0.9097	0.9605	0.9806	0.8886	1							
ΔK	0.7308	0.9246	0.8663	0.9257	0.8266	0.9370	1						
Total Phenolic Content	-0.4955	-0.7815	-0.6420	-0.7242	-0.7787	-0.8193	-0.8114	1					
Total Tocopherol Content	-0.5516	-0.7862	-0.6825	-0.7522	-0.7590	-0.8258	-0.8059	0.9712	1				
ABTS assay	-0.6686	-0.9000	-0.8187	-0.8852	-0.8964	-0.9337	-0.8946	0.9420	0.9358	1			
DPPH oil assay	-0.8067	-0.9410	-0.9067	-0.9583	-0.9017	-0.9751	-0.9476	0.8231	0.8071	0.9180	1		
DPPH hydro assay	-0.6740	-0.8832	-0.8162	-0.8770	-0.8895	-0.9377	-0.8989	0.9457	0.9151	0.9824	0.9277	1	
ORAC assay	-0.5367	-0.7590	-0.6743	-0.7362	-0.7544	-0.8144	-0.7819	0.9658	0.9909	0.9246	0.7899	0.9115	1

Table 15. Correlation matrix between chemical properties of Pomace Olive Oil before and after heating.

	Free Acidity	Peroxide Value	*p*-Anisidine Value	TOTOX	K232	K270	ΔK	Total Phenolic Content	Total Tocopherol Content	ABTS assay	DPPH oil assay	DPPH hydro assay	ORAC assay
Free Acidity	1												
Peroxide Value	0.9822	1											
p-Anisidine Value	0.9567	0.9594	1										
TOTOX	0.9677	0.9733	0.9985	1									
K232	0.6195	0.6929	0.6657	0.6752	1								
K270	0.8903	0.8958	0.9677	0.9600	0.6691	1							
ΔK	0.8319	0.8192	0.9000	0.8901	0.4356	0.9147	1						
Total Phenolic Content	-0.6913	-0.7133	-0.8265	-0.8099	-0.7159	-0.9166	-0.8354	1					
Total Tocopherol Content	-0.7774	-0.7884	0.8825	-0.8699	-0.6649	-0.9669	-0.8702	0.9557	1				
ABTS assay	-0.8324	-0.8515	-0.8992	-0.8957	-0.6938	-0.9081	-0.8359	0.8692	0.9038	1			
DPPH oil assay	-0.7820	-0.7803	-0.8851	-0.8705	-0.5545	-0.8693	-0.8576	0.8128	0.8508	0.9036	1		
DPPH hydro assay	-0.6438	-0.6561	-0.7034	-0.6987	-0.5697	-0.7818	-0.7970	0.8631	0.8084	0.7472	0.6855	1	
ORAC assay	-0.7645	-0.8156	-0.8592	-0.8563	-0.6961	-0.9253	-0.8343	0.9020	0.9210	0.8563	0.7379	0.7645	1

Table 16. Correlation matrix between chemical properties of Soybean Oil before and after heating.

	Free Acidity	Peroxide Value	*p*-Anisidine Value	TOTOX	K232	K270	ΔK	Total Phenolic Content	Total Tocopherol Content	ABTS assay	DPPH oil assay	DPPH hydro assay	ORAC assay
Free Acidity	1												
Peroxide Value	0.8486	1											
p-Anisidine Value	0.7634	0.9021	1										
TOTOX	0.7834	0.9254	0.9984	1									
K232	0.7388	0.8092	0.6786	0.7037	1								
K270	0.7403	0.8763	0.8545	0.8672	0.6972	1							
ΔK	0.6422	0.8081	0.7969	0.8075	0.6311	0.9588	1						
Total Phenolic Content	-0.7696	-0.9082	-0.9027	-0.9137	-0.7510	-0.9094	-0.8266	1					
Total Tocopherol Content	-0.8382	-0.9883	-0.8815	-0.9057	-0.7950	-0.8478	-0.8057	0.8532	1				
ABTS assay	-0.7822	-0.8473	-0.7025	-0.7297	-0.7304	-0.7681	-0.7315	0.8641	0.8297	1			
DPPH oil assay	-0.8372	-0.9456	-0.8172	-0.8436	-0.7957	-0.8273	-0.7721	0.9095	0.9295	0.9649	1		
DPPH hydro assay	-0.7477	-0.7865	-0.7539	-0.7668	-0.6029	-0.9041	-0.8835	0.8095	0.7913	0.7824	0.7881	1	
ORAC assay	-0.8181	-0.9422	-0.7523	-0.7861	-0.7668	-0.8531	-0.8294	0.8282	0.9519	0.9050	0.9489	0.8302	1

Table 17. Correlation matrix between chemical properties of Palm Oil before and after heating.

	Free Acidity	Peroxide Value	*p*-Anisidine Value	TOTOX	K232	K270	ΔK	Total Phenolic Content	Total Tocopherol Content	ABTS assay	DPPH oil assay	DPPH hydro assay	ORAC assay
Free Acidity	1												
Peroxide Value	0.8886	1											
p-Anisidine Value	0.8193	0.8009	1										
TOTOX	0.8656	0.8769	0.9901	1									
K232	0.7557	0.8806	0.7031	0.7704	1								
K270	0.8501	0.8485	0.9605	0.9695	0.7227	1							
ΔK	0.7400	0.7391	0.8894	0.8869	0.5450	0.9507	1						
Total Phenolic Content	-0.8787	-0.8762	-0.8904	-0.9198	-0.7836	-0.9019	-0.7545	1					
Total Tocopherol Content	-0.8819	-0.8447	-0.9343	-0.9476	-0.6783	-0.9243	-0.8087	0.9611	1				
ABTS assay	-0.8357	-0.9083	-0.8202	-0.8709	-0.9062	-0.8264	-0.6571	0.9163	0.8552	1			
DPPH oil assay	-0.8569	-0.8765	-0.9411	-0.9605	-0.7405	-0.9787	-0.9373	0.8628	0.8971	0.8137	1		
DPPH hydro assay	-0.8470	-0.9208	-0.9016	-0.9392	-0.8087	-0.8897	-0.7764	0.8892	0.9081	0.9053	0.9203	1	
ORAC assay	-0.8753	-0.9559	-0.7051	-0.7897	-0.7985	-0.7587	-0.6362	0.8734	0.8246	0.8823	0.7829	0.8540	1

3.12.2 Peroxide value

The two-way ANOVA analysis showed that in all the studied oils, the PV increase was not significantly influenced by temperature but was always very highly significantly influenced by the interaction between temperature and time (Table 2). PV was found to be very good and positively correlated with K270: 0.9097 EVOO, 0.8958 PO, 0.8763 SO and 0.8485 P, whereas a very high and negative correlation was found with AA-ABTS: -0.9000 EVOO, -0.8515 PO, -0.8473 SO and -0.9083 P (Tables 14-17).

3.12.3 *p*-Anisidine value

The one-way ANOVA analysis showed that very highly significant differences exist in all the studied oils between treatments. The two-way ANOVA analysis showed that in all the studied oils, the *p*-AnV increase was not significantly influenced by temperature or by time of heating but it was always very highly significantly influenced by the interaction between temperature and time (Table 3). In the three rectified oils, *p*-AnV was found to have negative and good or very good correlation with the parameters indicating antioxidant activity. In PO, *p*-AnV was found to be correlated with PV (0.9594), with TOTOX (0.9985) and with K270 0.9677). In SO, *p*-AnV was found to be correlated with PV (0.9021), with TOTOX (0.9984) and with total phenolic content (0.9027). In P, *p*-AnV was found to be correlated with TOTOX (0.9901), with K270 (0.9605) and with AA-DPPH Oil (-0.9411). In EVOO, a negative and very good correlation was found with ABTS assay (-0.8187), AA-DPPH-oil (-0.9067), AA-DPPH Hydro (-0.8162), (Tables 14-17).

3.12.4 TOTOX

The one-way ANOVA analysis showed very high significant differences between treatments. The two-way ANOVA analysis showed that in all the studied oils, the TOTOX variation was not significantly influenced by temperature or by time of heating but it was always very highly significantly influenced by the interaction between temperature and time (Table 4), according to two-way ANOVA results of *p*-AnV (Table 3). TOTOX showed a very good positive correlation with K270: 0.9806 in EVOO, 0.9600 in PO, 0.8672 in SO and 0.9695 in P (Tables 14-17).

3.12.5 K232

The two-way ANOVA demonstrated that K232 was not significantly affected by temperature in all oils, whereas the interaction between temperature and heating duration influenced very highly significantly ($p < 0.001$) EVOO and P (Table 5).
A low correlation with ΔK in all the rectified oils was found in K232: 0.4356 in PO, 0.6311 in SO and 0.5450 in P whereas K232 had a good correlation with ΔK in EVOO (Tables 14-17).

3.12.6 K270

Two-way ANOVA demonstrated that K270 was not influenced by temperature and heating duration in all the four studied oils, whereas a very high significant influence was found in the interaction between the two variables (Table 6).
Both K270 and *p*-AnV are used as indices to indicate a prolonged oxidation. K270 was found to be very well correlated with *p*-AnV in EVOO (0.9605), in PO (0.9677), in SO (0.8545) and in P (0.9605), (Tables 14-17).

3.12.7 ΔK

The one-way ANOVA analysis showed very high significant differences between treatments in all the four studied oils ($p < 0.001$). The two-way ANOVA analysis showed EVOO as the most influenced by heating duration and by the temperatures $p < 0.05$ for both the applied variables, whereas P was very highly significantly influenced ($p < 0.001$) by the interaction between the two treatments (Table 7). ΔK was found to have a very good correlation with K270 and the highest correlations were in the two seed oils: 0.9588 in SO and 0.9507 in P (Tables 14-17).

3.12.8 Total phenolic content

The one-way ANOVA analysis showed very highly significant differences between treatments. The two-way ANOVA analysis showed a different situation for each oil. EVOO was not influenced by temperature and by time, but it was very highly significantly influenced by their interaction. PO was very highly significantly influenced by temperature and by time ($p < 0.001$), but their interaction was not significant. The total phenolic content in SO was not influenced by time but was significantly influenced by temperature ($p < 0.05$) and very highly significantly influenced by their interaction ($p < 0.001$). In P, the interaction between time and temperature had no significant effect whereas temperature and time caused a highly significant effect ($p < 0.01$). Total phenolic content showed a negative very good correlation with K270 in EVOO (-0.8193), in PO (-0.9166), in SO (-0.9094) and in P (-0.9019), (Tables 14-17).

3.12.9 Total tocopherol content

One-way ANOVA analysis showed a very high significant lowering ($p < 0.001$) of the total tocopherol content during heating both at 180 and 220°C. The greatest lowering effect was produced at 220°C with a reduction accounting for -79.5% (EVOO), -72.5% (PO), -73.1% (S) and -63.4% (P) after 120 min of heating treatment (Table 9). The two-way ANOVA experiment demonstrated very high significant differences by the combined effects of temperature x time in all the four studied oils and not significant differences if only the temperature effect was considered (Table 9). A very good positive correlation (minimum 0.8532 in S) was always found with the total phenolic content and with the ORAC assay (minimum 0.8246 in P), (Tables 14-17).

3.12.10 Antioxidant Activity (ABTS assay)

The one-way ANOVA analysis showed very highly significant differences between treatments ($p < 0.001$). The two-way ANOVA analysis showed that in all the studied oils, the temperature significantly influenced the ABTS-AA ($p < 0.05$). Time of heating significantly influenced the ABTS-AA in all oils except in PO in which the significance was 0.057. The interaction between the two studied variables was not significant in all the studied oils except in EVOO in which a highly significant effect ($p < 0.01$) was found (Table 10).
ABTS-AA showed a negative very good correlation with PV in EVOO (-0.9000), in PO (-0.8515), in SO (-0.8473) and in P (-0.9083) and a positive very good correlation with total phenolic content: 0.9420 in EVOO, 0.8692 in PO, 0.8641 in SO and 0.9163 in P (Tables 14-17).

3.12.11 Antioxidant Activity (DPPH hydrophilic assay)

The one-way ANOVA analysis showed very highly significant differences between treatments ($p < 0.001$) in all oils except in PO in which a highly significant difference was found ($p < 0.01$). The two-way ANOVA analysis showed that temperature caused no significant difference in SO and P, in PO the significance was 0.053 and in EVOO the temperature caused significant differences ($p < 0.05$). The interaction between temperature and time caused no significant differences in the treatments of PO, whereas in the other oils the significance was $p < 0.05$ for P, $p < 0.01$ for EVOO and $p < 0.001$ for SO (Table 11).

3.12.12 Antioxidant Activity (DPPH oil assay)

One-way ANOVA evidenced very highly significant differences between samples ($p < 0.001$), (Table 12). Two-way ANOVA showed that the interaction between time and temperature influenced the AA-DPPH-oil as follows: EVOO ($p < 0.05$), SO ($p < 0.01$), PO and P ($p < 0.001$). No significant effect resulted from the temperature treatment in S and P, the same was for the time treatment in PO (Table 13).

3.12.13 Antioxidant Activity (ORAC assay)

The one-way ANOVA analysis showed very highly significant differences between treatments ($p < 0.001$) in all oils. The two-way ANOVA analysis showed that temperature caused no significant effect in EVOO and SO, whereas in PO and P a significant effect was found ($p< 0.05$). The time of heating showed no significant effect on EVOO, SO and P. The interaction between temperature and time of heating caused very highly significant effects ($p < 0.001$) in all the studied oils (Table 13).
ORAC assay showed the highest positive correlation with total phenolic content in EVOO (0.9658) and in PO (0.9020), (Tables 14-15). In SO the highest positive correlation was found with AA-DPPH-oil (0.9489), whereas ABTS was the highest correlated in P (0.8823), (Tables 16-17). The correlation between ORAC values and phenolic content was also studied by Ninfali *et al*. (2001) who found a positive correlation (R = 0.78925) by analyzing commercially available EVOOs.

4. CONCLUSIONS

The findings of this work suggest how to manage temperatures and heating duration during cooking with an extra virgin olive oil, a pomace olive oil, a soybean oil and a palm oil. The four studied vegetable oils showed four different behaviours in relation to temperature and heating duration. Extra virgin olive oil and palm oil showed the best performances in term of resistance to oxidation. All the heated oils showed a reduction in antioxidant activity when compared to control (unheated oil). A lower antioxidant activity was found in the heated oils because phenols and, in general, the antioxidants are destroyed during heating. The best cooking temperature was found to be at 180°C, which caused the lowest oil deterioration, as well as being less expensive if compared to 220°C. When extra virgin olive oil, pomace olive oil, soybean oil and palm oil are heated at 180°C they can be re-used for 120 minutes but if the heating temperature is 220°C, the suggested maximum time of use must be reduced to 60 minutes.

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PAPER

COMPOSITION AND THERMAL PROPERTIES OF QUATERNARY MIXTURES OF PALM OIL:PALM STEARIN:SOYBEAN OIL:COCOA BUTTER

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ABSTRACT

Pam oil (PO) is a semi-solid substance with potential functional lipid characteristics. A study was carried out to evaluate the effect of addition of soybean oil (SBO), palm stearin (PS) and cocoa butter (CB) on the solidification behavior of PO to formulate a mixture to become similar to lard (LD). A total of three mixtures were prepared: PO:PS:SBO:CB (38:5:52:5), PO:PS:SBO:CB (36:5:54:5) PO:PS:SBO:CB (34:5:56:5) (w/w), and identified by the mass ratio of PO to PS, CB and SBO. The fat mixtures were compared with lard in terms of the fatty acid and triacylglycerol compositions using gas chromatography and high performance liquid chromatography, thermal properties using differential scanning calorimetry (DSC) and solid fat content (SFC) using p-nuclear magnetic resonance (p-NMR). Although there were considerable differences between lard and the fat mixtures with regard to fatty acid and triacylglycerol compositions, some similarities were seen on their DSC thermal properties and solid fat content profile. Of the fat mixtures, PO:PS:SBO:CB (38:5:52:5) displayed closer similarity to lard by having least difference to SFC profile throughout the temperature range and a common DSC thermal transition at around -3.59°C.

Keywords: palm oil, DSC, lard substitute, cocoa butter, soybean oil, thermal analysis

1. INTRODUCTION

Animal fats have traditionally been used as medium of frying in many types of foods. They were used in food applications mainly due to economic reasons since voluminous amounts of animal fats are discarded by the industry. Flavor imparted on foods by animal fats is an important reason for animal fat use. For instance, in some food cultures, vegetable oils used for frying are blended with small amounts of lard to impart characteristic flavors. Although the use of animal fats such as lard, tallow, etc. is already popular, the bad effects of the consumption are also being studied since the consumer perception begins to be negative with regard to the use of animal fat. As a result, there has been a great deal of interest among researchers to investigate various plant-based substitutes as alternative for lard (MARIKKAR and NOORZIYANNA YANTY, 2018; HSU and YU, 2002). When exploring these plant-fats, comparing their composition and thermal properties with those of LD was an important aspect of the investigations. For example, FLOTER (2009) highlighted the importance of studying physical properties data in product development. Separately, GIVEN (1990) also emphasized the influence of fat and oil physicochemical properties on the expression of functionality in baked goods.
The special properties of LD are large due to its peculiar nature of TAG composition. As reported previously by SILVA *et al*. (2009), LPO, OPO and SPO were the predominating TAG molecular species of LD with oleic, palmitic and stearic acids occurring in higher proportions. Owing to this reason, LD alternative fats were formulated using plant fat mixtures having these TAG molecular species. Accordingly, a replacement for LD was investigated using binary fat mixtures of mee fat and palm stearin as well as binary fat mixtures composed of engkabang fat and canola oil (YANTY, 2016; NUR ILLIYIN *et al*., 2013). In a separate experiment, YANTY (2016) found that ternary blends of avocado (Avo) fat, PS and CB also satisfied this requirement. As a notable feature, these fat mixtures displayed solidification behavior closely similar to that of LD. For our knowledge, the compatibility of quaternary fat mixtures comprising palm oil (PO), PS, soybean (SBO) and cocoa butter (CB) has not been considered for the said purpose. According to previous studies, PO contained approximately 40% oleic, 10% linoleic, 45% palmitic and 5% stearic acids (TAN and CHE MAN, 2000). As solidification values of PO was always higher than that of LD throughout the temperature region, addition of liquid oils such as SBO would be necessary. SBO has very higher amount of oleic and lineic acids, and hence the blending would tend to affect the proportion of palmitic and stearic acid contents. Owing to this reason, inclusion of small amounts of PS and CB would be needed to maintain the required proportions of palmitic and stearic acids in the final mixture. In this study, three quaternary plant-based fat blends (PO, SBO, PS and CB) were formulated to make comparison to lard with respect to their composition, DSC thermal properties, and solidification profiles.

2. MATERIALS AND METHODS

2.1. Materials

LD was extracted using three batches of adipose tissues of swine collected from local slaughter houses as described in previous reports. Samples of PO and PS were obtained as generous gift from Malaysian Palm Oil Board (MPOB). CB and SBO were purchased from Malaysian Cocoa Board and a local supermarket, respectively. All chemicals used in this experiment were of analytical or HPLC grade.

2.2. Preparation of quaternary mixtures

The fat samples were melted at 70°C for 1 h before mixing. A total of three fat mixtures were prepared: PO:PS:SBO:CB (38:5:52:5), PO:PS:SBO:CB (36:5:54:5) PO:PS:SBO:CB (34:5:56:5) (w/w), and identified by the mass ratio of PO to PS, CB and SBO. All samples were kept under frozen storage at –20°C. Prior to analyses, the fat mixtures were removed from frozen storage, and then left static at room temperature for 1 h before being warmed at 70° C until they became completely molten.

2.3. Determination of SMP and IV

SMP and IV of the fat samples were determined according to AOCS method Cc.3.25, and AOCS method Cd Id–92, respectively (AOCS, 1999).

2.4. Determination of FA composition

Fatty acid methyl esters were prepared by dissolving 50 mg portion of oil in 0.8 mL of hexane and adding 0.2 mL portion of 1 M solution of sodium methoxide (PORIM, 1995). The top hexane layer was injected on an Agilent 6890N gas chromatograph (Agillent Technologies, Singapore) equipped with a polar capillary column RTX-5 (0.32 mm internal diameter, 30 m length, and 0.25 μm film thickness; Restex Corp., Bellefonte, PA) and a flame ionization detector (FID). Split injection was conducted with a split ratio of 58:1, nitrogen was used as a carrier gas at a flow-rate of 1.00 mL/min. The temperature of the column was 50°C (for 1 min), and programmed to increase to 200°C at 8°C/min. The temperatures of the injector and detector were maintained at 200°C. The identification of the peaks of the samples was done with reference to a chromatographic profile containing a set of fatty acid methyl ester standards. The percentage of fatty acid was calculated as the ratio of the partial area to the total area (NUR ILLIYIN *et al.*, 2013).

2.5. Determination of TAG composition

The TAG compositions of samples were determined according to the method described by YANTY (2016) using Waters Model 510 liquid chromatography equipped with a differential refractometer Model 410 as the detector (Waters Associates, Milford, MA). The analysis of TAG was performed on a Merck Lichrosphere RP-18 column (5 μm; 12.5 cm × 4 mm i.d.; Merck, Darmstadt, Germany), which was maintained at 30°. The mobile phase was a mixture of acetone:acetonitrile (63.5:36.5) and the flow rate was 1.5 mL/min. The injector volume was 10 μL of 5% (w/w) oil in chloroform. Each sample was chromatographed three times, and the data were reported as peak area percentages. The identification of the peaks of the samples was done using a set of TAG standards purchased from Sigma-Aldrich (Deisehofen, Germany) as well as the TAG profiles of lard (NUR ILLIYIN *et al.*, 2013), PO (TAN and CHE MAN, 2000), PS (TAN and CHE MAN, 2000), CB (SEGALL *et al.*, 2005) and SBO (TAN and CHE MAN, 2000) reported previously.

2.6. Thermal analysis by DSC

Thermal analysis was carried out on a Mettler Toledo differential scanning calorimeter (DSC 823 Model) equipped with a thermal analysis data station (STARe software, Version 9.0x, Schwerzenbach, Switzerland). Nitrogen (99.999% purity) was used as the purge gas at a rate of ~20 mL/min. Approximately, 4-8 mg of melted sample was placed in a standard DSC aluminum pan and then hermetically sealed. An empty, hermetically sealed

DSC aluminum pan was used as the reference. The oil/fat samples were subjected to the following temperature program: 70°C isotherm for 1 min, cooled at 5°C/min to -70°C. The samples were held at -70°C isotherm for 1 min, and heated at 5°C/min to reach 70°C (YANTY, 2016)

2.7. Determination of SFC

SFC was measured according to AOCS method Cd 16b-93 (AOCS, 1999) using a Bruker Minispec (Model Mq 20) pulse nuclear magnetic resonance (pNMR) spectrometer (Karlsruhe, Germany). The sample in the NMR tube was melted at 70°C for 15 min, followed by chilling at 0°C for 60 min, and then held at each measuring temperature for 30 min prior to measurement. Melting, chilling, and holding of the samples were carried out in pre-equilibrated thermostatic glycol containing baths, accurate to 0.1°C. SFC measurements were taken at 5°C intervals over the range of 0-70°C.

2.8. Statistical analysis

All analyses were carried out in triplicate and the results were expressed as mean value ± standard deviation. Data were statistically analyzed by one-way analysis of variance (ANOVA), by using Tukey's Test of MINITAB (version 15) statistical package at 0.05 probability level.

3. RESULTS AND DISCUSSION

3.1. FA composition, SMP, and IV

FA composition of PO, PS, SBO, CB, all three quaternary mixtures and LD were presented in Table 1. Values showed the great variability of the samples, which might influence melting point, iodine values and the shape of DSC thermal curves. According to Table 1, the most dominant FA of PO was palmitic (43.99%), followed by oleic (39.24%), and linoleic (10.25%) acids. PS, the hard stearin of PO contained palmitic (78.9%) as the most dominant fatty acid followed by oleic (10.6%), and stearic (6.32%) acids. In the meantime, CB was found to possess stearic (37.84%) as the predominant FA followed by oleic (32.83%) and palmitic (25.34%). SBO, on the other hand, contained linoleic acid (53.93%) as the most predominant FA, followed by oleic (23.87%), palmitic (11.33%) and linolenic (6.46%) acids. These values were found to be comparable to those reported previously by TAN and CHE MAN (2000). They found that SBO contained linoleic (53.3%) as the most predominant FA, followed by oleic (23.6%), palmitic (12.6%) and linolenic (6.3%) acids. In fact, additions of PS, SBO and CB into PO caused significant ($p<0.05$) increments in the proportions of stearic, linoleic, and linolenic acids with concurrent decreases in the amounts of palmitic and oleic acids. Among these FAs, linoleic acid was found to be increased considerably while palmitic and oleic acids were found to decreased. The increment in the proportion of linoleic acid (30.78 to 34.44) of the quaternary mixtures was due to the presence of higher proportion of linoleic acid in SBO. This was far higher than the proportion of linoleic in LD or any other animal fats. However, the proportions of palmitic and oleic in the three mixtures were somewhat lower than the corresponding amounts found in LD. When compared to LD, the palmitic and linoleic acid contents of the quaternary mixtures were significantly ($p<0.05$) higher while stearic and oleic acid contents were significantly ($p<0.05$) lower. With the decreasing amount of PO in the mixtures, the total SFA content was tended to decrease (from 36.76 to 34.40 %) while the

total USFA content was found to increase (from 63.24 to 65.60 %). Among the mixture, total USFA (~63%) and SFA (~37%) contents of the PO:PS:SBO:CB (38:5:52:5) were found to be roughly similarly to the total USFA (~61%) and SFA (~39%) contents of LD.
According to Table 1, the SMP of PO, PS, CB and LD were 30.5, 59.5, 34.25 and 27.5°C, respectively. This measurement was not done for soybean oil because this method does not apply to this type of oil. Additions of PS and CB into PO were found to cause significant ($p<0.05$) increases in SMP values (from 38.0 to 41.25°C) when compared to that of original PO. This could be due to the fact that CB and PS were crystalline solid fats and had comparably higher proportions of palmitic and stearic acids (Table 1). With regard to the degree of unsaturation, the IV of PO, PS, SBO and CB were 54, 14, 136.85 and 34, respectively. The IV of quaternary mixtures of PO:PS:SBO:CB were found to be in the range of 92.26 to 96.95. All fat mixtures of this study displayed significantly higher ($p<0.05$) IV than either PO (54.00) or LD (73.76). Based on these results, none of the quaternary mixtures of this study was found to have a SMP and IV closely similar to those of LD. The changes in SMP and IV of these mixtures as noted before (Table 1) could be mainly due to the changes in FA compositions.

3.2. TAG composition

The TAG compositions of PO, PS, SBO, CB, quaternary mixtures of PO:PS:SBO:CB were compared to that of LD as shown in Table 2. PO composed of PPO (31.61%), POO (24.76%), PPL (10.19%) and POL (9.96%) as dominant TAG molecules. These values were comparably similar to the ranges reported previously (MARIKKAR and GHAZALI, 2011). On the other hand, PS contained PPP (68.66%), PPO (15.23%), and StOP (11.07%) as major TAG molecules. The major TAG molecules of CB were SOP (40.78%), SOS (29.35%) and PPO (18.08%). SBO, on the other hand was found to possess LLL (23.56%), OLL (17.77%), PLL (15.82%) and POL (13.69%) as the major TAG molecules. After addition of PS, SBO and CB into PO, some of the TAG molecules were found to increase (e.g. PPL, OOL, POL, PPP and SOS) while others were tended to decrease (e.g. MMM, MPL, PPL, OOO, POO, PPO, SOO, SPO and PPS). The increments in the proportions of PLL, OOL and POL could be due to the presence of SBO in the mixture. There were significant increases in the proportions of PPP, and SOS as these were major TAG species of PS (NOR AINI and MISKANDAR, 2007) and CB (LIU *et al*., 2007; SEGALL *et al*., 2005), respectively. TAG molecular species namely, LLnLn, LLLn, OLnLn, LLL, PLLn, OLL, POLn and SSS were also found in the mixtures after addition of PS, SBO and CB into PO. Among the TAG molecular species, PPO (ranging from 13.48 to 12.09%) and POO (ranging from 10.87 to 9.70%) were found to reduce dramatically in the mixtures when compared to those of original PO (31.61 and 24.76%, respectively). In addition, UUU and StStSt TAG molecules were found to increase with respect to the original sample of PO. For instance, the UUU TAG was increased (34.89 to 37.14%) when compared to that of the original sample of PO (12.68%). The StStSt TAG molecules were also found to decrease slightly (24.86 to 22.47%). In the meantime, the amount of PPO in the mixtures was found to be somewhat similar to those of LD. When compared to LD, the amount of UUU and StStSt TAG of the PO:PS:SBO:CB mixtures were found to be higher with concurrent decreases of UUSt and UStSt TAG molecules. The increasing proportions of UUU TAGs in the fat mixtures could have led to the occurrence of greater amounts of oleic and linoleic acids as shown in the overall FA distribution (Table 1). Among them, UStSt TAG content of PO:PS:SBO:CB (38:5:52:5) was found to be closely (24.86%) comparable to that of LD (26.60%).

Table 1. Basic physico-chemical characteristics and fatty acid composition (%) of PO, PS, SBO, CB, quaternary mixtures of PO:PS:SBO:CB and LD.

	PO	PS	SBO	CB	PO:PS:SBO:CB (38:5:52:5)	PO:PS:SBO:CB (36:5:54:5)	PO:PS:SBO:CB (34:5:56:5)	LD
SMP	30.50±0.71^{e}	59.50±0.71^{a}	nd	34.25±0.35^{d}	41.25±0.35^{b}	39.25±0.35^{b,c}	38.00±0.71^{c}	27.50±0.71^{f}
IV	54.00±0.00^{f}	14.00±0.01^{h}	136.85±0.21^{a}	34.00±1.41^{g}	92.26±0.74^{d}	94.36±0.08^{c}	96.95±0.10^{b}	73.76±0.34^{e}
FA								
C12:0	0.33±0.01^{a}	0.20±0.14^{a,b}	n.d	n.d	0.11±0.01^{b}	0.10±0.01^{a,b}	0.10±0.01^{b}	0.09±0.01^{b}
C14:0	1.10±0.00^{c}	1.65±0.07^{a}	n.d	n.d	0.54±0.01^{d}	0.40±0.01^{e}	0.27±0.02^{f}	1.24±0.01^{a}
C16:0	43.99±0.20^{b}	78.90±0.28^{a}	11.33±0.13^{e}	25.34±0.04^{c}	27.88±0.01^{c}	26.69±0.07^{c}	25.72±0.03^{c}	22.68±0.48^{d}
C16:1	0.18±0.01^{b}	n.d	n.d	n.d	0.11±0.00^{b,c}	0.08±0.01^{c,d}	0.04±0.01^{d}	1.42±0.05^{a}
C18:0	4.36±0.06^{e}	6.32±0.17^{d}	4.42±0.01^{e}	37.84±0.14^{a}	7.83±0.04^{c}	7.86±0.02^{c}	7.87±0.01^{c}	12.70±0.28^{b}
C18:1	39.24±0.20	10.90±0.00	23.87±0.00^{e}	32.83±0.26	29.39±0.01^{b}	28.45±0.07^{c}	27.56±0.01^{d}	38.24±0.13^{a}
C18:2	10.25±0.06	1.58±0.60	53.93±0.02^{a}	2.92±0.06	30.78±0.05^{d}	32.90±0.10^{c}	34.44±0.09^{b}	20.39±0.04^{e}
C18:3	0.19±0.01	n.d	6.46±0.10^{a}	n.d	2.96±0.02^{d}	3.28±0.01^{b}	3.56±0.02^{b}	0.98±0.01^{e}
C20:0	0.36±0.01^{c}	0.46±0.08^{c}	n.d	1.07±0.01^{a}	0.43±0.00^{c}	0.47±0.01^{c}	0.48±0.01^{c}	0.67±0.01^{b}
Others	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.59

Each value in the table represents the mean of three determinations. Means within each row bearing different superscripts are significantly ($p<0.05$) different. Abbreviations: PO, palm oil: PS, palm stearin: SBO, soybean oil: CB, cocoa butter; LD, lard; FA, fatty acid; SMP, slip melting point; IV, iodine value; n.d, not detected.

Table 2. TAG composition of PO, PS, SBO, CB, quaternary mixtures of PO:PS:SBO:CB and LD.

TAG	PO	PS	SBO	CB	PO:PS:SBO:CB (38:5:52:5)	PO:PS:SBO:CB (36:5:54:5)	PO:PS:SBO:CB (34:5:56:5)	LD
LLnLn	n.d	n.d	1.31±0.03^{a}	n.d	0.70±0.01^{b}	0.70±0.00^{b}	0.74±0.01^{b}	n.d
LLLn	n.d	n.d	7.66±0.01^{a}	n.d	3.93±0.01^{c}	4.10±0.01^{b,c}	4.29±0.01^{b}	1.54±0.21^{d}
OLnLn	n.d	n.d	0.02±0.00^{a}	n.d	0.01±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}	n.d
LLL	n.d	n.d	23.56±0.03^{a}	n.d	12.23±0.00^{d}	12.72±0.01^{c}	13.15±0.01^{b}	0.68±0.21^{e}
PLLn	n.d	n.d	3.64±0.01^{a}	n.d	1.90±0.01^{d}	1.97±0.00^{c}	2.05±0.01^{b}	n.d
OLL	n.d	n.d	17.77±0.01^{a}	n.d	9.28±0.01^{d}	9.61±0.00^{c}	9.98±0.01^{b}	4.68±0.08^{e}
MMM	0.21±0.01^{a}	n.d	n.d	n.d	0.16±0.01^{b}	0.17±0.00^{b}	0.17±0.01^{b}	n.d
PLL	2.08±0.03^{f}	n.d	15.82±0.01^{a}	0.27±0.00^{g}	8.23±0.04^{d}	8.56±0.03^{c}	8.84±0.01^{b}	7.05±0.06^{e}
MPL	0.54±0.01^{a}	n.d	n.d	n.d	0.19±0.01^{b}	0.18±0.00^{b,c}	0.17±0.00^{c}	n.d
POLn	n.d	n.d	0.13±0.01^{a}	n.d	0.06±0.00^{b}	0.07±0.01^{b}	0.08±0.01^{b}	n.d
OOL	1.62±0.02^{d}	n.d	8.72±0.23^{a}	n.d	5.58±0.00^{c}	5.73±0.01^{c}	5.85±0.01^{c}	6.93±0.04^{b}
POL	9.96±0.01^{e}	0.32±0.03^{g}	13.69±0.02^{b}	0.85±0.01^{f}	11.31±0.02^{d}	11.86±0.01^{c}	12.15±0.11^{c}	20.00±0.27^{a}
PPL	10.19±0.01^{a}	1.03±0.10^{h}	2.25±0.01^{f}	1.55±0.00^{g}	5.02±0.01^{b}	4.73±0.01^{c}	4.52±0.01^{d}	2.62±0.04^{e}
OOO	3.97±0.02^{b}	0.13±0.01^{f}	2.02±0.13^{d}	0.69±0.01^{e}	3.14±0.00^{c}	3.13±0.01^{c}	3.12±0.01^{c}	4.33±0.21^{a}
POO	24.76±0.01^{a}	2.22±0.02^{g}	1.11±0.02^{e}	2.27±0.02^{f}	10.87±0.04^{c}	10.28±0.07^{d}	9.70±0.04^{e}	20.67±0.11^{b}
PPO	31.61±0.01^{a}	15.23±0.03^{c}	0.56±0.03^{h}	18.08±0.01^{b}	13.48±0.20^{d}	12.73±0.05^{e}	12.09±0.02^{f}	10.63±0.01^{g}
PPP	4.77±0.03^{c}	68.66±0.13^{a}	n.d	0.26±0.01^{f}	4.90±0.04^{b}	4.75±0.02^{c}	4.46±0.01^{d}	0.38±0.00^{e}
SOO	2.72±0.02^{c}	0.26±0.06^{g}	1.23±0.00^{f}	2.98±0.00^{b}	1.85±0.01^{e}	1.92±0.01^{e}	2.10±0.01^{d}	3.62±0.04^{a}
SPO	5.65±0.01^{d}	11.07±0.01^{c}	0.54±0.02^{g}	40.78±0.10^{a}	4.58±0.02^{e}	4.35±0.01^{f}	4.15±0.01^{f}	12.52±0.12^{b}
PPS	0.92±0.01^{a}	0.68±0.04^{c}	n.d	0.41±0.01^{d}	0.86±0.01^{a,b}	0.84±0.00^{a,b}	0.82±0.00^{b}	0.81±0.00^{b}
SOS	0.52±0.01^{e}	n.d	n.d	29.35±0.01^{a}	1.59±0.01^{b}	1.57±0.01^{b,c}	1.54±0.00^{c}	0.83±0.01^{d}
SSS	n.d	0.41±0.01^{a}	n.d	0.40±0.04^{a}	0.02±0.00^{c}	0.02±0.00^{c}	0.02±0.00^{c}	1.31±0.01^{b}
Others	0.48±0.01	n.d	n.d	2.11±0.14	n.d	n.d	n.d	1.41±0.33
UUU	12.68	0.13	61.06	0.69	34.89	36.00	37.14	18.16
UUSt	40.06	2.80	35.59	6.37	34.22	34.66	34.92	51.34
UStSt	47.97	27.33	3.35	89.76	24.86	23.56	22.47	26.60
StStSt	5.90	69.75	n.d	0.66	5.94	5.78	5.47	2.50

Each value in the table represents the mean of two determinations. Means within each row bearing different superscripts are significantly ($p<0.05$) different. Abbreviations: TAG, triacglycerol; PO, palm oil; PS, palm stearin; SBO, soybean oil; CB, cocoa butter; LD, lard; O, oleic; P, palmitic; L, linoleic; Ln, linolenic; St, stearic; U, unsaturated; S, saturated; n.d, not determined.

3.3. Thermal characteristics

The cooling behaviors of PO, PS, SBO, CB, PO:PS:SBO:CB mixtures and LD were compared in Fig. 1a. The cooling profile of LD (curve A) is characterized by two widely separated transitions: a high (a_1, a_2) and low (a_3) temperature regions. This was roughly similar to the findings reported previously (MARIKKAR and YANTY, 2014). According to Fig. 1a, the cooling profile of PO (curve E) had four cooling transitions; one major sharp peak at 18.8°C (e_1) and one broader peak at 5.0°C (e_2), with a shoulder peak at -4.2°C (e_3) which was in accordance with the previous findings (TAN and CHE MAN, 2000). In addition to these, a minor peak was also appeared in the lower-temperature region at around -42°C (e_4). The cooling thermograms of PS (curve F) and CB (curve H) had one major sharp peak at around 42.8 (f_1) and 13.4°C (h_2), respectively. In the case of CB, additional small peak (f_4) was also found at -30.7°C. As SBO was a liquid oil, its DSC curve (curve G) had three cooling transition at low-temperature region (below 0°C); the first peak was found at -9.2°C (g_1), a broader peak at -37.7°C (g_2) and a small peak at -64.5°C (g_3). These agreed with the results reported in other studies (TAN and CHE MAN, 2000). Additions of PS, SBO and CB into PO brought considerable changes to cooling profiles of the three PO:PS:SBO:CB mixtures. Only two thermal transitions were displayed by the mixtures; a major sharp peak at around 21°C and a minor peak at around -1°C. With respect to original PO curve, the peak-maxima of the thermal transitions of quaternary mixtures were also found to have shifted slightly. These changes in the profiles of mixtures could be attributed to the changing SFA to USFA ratio as noted previously in Tables 1 and 2. This has been in accordance with the findings reported by others (NUR ILLYIN *et al*., 2013).

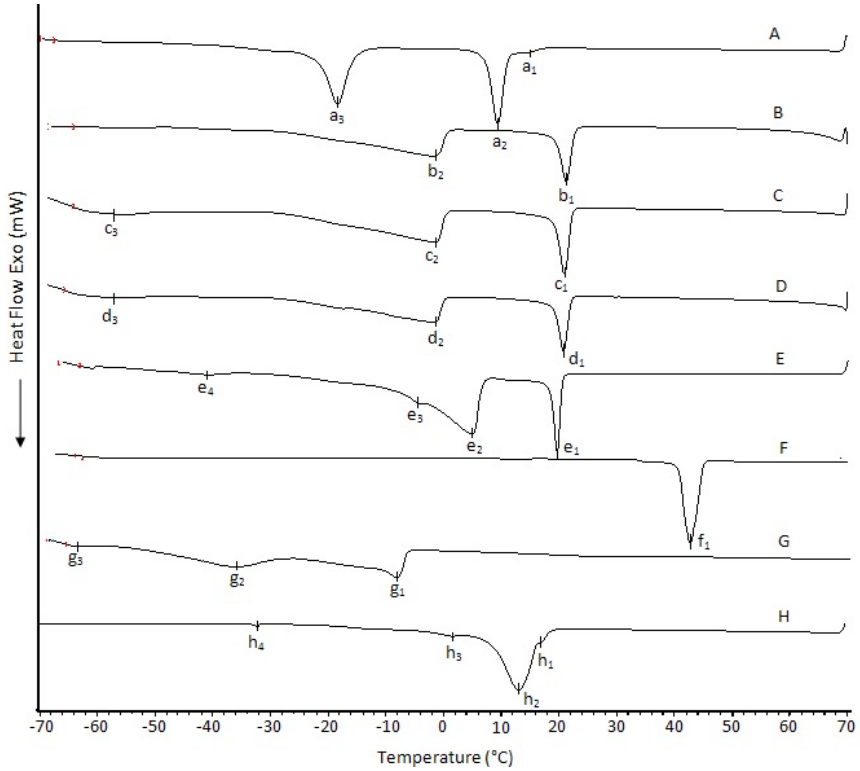

Figure 1a. DSC cooling thermograms of LD (A), quaternary mixtures of PO:PS:SBO:CB (B=38:5:52:5; C= 36:5:54:5; D=34:5:56:5), PO (E), PS (F), SBO (G) and CB (H).
Abbreviations: LD, lard; PO, palm oil; PS, palm stearin; SBO, soybean oil; CB, cocoa butter.

On the other hand, the major and minor sharp peaks of LD were found at 10.3 and -18.0°C, respectively. The high-melting cooling transitions of PO:PS:SBO:CB mixtures were found to be little higher (22.3°C) when compared to that of LD (18°C). These results suggested that none of the quaternary mixtures of PO:PS:SBO:CB had thermal transitions exactly matching with the cooling thermogram of LD. However, the peak corresponding to CB 13.4°C (h_2) showed a closer similarity to the high melting transition of LD (10.3°C).

The melting behaviors of PO, PS, SBO, CB, PO:PS:SBO:CB mixtures and LD were depicted in Fig. 1b. The melting profile of LD (curve A) has five endothermic transitions, which could be classified into two distinct regions namely, low-melting region below 0°C (a_1, a_2) and high-melting region above 0°C (a_3, a_4, a_5). The native PO sample (curve E) had seven endothermic transitions; two major endothermic regions, corresponding to low-melting fraction known as olein and high-melting fraction known as stearin. These were largely confirmatory with the findings reported previously. The high-melting region (above 10°C) consisted of a plateau with a pair of shoulder peaks (c_6 and c_7), while the low-melting region (below 10°C) contained five overlapping peaks (c_1, c_2, c_3, c_4 and c_5). PS (curve F) and CB (curve H), on the other hand, had one major sharp peak at 59.0 (f_1) and 20.4°C (h_2). In addition, CB had one shoulder peak at 14.8°C (h_1). In the meantime, SBO (curve G) had four endothermic transitions at low-temperature region; the profile was comparably similar to that reported previously by TAN and CHE MAN (2000). This could be due to the fact that SBO was a liquid oil that contained a high amount of USFA (Table 1) and UUU TAG molecules (Table 2). The major peak was found at -27.7 (g_2)°C with two shoulder peaks at -20.1 (g_3) and -6.5°C (g_4). The minor peak was found at -38.2°C (g_1). Generally, the melting profile of mixtures (curve B, C, D) has six endothermic transitions, which could be classified into three distinct regions namely, low-melting region below -20°C (peaks at position 1 and 2), middle melting region between -10 to 20°C (peaks at position 3, 4 and 5) and high-melting region at around 40°C (peaks at position 6).

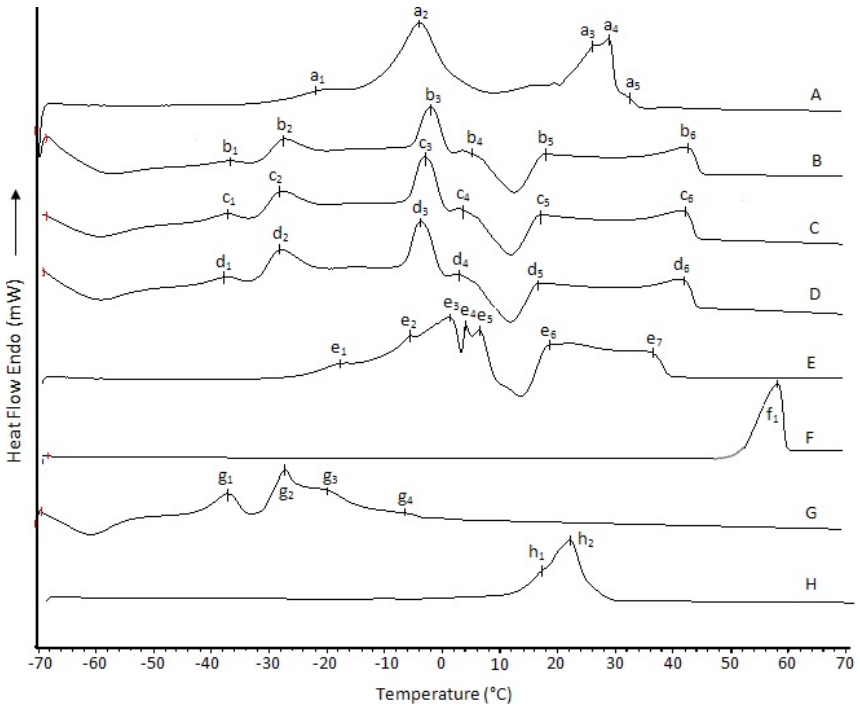

Figure 1b. DSC melting thermograms of LD (A), quaternary mixtures of PO:PS:SBO:CB (B=38:5:52:5; C= 36:5:54:5; D=34:5:56:5) PO (E), PS (F), SBO (G) and CB (H).
Abbreviations: LD, lard; PO, palm oil; PS, palm stearin; SBO, soybean oil; CB, cocoa butter.

According to Fig. 1b, the thermal profiles displayed by the quaternary mixtures were considerably different from the melting profile of original sample of PO. The melting profiles of mixtures were found to have one additional minor peak (b_2, c_2 and d_2) with a shoulder peak (b_1, c_1 and d_1) at below -20°C could be attributed to the presence of SBO, which had all of its thermal peaks in the low-melting region. With respect to the original sample, T_{endset} of all quaternary mixtures were found to be shifted to higher temperature region after addition of PS and CB into PO. When compared to LD (T_{endset} =37.5°C), all three quaternary mixtures had higher end-set of melting (T_{endset}) (at around 44°C) and lower on-set of melting T_{onset} (at around -45°C). Although there were much differences in melting transitions between lard and the mixtures, a closer similarity between them was seen at the peak-maximum of (b_3, c_3 and d_3) and (a_2) at -3.59°C.

3.4. Solidification behavior

A comparison of the SFC profiles of the quaternary fat mixtures and LD was given in Fig. 2. The SFC of LD and PO at 0 ºC was 30.8 and 68.63%, respectively and tended to decrease gradually until they become 0% at 40 and 55 ºC, respectively. As mentioned previously by YANTY (2016), the SFC values of PS and CB were found to drop dramatically at 25 ºC and above 55 ºC, respectively. This unique behaviour of PS and CB was largely in agreement with the observed thermal events in their respective DSC curves where the occurrence of single sharp peak was indicative of the meltdown of the entire TAG groups within a narrow temperature range. This rapid meltdown behaviour of these fats was also further discussed in other reports (YANTY, 2016).

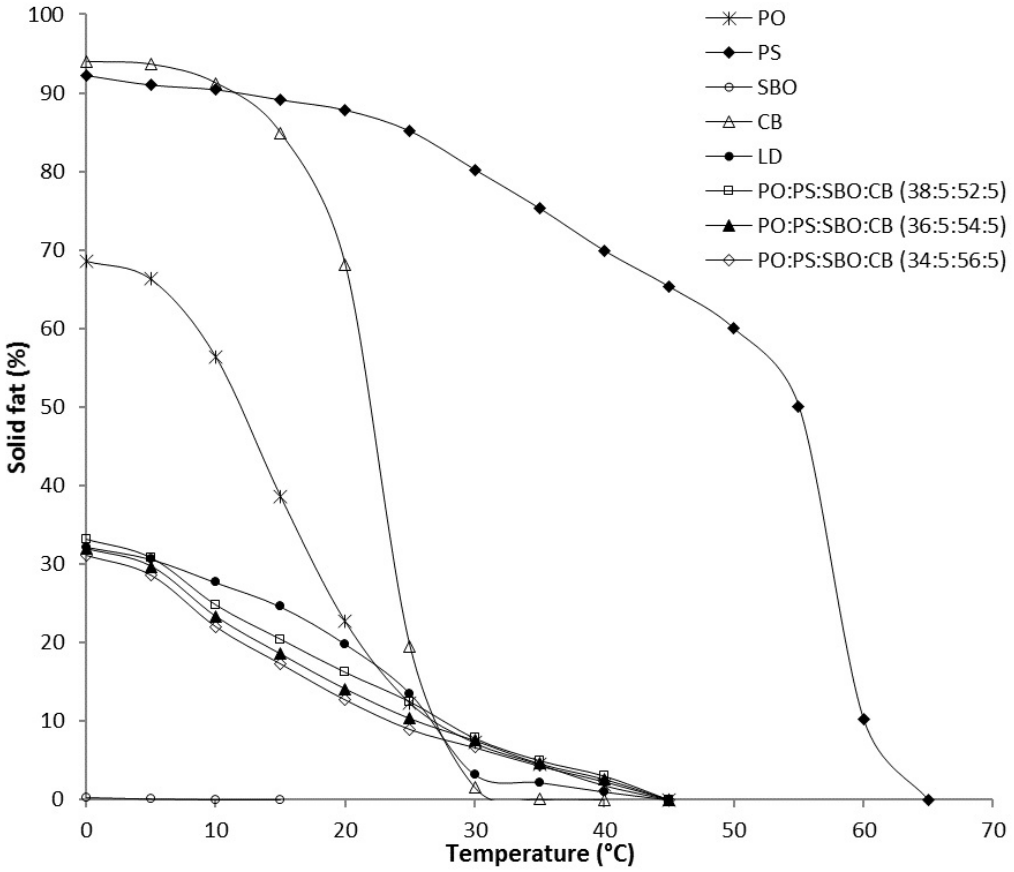

Figure 2. Solid fat content of PO, PS, SBO, CB, quaternary mixtures of PO:PS:SBO:CB and LD. Abbreviations: PO, palm oil; PS, palm stearin; SBO, soybean oil; CB, cocoa butter; LD, lard.

However, the SFC value of SBO at 0 ºC was found to be 0.31% and become 0% at 10 ºC. This phenomenon could be due to the presence of high amount of USFAs (Table 1) and UUU TAG molecules (Table 2) in SBO. The SFC value of PO was found to be higher than that of LD within the temperature range between 0 and 20°C. Addition of SBO into PO could probably reduce the amount of SFC in this temperature region. However, addition of 50 % of SBO into PO was resulted in a big slope (below SFC values of LD) within this temperature ranges and the SFC values tended to be higher than that of LD from 30 to 55°C. Although additions of PS into PO:SBO mixtures were tended to increase the SFC values at 0 to 20°C, the SFC values were still higher than those of LD at 30 and 35°C.
According to another set of SFC data not shown here, addition of CB into PO:SBO mixtures did not change the SFC values above 30°C. Hence, it was assumed that blending PO with an appropriate amount of PS, SBO and CB would help to adjust SFC values of PO to become closer to that of lard at almost all temperature regions. The SFC values of three quaternary mixtures in Fig. 2 were found to be lower than that of LD in between 0 to 25°C. However, the SFC values of the mixtures were tended to be higher than that of lard above 30°C due to a presence of PS. Out of the three quaternary mixtures, PO:PS:SBO:CB (38:5:52:5) was found to have SFC value somewhat closer to LD at 0, 5 and 25° C. The calculations presented in Table 3 also showed that PO:PS:SBO:CB (38:5:52:5) was found to have the least difference to LD in terms of SFC values throughout the temperature range. Hence, this mixture was found to be the most compatible to LD in term of solidification behavior.

Table 3. Comparing least difference of SFC values of LD and PO:PS:SBO:CB mixtures.

Temp (°C)	PO:PS:SBO:CB (38:5:52:5)+/- LD	PO:PS:SBO:CB (36:5:54:5)+/- LD	PO:PS:SBO:CB (34:5:56:5)+/- LD
0	0.96	-0.25	-1.13
5	0.17	-0.92	-2.08
10	-2.87	-4.35	-5.68
15	-4.15	-6.04	-7.31
20	-3.59	-5.71	-7.1
25	-1.05	-3.2	-4.58
30	4.63	4.33	3.44
35	4.28	3.69	3.62
40	2.02	1.61	1.29
Total	**0.4**	**-10.84**	**-19.53**

Abbreviations: PO, palm oil; PS, palm stearin: SBO, soybean oil; CB, cocoa butter; Temp, Temperature.

4. CONCLUSIONS

This study demonstrated the possibility of producing a fat mixture to mimic some of the compositional and thermal properties of LD by blending PO with PS, SBO and CB in appropriate ratios. Among the three different mixtures formulated, PO:PS:SBO:CB (38:5:52:5) was found to have the closest similarity to LD in terms of some DSC parameters and SFC behavior. The SFC values of this mixture were found to display the least difference to those of LD throughout the temperature range. Particularly, the closest compatibility in terms of SFC values was seen at 0, 5 and 25°C. In terms of composition, the USFA and SFA contents of this mixture were least difference to those of LD.

ACKNOWLEDGEMENTS

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PAPER

GC-C-IRMS CHARACTERIZATION OF SYNTHETIC BIS(METHYL-THIO)METHANE IN TRUFFLE FLAVORINGS

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ABSTRACT

Bis(methyl-thio)methane (BMTM), the molecule which provides "white truffle-like" flavor was characterized by physico-chemical methods. Analysis by GC-C-IRMS of eight samples of synthetic BMTM from various raw material suppliers allowed the investigation of the $\delta^{13}C$ values. More, ten samples purchased on the Italian flavoring market, declared as synthetic BMTM principal component diluted in olive oil were analyzed by GC-C-IRMS. The results of both sample groups allowed us to define the range of $\delta^{13}C$ values of synthetic BMTM.

We verified if the simple proposed extraction method allows to measure the $\delta^{13}C$ value of BMTM also identified in seasonings of the Italian market declared on label as "white truffle flavored olive oil". In all twenty samples purchased on the market, the data strictly corresponded with synthetic BMTM as the principal component.

Measurements by ^{1}H NMR made on synthetic BMTM and BMTM extracted from "white truffle-like flavor" confirmed that the adopted extraction method using methanol-d4 determined the isotopic distribution of $^{13}C/^{12}C$ ratio in two characteristic sites of this molecule.

Keywords: white truffle flavoring, BMTM, GC-C-IRMS, ^{1}H NMR

1. INTRODUCTION

Tuber magnatum (white truffle), *Tuber aestivum* (summer truffle), and *Tuber melanosporum* (black truffle) are the most well-known species belonging to the genus *Tuber F.H*. These species have been the object of numerous studies using various analytical systems for the identification of the compounds that provide the distinctive aroma of these fungi (BELLESIA *et al.*, 1996; DÍAZ *et al*., 2002; DÍAZ *et al*., 2003; TALOU *et al*., 1989).
Several reports have considered the constituents responsible for the typical aroma and have also studied the quantitative and qualitative fluctuations of these compounds, depending upon truffle type and geographical origin (COSTA *et al*., 2015; FIECCHI *et al*., 1967; GIOACCHINI *et al*., 2008; MAURIELLO *et al*., 2004; PELUSIO *et al*., 1995). Presently, pure natural BMTM derived from truffles is not available on the raw material flavor market because the levels of BMTM in truffles are too low for satisfactory isolation of a significant quantity of this molecule (SCHMIDBERGER and SCHIEBERLE, 2017). Also, the commercial cost of the natural BMTM from the white truffle would be unsustainable because the raw material is very expensive (BORSINO DEL TARTUFO, 2018).
Therefore, due to the non-feasibility of BMTM isolation from the natural matrix, no measurements using Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) have been previously carried on.
The European Union (EU) Regulation 1334/2008 allows the citation of the flavoring source (in our case "white truffle") only if the flavoring components are obtained exclusively or by at least 95% (w/w) from the source material referred to and the other maximum 5% can derive only from other natural sources. Therefore, the citation in the label "natural flavoring", without the citation of a defined natural source, may only be used if all the flavoring components derive from natural sources. If one or more synthetic compounds are present in a flavoring formulation, the label must report only the term "flavor" without any reference to a food, food category or a vegetable or animal flavoring source (REG. EU 1334, 2008).
The corresponding naturally-occurring identical compound, easily synthesized by the oil industry and supported in olive oil (PACIONI et al., 2014), is used as a flavoring agent for truffle flavored food products. Among the "white truffle-like" flavored foods, extra virgin olive oil, flavored with bis(methyl-thio)methane (BMTM), used as a seasoning, occupies the most important position in the market.
Using a simple extraction method and the GC-C-IRMS analytical technique, we aimed to ascertain the reliability of characterizations of the synthetic BMTM molecule present in "white truffle-like" flavors and seasonings. Following the EU Regulation 1334/2008 before cited, the identification of synthetic BMTM in the formulation of flavors and seasonings does not allow the use on the label the term "natural white truffle flavor", or "natural flavor" or "white truffle flavor", but only the term "flavor".
Synthetic BMTM characterization by GC-C-IRMS, confirmed by ^{1}H-NMR, allowed us to demonstrate the identity of the BMTM molecule extracted from "white truffle-like" flavors purchased on the Italian flavoring market and from olive oil seasonings available on the Italian consumer market.

2. MATERIALS AND METHODS

2.1. Solvents

Methanol (anhydrous) (99.8) and methanol d-4 were purchased from Sigma Aldrich (Milan, Italy).

2.2. Samples analyzed by GC-C-IRMS

- Eight samples of BMTM ($C_2H_8S_2$) declared to be synthetically derived were obtained. The first purchased was a standard (> 99%) from Sigma Aldrich (Italy) and the others were purchased on the flavor raw material market (FCI, Frutarom, Moellhausen, Penta, Treatt, Sterling, Sigma Aldrich).
- Ten samples of "white truffle-like" flavors purchased on the Italian flavorings market and declared as synthetic BMTM diluted in olive oil.
- Twenty "white truffle-like" seasonings purchased on the Italian market and consisting of flavored extra virgin olive oil, or olive oil, or sunflower oil. All the seasonings were declared to contain olive oil or extra-virgin olive oil except the seasoning n. 4, which contains sunflower oil. The product n. 8 reported on the label "natural flavor". The product n. 13 reported on the label "white truffle natural flavor". The products n. 6, 11, 16, and 19 reported on the label "white truffle flavor". The other products reported on the label "flavor".

2.3. Samples analysis by ^{1}H NMR

Two samples were analyzed, one corresponding to the standard BMTM (sample n.1 in Table 1) and another corresponding to a "white truffle-like" flavoring (sample n.1 in Table 2).

2.4. Samples preparation

For GC-C-IRMS analysis, samples of synthetic BMTM (Table 1) were dissolved in anhydrous methanol (99.8) at a concentration of 60 μL mL^{-1}. The mix was vortexed for 1 min.
For samples of "white truffle-like" flavoring (Table 2) an aliquot of 3 mL was vortexed with 1 mL of methanol for 5 min and then centrifuged at 5000 rpm for 5 min. The methanol layer was isolated by a Pasteur pipette and utilized for the analysis.
For samples of seasonings, an aliquot of 5-10 mL was vortexed with 1 mL of methanol for 5 min and then centrifuged at 5000 rpm for 5 min. The methanol layer was isolated by a Pasteur pipette and utilized for the analysis.
For NMR analysis, a sample of standard BMTM (sample n.1 in Table 1) was dissolved in methanol d-4 at a concentration of 60 μL mL^{-1}. The mix was vortexed for 1 min. For sample of a "white truffle-like" flavoring (sample n.1 in Table 2), an aliquot of 3 mL was vortexed with 1 mL of methanol d-4 for 5 min and then centrifuged at 5000 rpm for 5 min. The methanol layer, isolated by a Pasteur pipette, was utilized for the analysis.

2.5. GC-C-IRMS analysis

The system consisted of an Agilent Technology 7890A gas chromatograph equipped with a G4513A autosampler (Agilent Technology, Germany) and coupled to an IsoPrime stable IRMS GC5 (Isoprime, Cheadle, UK) via a combustion interface under a continuous flow of helium. The combustion interface consisted of a ceramic furnace with a copper oxide and platinum catalyst at 850°C. The carbon stable isotope ratio was determined by referring to the international standard Vienna PeeDee Belemnite ($\delta^{13}C_{VPDB}$) with a defined ^{13}C content. The CRM used for the GC-C-IRMS multipoint calibration were *n*-undecane ($\delta^{13}C$: -26.11‰, Chiron C0414.11-150-CY), *n*-pentadecane ($\delta^{13}C$: -30.22‰, Chiron C0418.15-150-CY) and *n*-hexadecane ($\delta^{13}C$: -34.87‰). The hexadecane delta value was obtained by EA-IRMS using the following primary standards: glucose ($\delta^{13}C$: -10.76‰, Sigma Aldrich BCR657),

polyethylene ($\delta^{13}C$: -32.15‰, IAEA IAEA-CH-7) and lithium carbonate ($\delta^{13}C$: -46.60‰, NIST RM 8545).
Isotope ratios were expressed as values (‰) and calculated on the basis of the following equation:

$$\delta i\, E = \frac{(i\, R_{SA} - i\, R_{REF})}{i\, R_{REF}}$$

where "i" is the mass number of the heavier isotope of element E (in this case ^{13}C), R_{SA} is the respective isotope ratio of the sample and R_{REF} is the relevant internationally recognized reference material. The delta values were multiplied by 1000 and expressed in units "per mill" (‰) (COPLEN, 2011).
The GC was operated using a HP-5 capillary column, 30 m x 0.32 mm i.d., 0.25 μm film thickness (Agilent – Italy). Helium was used as the carrier gas at a flow rate of 1.2 mL. The oven temperature program was initially 50°C (held for 1 min), then increased to 150°C at a rate of 5°C min^{-1}, then increased to 250°C at a rate of 20°C min^{-1} (held for 1 min). The injector temperature was 220°C and the injection volume was 1 mL (split 1:10). Data were collected in triplicate.

2.6. NMR analysis

The ^{1}H NMR spectra were measured on a Bruker spectrometer AVIII400 equipped with a SampleXpress sample changer and using a BBIz probe. The spectra were acquired using a 30° excitation pulse width of 2.66 μs, relaxation delay of 40 sec, and acquisition time of 5 s at 298 K. T1 relaxation times of the quantified peaks were measured with inversion-recovery experiments to assure that no bias from a short D1 would arise. The longest T1 measured for the standard BMTM (sample n.1 in Table 1) was 6.96 s, found for the CH_2 peak, while for the "white truffle-like" flavoring (sample n.1 in Table 2) we observed 6.02 sec for the same peak as the longest T1. Thus, the chosen D1 was sufficient to ensure a complete relaxation of the magnetization and to give fully quantitative results. The spectral width was 40 ppm centered at 6.5 ppm to ensure that the baseline was perfectly flat, as this is a precondition to correct integration and quantification of the peaks. For each spectrum, 64 scans were summed. The acquisitions were performed in a fully automated way. The spectra were processed with 260 K points and an exponential multiplication of 0.3 Hz. The data were acquired, processed, and analyzed using the software program Topspin 3.5 from Bruker Biospin.
Phasing and baseline correction were performed manually. In the case of the standard BMTM (sample n.1 in Table 1), the two main peaks of the H_1-C_{12} were considered together with both satellites for each peak. However, in the case of the "white truffle-like" flavoring (sample n.1 in Table 2), only one satellite per peak was considered due to the superposition with other signals in the matrix. The intensity of the peak was doubled for the calculation of the isotopic ratio.

3. RESULTS

3.1. GC-C-IRMS

The samples of commercially available synthetic BMTM could be readily prepared for GC-C-IRMS analysis by simple dilution in anhydrous methanol (Table 1).

Similarly, the extraction method adopted for samples reported in Tables 2 and 3 allowed us to isolate BMTM as a methanolic extract for use in GC-C-IRMS measurements.
For samples reported in Tables 1 and 2, corresponding to BMTM declared from synthesis and "white truffle-like" flavors purchased on the Italian flavorings market, respectively, the $\delta^{13}C$ values varied in the tight range of -42.24 and -43.40‰.
The above cited range, deduced from analysis of a sufficiently large number of samples (standards and flavorings) produced with "BMTM" certified from synthesis, was very useful for characterization of the "synthetic" authenticity of the molecule subjected to $\delta^{13}C$ measure. In addition, the same data, as expected, was not consistent with the carbon isotope ratio natural abundance ranges, as documented in literature data (VAN LEEUWEN *et al*., 2014).
Data obtained for seasonings of Table 3 are also included in the range between -42.34 and -43.26‰, and was not significantly different from the values corresponding to the synthetic samples cited above.

Table 1. GC-C-IRMS data produced by eight samples declared as BMTM from synthesis and available on the flavoring market.

Sample	$\delta^{13}C$ ‰ (mean)	s
1	-43.35	0.43
2	-42.30	0.35
3	-43.05	0.34
4	-42.47	0.32
5	-42.24	0.35
6	-43.40	0.34
7	-42.26	0.34
8	-43.14	0.41

Table 2. GC-C-IRMS data produced by ten samples, all declared as "white truffle-like" flavors, purchased on the Italian flavorings market and consisting of synthetic BMTM as the principal component diluted in olive oil.

Sample	$d^{13}C$ ‰ (mean)	s
1	-42.50	0.58
2	-42.43	0.53
3	-43.12	0.41
4	-42.40	0.34
5	-42.55	0.38
6	-42.64	0.47
7	-43.18	0.52
8	-42.38	0.45
9	-42.46	0.37
10	-42.52	0.46

Table 3. GC-C-IRMS data produced by twenty samples of white truffle flavored oils purchased on the Italian market (glass pack 40-250 mL).

Seasoning	$\delta^{13}C$ ‰ (mean)	s
1	-42.34	0.42
2	- 43.08	0.34
3	-42.81	0.52
4	-42.63	0.36
5	-43.16	0.38
6	-42.86	0.48
7	-43.02	0.35
8	n.d.	----
9	-42.53	0.46
10	-43.12	0.43
11	-43.06	0.37
12	-43.18	0.51
13	-42.68	0.45
14	-42.48	0.39
15	-43.04	0.37
16	-43.10	0.41
17	-42.66	0.56
18	-42.74	0.46
19	-43.26	0.38
20	-42.45	0.42

3.2. ^{1}H NMR characterization of BMTM

The aim of our study was to develop an approach using NMR methodology to characterize the BMTM synthetic molecule of a standard sample and to verify the possibility of using the extraction method referred in "2.4 Sample preparation" to characterize the synthetic BMTM present in a "white truffle-like" flavor. We measured the values $^{13}C/^{12}C$ for the two sites corresponding to CH_2 and CH_3 in the BMTM molecule extracted from the two samples. Our data showed the structural isotopic distribution of $^{13}C/^{12}C$ in the characteristic sites of these two molecules.

3.2.1 BMTM from synthesis (sample 1, Table 1)

The ^{1}H NMR spectra of the synthetic standard BMTM were acquired using two aliquots of the same sample diluted in methanol-d4 and in replicates to evaluate the experimental CV% (coefficient of variation %) from 8 trials.
The BMTM ^{1}H NMR spectrum in methanol-d4 displayed two singlets, resonating at 3.68 and at 2.15 ppm. The signals are attributed to the CH_2 (3.68 ppm) and the CH_3 (2.15 ppm). The ^{13}C satellites of the two peaks were easily recognizable and they appeared as doublets with a heteronuclear J coupling of 149.9 Hz for the CH_2 and of 138.5 Hz for the CH_3. The doublets were centered at the isotropic resonances of the peaks. As expected, the intensities of the satellites were significantly lower than those of the main peaks, which were attributed to the ^{1}H attached to the ^{12}C.

The ratio between the intensities of satellites due to the ^{1}H - ^{13}C heteronuclear coupling and that of the main peak allowed us to extract the integral ratio between ^{13}C and ^{12}C in a site specific manner.
The ^{1}H NMR spectrum (Fig. 1) and the results (Table 4) indicated that the ratio of $^{13}C/^{12}C$ gave the same value for both sites (CH_2 and CH_3). This value is in agreement with the expected $^{13}C/^{12}C$ global ratio.

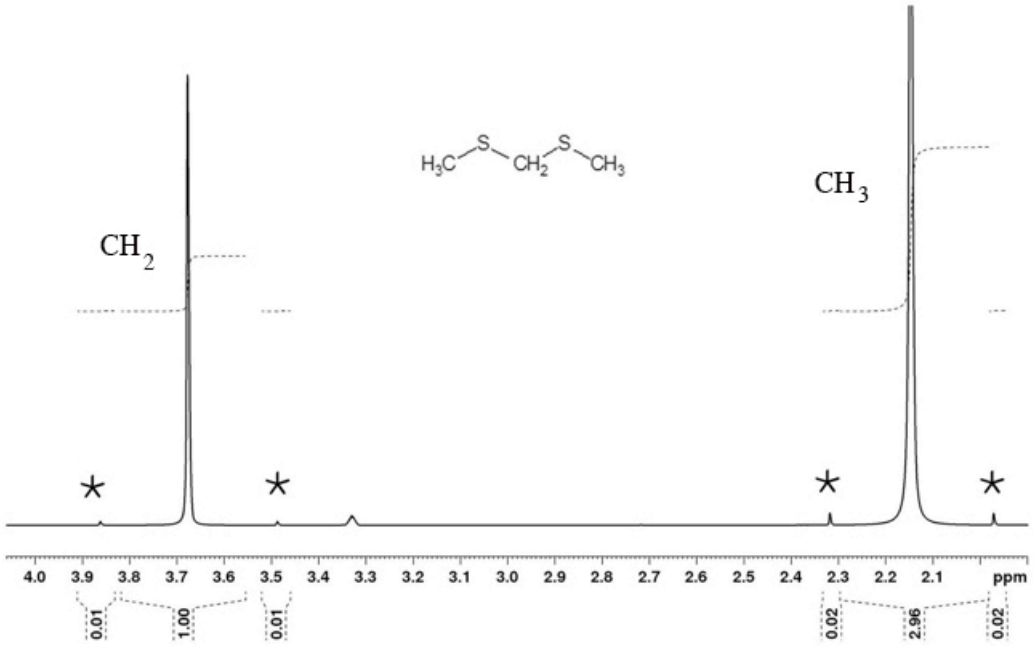

Figure 1. ^{1}H spectrum of the BMTM from synthesis, available on the flavor raw material market (sample 1, Table 1). The asterisk indicates the ^{13}C satellites. The dotted lines indicate the integral regions.

Table 4. Data %^{13}C measured by ^{1}H NMR spectra in replicates of two aliquots for each sample: synthetic BMTM standard (sample 1 in Table 1), synthetic BMTM from "white truffle-like" flavor (sample 1 in Table 2).

		% ^{13}CH			
		BMTM synth. std.		BMTM synth. flav.	
replicates		$-CH_2-$	$-CH_3$	$-CH_2-$	$-CH_3$
aliquot 1	1	1.02	1.06	1.01	0.99
	2	1.03	1.02	1.07	0.99
	3	1.05	1.05	1.03	1.06
aliquot 2	1	1.05	1.05	0.99	1.02
	2	1.02	1.00	0.99	0.99
	3	1.00	1.01	0.99	1.04
	4	1.01	1.00	0.99	1.02
	5	1.03	0.99	1.03	1.05
mean		1.03	1.02	1.01	1.02
CV%		0.02	0.03	0.03	0.03

3.2.2 BMTM extracted from synthetic white truffle-like flavoring (sample1, Table 2)

The same approach described for the synthetic BMTM standard was applied to the synthetic BMTM in "white truffle-like" flavoring. As in the case of the molecule BMTM from synthesis, the spectrum was acquired in 8 replicates derived from two extracts produced from two aliquots of synthetic BMTM from "white truffle-like" flavoring. The ^{1}H spectra of the two aliquots extracted with methanol-d4 showed similar features to that of the synthetic BMTM standard, with the two singlets in the same position (3.67 ppm for CH_2 and 2.14 ppm for CH_3) and the same heteronuclear J couplings (149.9 Hz for the CH_2 and of 138.5 Hz for the CH_3). The 0.01 ppm shift is attributable to a matrix effect and it is reproducible in all the replicates. The spectrum acquired is reported in Fig. 2 and also in this case, as reported in Table 4, the ratio of $^{13}C/^{12}C$ gave the same value for both sites (CH_2 and CH_3).
As shown in Fig. 2, the presence of a potentially interfering substance often found in "white truffle" flavor (identifiable by GC/MS as methylsulfinyl(methylthio)-methane) did not hinder the identification of ^{13}C satellites and the % ^{13}C values were strictly comparable to data obtained for synthetic BMTM standard.
To clarify, the ^{1}H NMR spectrum of the sulfoxide is easily recognizable by these features: one singlet at 2.71 ppm attributable to one terminal methyl; one singlet at 2.33 ppm attributable to the second terminal methyl; two doublets centered at 3.98 ppm and at 3.83 ppm with a J=13.8 Hz attributable to the CH_2 protons that became diastereotopic upon asymmetric oxidation.

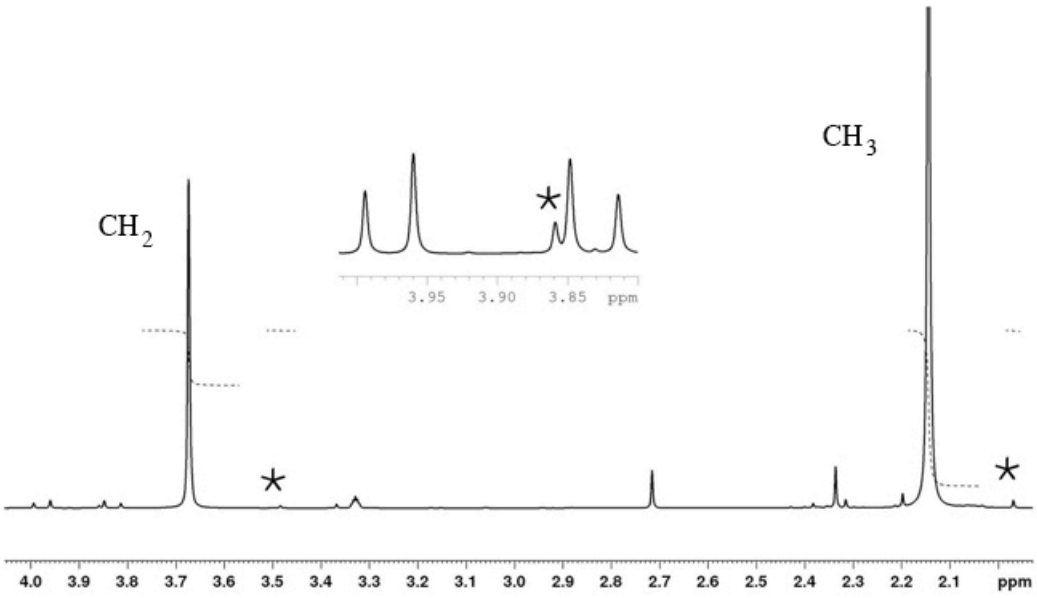

Figure 2. ^{1}H spectrum of the "white truffle" flavor purchased on the Italian flavor market and consisting of synthetic BMTM as the principal component diluted in olive oil. The asterisk indicates the ^{13}C satellites. The dotted lines indicate the integral regions. The inset shows the expansion of the 4.05-3.75. These peaks are attributed to the CH_2 protons in a molecule identified as methylsulfinyl(methylthio)-methane.

4. DISCUSSION

The simple extraction method, adopting methanol as the solvent, is useful for application to provide data by GC-C-IRMS of a synthetic BMTM standard, in flavoring from the raw material market (see Table 1), and in Italian flavoring from the commercial market (see Table 2). The $\delta^{13}C‰$ data collected for all these matrices ranged between -42.24 (σ 0.35) and -43.40‰ (σ 0.34). Clearly, the range is external to the natural abundance of the carbon isotope ratio, and allows one to identify the synthetic origin of BMTM if this molecule is used to produce seasonings made by the addition to olive oil or other vegetable oils.

The $\delta^{13}C‰$ data obtained with the same simple extraction method on twenty seasonings purchased on the Italian market allowed us to deduce that the synthetic BMTM molecule is clearly identifiable. In fact, the $\delta^{13}C‰$ values reported in Table 3, except for the sample n. 8, which does not contain BMTM, exhibited data in the range -42.34 and -43.26‰, identified as characteristic of synthetic BMTM.

Most seasoning samples – representing about 75% of the total seasoning samples examined (namely, n. 1-5, 7, 9, 10, 12, 14-15, 17-18 and 20 in Table 3) are compliant with the EU Regulation 1334/2008 because they contain synthetic BMTM and on the label only the term "flavor" is correctly used.

Samples n. 6, 11, 13, 16, and 19 in Table 3 are not compliant with the EU Regulation 1334/2008 because they contain synthetic BMTM and report on label the reference to the term "truffle". Specifically, the term "white truffle flavor" was used for samples 6, 11, 16, 19, while "natural white truffle flavor" for sample 13.

For the sample n. 8 in Table 3, the only one resulting as not containing BMTM, and reported on label as produced with "natural flavor", the judgment of compliance or otherwise with the EU Regulation 1334/2008 does not depend from the BMTM identification (natural or synthetic), but from the origin of all the molecules constituting the flavor. In fact, a natural flavor used for a seasoning can be realized totally with natural molecules. In this case, the final judgment is not linked to the BMTM identification, but from the naturalness of all the components of the flavor used.

There was no evidence of data that did not fall within the range characteristic of synthetic BMTM, and it is justified by being anyhow unavoidable due to the lack of commercial availability of natural BMTM in the raw material flavor market.

Adopting the same extraction method, but using methanol d-4 as the solvent, we demonstrated the feasibility of ^{1}H NMR measures to calculate % $^{13}C/^{12}C$ in two characteristic sites -CH_2- and -CH_3. This investigation, applied to a declared synthetic BMTM standard (sample 1 in Table 1) characterizes the integral ratio between ^{13}C and ^{12}C in a site specific manner. In fact, the ^{1}H NMR spectrum (Fig. 1) and the results (Table 4) indicated that the ratio of $^{13}C/^{12}C$ gave the same value for both sites (CH_2 and CH_3), in agreement with the expected $^{13}C/^{12}C$ global ratio.

Data produced from a synthetic BMTM standard were not statistically different from the corresponding data produced for a "white truffle-like" flavor, as shown in Table 4.

5. CONCLUSIONS

The data reported in this paper are the first GC-C-IRMS and ^{1}H NMR contributions to the characterization of synthetic BMTM available on the flavoring market and in some "white truffle-like" flavors. All the analyzed seasoning (except one sample not containing BMTM) produced GC-C-IRMS data of BMTM in agreement with the values of the synthetic molecule.

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PAPER

THE EFFECT OF THE ADDITION OF WATER, SOY PROTEIN, INULIN, AND MALTODEXTRIN ON THE QUALITY OF DOUGH AND GLUTEN-FREE BREADS

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ABSTRACT

The main aim of the study was to improve the textural properties of dough and the physicochemical properties of gluten-free breads derived from mixtures. The mixtures contained hydroxypropyl cellulose, soy protein isolate, inulin, and maltodextrin. During experiments the amount of water in dough was changed in the range from 80 to 100 g of the total weight of the mixtures. The textural properties of gluten-free dough (TPA's test) were measured. Bread volume, porosity, moisture, and hardness of crumb in the obtained breads were determined.

The obtained results show that the best positive impact on the textural properties of the gluten-free dough has the addition of 0.5 g/100 g of hydroxypropyl cellulose, 5.0 g/100 g of soy protein isolate, and 10.0 g/100 g of maltodextrin, with 100 g of water/100 g mixture. The results also suggest that water content is strongly associated with bread staling. Despite differences in their sensory properties, the breads were accepted by consumers.

Keywords: hydrocolloids, soy protein, inulin, maltodextrin, gluten-free bread, bread quality

1. INTRODUCTION

Coeliac disease (gluten sensitive enteropathy) is a chronic gluten intolerance. So far, the only effective way of the coeliac disease treatment was to follow the strict restrictions of a gluten-free diet.

The food that fulfills the gluten-free standards differs unfavorably from the regular food in terms of sensory quality. Such food usually has a lower nutritional value and its production process causes several technological problems (MOHAMMADI *et al.*, 2014).

In a typical bread production process, during the process of dough kneading, gluten-free flour with water cannot create the elastic structure of the dough. Gluten-free dough weakly keeps carbon dioxide during the process of growing and baking, which results in a small volume of bread (RAKKAR, 2007; PACIULLI *et al.*, 2016). The lack of gluten, which is responsible, among others, for the dough flexibility, makes gluten-free breads smaller (in volume) with an adverse taste, unpleasant smell and worse structure (MIR *et al.*, 2016). The crumb of such bread tends to crumble, and the process of staling is very fast (FURLAN *et al.*, 2015). The specificity of gluten-free ingredients does not allow to enrich the dough with adequate quantities of water, which could ensure the proper level of moisture. Hence, the gluten-free breads, especially those produced with a large addition of raw starch, tend to lose their freshness very fast. In such case, the bread with very good properties after baking concerning its moisture, cohesion and cutting, crumbles and becomes dry on the next day (GALLAGHER *et al.*, 2003).

Because of the elimination of gluten, which is the most important ingredient in the production process of traditional bread responsible for the bread structure-forming, for gluten-free breads it is necessary to use hydrocolloids with the ability to bind water, which serve in a similar way as gluten in wheat dough (MANCEBO *et al.*, 2015; MIR *et al.*, 2016). Polysaccharide hydrocolloids such as xanthan gum, guar gum, pectin, cellulose, and their mixtures are often used as structure-forming components in gluten-free bread concentrates (AHLBORN *et al.*, 2005; SCIARINI *et al.*, 2010; MASURE *et al.*, 2016). Moreover, several scientists (CATO *et al.*, 2004; SIVARAMAKRISHNAN *et al.*, 2004; ONYANGO *et al.*, 2009) use different types of modified cellulose as a structure forming factor. The substantial disposal of a nutritional value in this type of food, however, caused the necessity to find such additives that combine structure-forming and active prohealth features. Such replacement may be represented by inulin or soy proteins. In combination with hydrocolloids and maltodextrin they can create breads similar to the ones that contain gluten in terms of both physicochemical and sensory features.

Inulin is an oligosaccharide belonging to fructans. What is important, the molecules of fructose in inulin are linked by β-2-1 glycoside bind (GUARNER, 2007). It appears that inulin, in addition to the numerous prohealth properties, can also be used as a texture-making factor – that stems from its capacity for absorbing water and for gelation, and also from its ability to create emulsions and condensation (BROWN and TUOHY, 2006; ZIOBRO *et al.*, 2013). The trials to enrich gluten-free bread by inulin have been carried out by GALLAGHER *et al.* (2003).

The main feature of maltodextrin is the value of its glucose equivalent DE (i. e. dextrose equivalent), which indicates the percentage of reductable sugars, expressed as glucose, calculated for a dry product. By the term maltodextrin, one can understand starch hydrolysates with DE below 20. Maltodextrin, due to its attractive rheological properties, is widely used in food processing (WITCZAK *et al.*, 2010).

Water plays an important role in the physical and chemical changes that occur during the production process of dough and bread. Depending on the amount of the added water the various quality characteristics of dough (CHIN *et al.*, 2005; HERA *et al.*, 2014) and bread (OSELLA *et al.*, 2007) can be affected. Hence, the aim of our work was to assess the textural

properties of dough and gluten-free breads obtained with the addition of the structure-forming additives and different amounts of water added to the dough. It is worth noting that many research on the influence of formulation, including water content, on the quality of gluten-free dough and breads were carried out. But still gluten-free commercial breads are not as good as traditional breads. Thus, it is necessary to conduct further research.

2. MATERIALS AND METHODS

2.1. Preparation of mixtures

Mixtures contained 100g of dry dough ingredients. The mixtures of gluten-free breads were produced from corn flour and rice flour used in the ratio of 1:2. In each sample, the gluten-free mixture had the same quantity of yeast, salt, and sugar. As structure-forming additives, hydroxypropyl cellulose - HPC (Klucel, type M CS), soy protein isolate - SPI (795), maltodextrin (DE 16, Jaskulski Aromas JAR), and inulin (HPX, Hortimex) were used (Table 1). The first sample contained only HPC, while the next samples were enriched by SPI, inulin, and maltodextrin. Accordingly, we introduced the following sample codes: HPC; HPC-SPI; HPC-SPI-I; HPC-SPI-M. Our samples were chosen basing on preliminary results.

Table 1. The composition of the gluten-free bread mixture.

Additives	Content (g/100 g) Sample code			
	HPC	HPC-SPI	HPC-SPI-I	HPC-SPI-M
Corn flour	30.2	28.5	25.2	25.2
Rice flour	60.3	57.0	50.3	50.3
Yeast instant	2.4			
Sugar	5.1			
Salt	1.5			
Hydroxypropyl cellulose	0.5			
Soy protein isolate	—	5.0	5.0	5.0
Maltodextrin	—	—	—	10.0
Inulin	—	—	10.0	—

2.2. Bread baking

The amount of water in the dough was 80, 90 and 100 g with 100 g of the mixture. Firstly, the dough was mixed in a mixer for 5 minutes, and, then, it was kept for 30 min in a plastic bowl in the temperature of 40°C. Subsequently, after 30 minutes, the dough was moved into shaped bowls where the fermentation process was continued for the next 10 minutes until the optimum volume was reached. Baking was carried out in the combi-streamer oven by UNOX (type XBC, model XBC 404) for 23 min in 175°C on the third level of vaporization. The breads were cooled down to the ambient air temperature. Specifically, they were packed into plastic bags that remained open and, in order to make

measurements, were kept in the room temperature for two days. Two breads were produced from one mixture. Six repetitions were made for each measurement.

2.3. Texture of gluten-free dough

The determination of the texture of gluten-free dough (test TPA-Texture Profile Analysis) was done using texture Analyzer TA-XT2 (Stable Micro System) connected to the PC. Raw dough samples were placed in glass vessels in the form of cylinders of 60 mm in diameter and 30 mm in height. The dough was double compressed to 1/3 of the height of the sample using a cylindrical head with an attachment in the shape of cylinder of 25 mm in diameter. The value of pressure used to compress the sample was equal to 250 N. The speed of measurement was equal to the speed of the head - 1 mm s^{-1}. During the study, curves in a force-shift of the stem system were obtained. On their basis, such features as hardness, cohesiveness, springiness, gumminess, and adhesiveness were specified. The TPA test was performed immediately after the mixer dough kneading had been completed.
The hardness is defined as the maximum value of the force needed to deform the dough during the first squeezing. The cohesiveness is defined as a ratio of the work needed to reach the point of hardness for the first compress to the work needed to reach the point of hardness for the second compress. The springiness is the difference between the distance at the point of bearing and the distance at the point of contact during the second cycle test. The gumminess is the product of the hardness of the first cycle and the cohesiveness. The adhesiveness is the area over the negative part of return force between the point of relaxation and the point of loss of contact (Stable Micro System, 1997).

2.4. Analysis of gluten-free breads

2.4.1 Physicochemical properties

The rapeseed displacement method 10-05 was applied to measure the bread volume. The Sa - Wa apparatus, produced by Sadkiewicz Instruments, was used to measure bread volume. The approved method 44-15A (AACC International, 2000) was applied to determine the bread moisture. The porosity of bread crumb was assessed in the same way as in, for example, ROMANKIEWICZ *et al.* (2017). It was performed by determination of the differences between the volumes of uncompressed and compressed crumb, which was devoiced of pores by kneading. The 27 cm^3 volume cylinders were cut from the bread crumb and were kneaded. Next, they were immersed in oil (edible oil was poured into cylinders up to a volume of 30 cm^3) in order to determine the volume. The height / diameter ratio (H/D ratio) was determined as well.

2.4.2 Textural properties

The crumb hardness was measured using texture Analyzer TA-XT2 (Stable Micro System, 1997). Twenty-millimeter thick slices were cut from the center of the analyzed loaves. Then, the slices, were compressed and relaxed. As previously, the cylindrical head with the attachment in the shape of a cylinder of a diameter of 25 mm was used. During the measurements, the speed of movement of the head was 1 mm s^{-1} and the sample was penetrated to the depth of 9 mm with the force of 250 N. We studied the moisture and the hardness of crumb 24 and 48 hours after baking.

2.4.3 Sensory assessment of gluten-free bread

Consumer quality assessment was performed using hedonic rating scale (100 mm - subsequently adopted as 100 arbitrary units). The boundary markings were from "very undesirable" (0 units) to "very desirable" (100 units). A team of 40 trained panelists conducted the sensory evaluation of the breads. The quality assessment was determined separately for each bread. The assessment was performed during several sessions, where each time 6 samples were evaluated. Based on the scores of the evaluation panel mean values were calculated (BARYŁKO-PIKIELNA, 1975).

2.4.4 Statistical analysis

All tests were performed at least in three replicates. The statistical analysis of the obtained data was performed applying Statgraphics Plus. The differences between the means were based on one-way ANOVA. The significance level (α) was set to 0.05 and the multiple comparisons of the means were conducted using Tukey's HSD test.

3. RESULTS AND DISCUSSION

3.1. Analysis of textural properties of gluten-free dough (TPA's test)

Table 2 presents the results of the textural features analysis of the gluten-free dough. The statistical analysis shows that the textural properties of the dough depend significantly on the amount of water addition, as well as on the type of structure forming additives (the results of two-way ANOVA may be obtained from the authors upon request). An increase of water amount, no matter what type of structure-forming additive was used, caused a significant decrease of the dough hardness, gumminess and adhesiveness. However, no significant impact of the amount of water on the springiness and cohesiveness of the dough was observed. The analysis of the impact of structure-forming additives on the textural properties indicates that a simultaneous use of HPC and SPI leads to an increase of the dough hardness, a significant increase of its gumminess and adhesiveness with 80 g and 90 g of water addition per 100 g of the mixture respectively, and a small decrease of its cohesiveness. The springiness of the dough samples, with and without the SPI addition, was on the same level for the same water addition respectively in whole analyzed range. One can conclude that the introduction of SPI into the gluten-free dough had no positive impact on the textural properties of the analyzed dough samples, but it is important to remember that the SPI additive significantly increased the amount of protein in the gluten-free bread, and, in consequence, increased its nutritional value.
There are a number of publications showing that the properties of mixtures of some biopolymers can be significantly different from the properties of individual components (MOHAMMADI *et al.*, 2014; DIOWKSZ *et al.*, 2009). The usage of polysaccharides composition allows to observe new functional properties or to change the rheological properties of food products (DIOWKSZ *et al.*, 2009). The phenomenon may be observed during the creation of the ternary systems of protein-polysaccharide-water. Depending on the proportion of the mixed ingredients, their structure, molecular weight, and the nature of the specific polysaccharides and proteins, distinct properties of mixtures, often beneficial, can be noticed. It is the case of, for instance, solubility, viscosity, susceptibility to an action of enzymes, gelation, denaturing temperature (MIR *et al.*, 2016).

Table 2. The parameter values of the texture of gluten-free dough with different addition of water.

Sample code	Amount of added water (g/ 100 g mixture)	Texture parameters				
		Hardness (N) $\bar{x}$ ±SD	Cohesiveness (-) $\bar{x}$ ±SD	Springiness (mm) $\bar{x}$ ±SD	Gumminess (N) $\bar{x}$ ±SD	Adhesiveness (N·mm) $\bar{x}$ ±SD
HPC	80	0.434±0.015^{c}	0.675±0.049^{a}	9.637±0.068^{a}	0.294±0.031^{b}	0.932±0.121^{c}
	90	0.220±0.017^{b}	0.710±0.073^{a}	9.252±0.330^{a}	0.157±0.022^{a}	0.353±0.044^{b}
	100	0.162±0.013^{a}	0.730±0.052^{a}	9.522±0.036^{a}	0.117±0.005^{a}	0.167±0.039^{a}
HPC-SPI	80	1.118±0.021^{c}	0.651±0.007^{a}	9.663±0.029^{b}	0.728±0.014^{c}	3.205±0.040^{c}
	90	0.474±0,044^{b}	0.660±0.027^{a}	9.587±0.033^{b}	0.313±0.032^{b}	1.141±0.170^{b}
	100	0.245±0.027^{a}	0.646±0.023^{a}	9.458±0.084^{a}	0.158±0.012^{a}	0.398±0.091^{a}
HPC-SPI-I	80	0.485±0.070^{c}	0.798±0.038^{a}	9.741±0.030^{a}	0.388±0.064^{c}	1.272±0.301^{c}
	90	0.287±0.077^{b}	0.800±0.088^{a}	9.762±0.053^{a}	0.226±0.040^{b}	0.548±0.317^{b}
	100	0.154±0.007^{a}	0.811±0.064^{a}	9.959±0.035^{b}	0.125±0.012^{a}	0.040±0.019^{a}
HPC-SPI-M	80	0.342±0.014^{c}	0.579±0.016^{ab}	9.818±0.055^{a}	0.198±0.004^{c}	0.248±0.027^{c}
	90	0.222±0.016^{b}	0.638±0.044^{b}	9.879±0.081^{ab}	0.141±0.004^{b}	0.075±0.047^{b}
	100	0.132±0.010^{a}	0.543±0.038^{a}	9.975±0.016^{b}	0.072±0.005^{a}	0.050±0.001^{a}

HPC - Hydroxypropyl cellulose, SPI - Soy protein isolate.

$\bar{x}$ - mean value / SD – standard deviation.

mean values denoted by different superscripts (a) to (c) in the same column for the same addition differ significantly from each other ($\alpha = 0.05$).

It was found out that the best effect on the textural properties of the gluten-free dough had the addition of the mixture of HPC, SPI and maltodextrin. Thus, the dough obtained using the mixture of these additives had the highest springiness and the lowest hardness, gumminess and adhesiveness. The best effect of these compounds was noticed for the highest level of water addition. The addition of inulin instead of maltodextrin enables obtaining the most compact dough for each amount of water. The lowest rates can be noted for the dough containing the mixture of HPC and SPI for each amount of water, which were characterized by the highest level of hardness, gumminess and adhesiveness. DIOWKSZ *et al.* (2009) evaluated the impact of the enrichment of gluten-free bread by fibre creams of different composition on the rheological properties of the gluten-free dough. The authors argued that the use of lupin fibre with addition of inulin favorably reduced the hardness of the cream. The addition of inulin to lupin fibre substantially changed the rheological properties of the mixture. The significant influence of inulin on the quality of the gluten-free dough was not noticed, probably because of its small addition in relation to the total weight of the dough (from 1-3 %) and the properties of the dough were determined by lupin fibre or soy fibre (from 17-20 %). WITCZAK *et al.* (2010) claim that the rheological properties of gluten-free dough may be modified by the addition of maltodextrin, which weakens its structure and increases its ability of deformation. The dough with the addition of inulin is less flexible and more viscous. As a result, the addition of inulin is followed by the decrease in water amount. This effect is associated with the ability of inulin to form gels. The macromolecules of inulin zones are present in the cross-linked form and therefore they hold large quantities of water. During the folding of gel molecules, water is hold and does not bind (CAPRILES and AREAS 2013; ZIOBRO *et al.*, 2013; TSATSARAGKOU *et al.*, 2016).

3.2. Analysis of physicochemical properties of gluten-free breads

The properties of the porous 3D structure of crumb and the resulting volume of bread are next to the taste and smell basic features of the bread quality (DIOWKSZ *et al.,* 2009). The research shows that the volume of the gluten-free bread was differentiated not only in terms of structure-forming additives but also with reference to the amount of water addition, which also results from the different absorption capacity of the applied additives. On the basis of the obtained results (Fig. 1A), it was found out that the best influence on the volume of the gluten-free bread has the addition of the mixture of HPC and SPI and 100 g of water per 100 g of the mixture. The addition of SPI to the bread containing only HPC caused the increase of the volume of bread with 90 and 100 g of water per 100 g of the mixture. Further extension of the recipe to include inulin, only in the case of the bread with the addition of 90 g of water per 100 g of the mixture (which amounted to 178 cm^3/100 g of the product), remitted the increase of its volume. The replacement of inulin by maltodextrin proved beneficial only for 80 g of water per 100 g of the mixture, when the volume grew for the bread with the mixture of HPC and SPI with an increase of water content from 80 to 100 g per 100 g of the mixture. In the case of the bread containing only HPC and the bread with the addition of inulin and maltodextrin with the increase of water content in the mixture in the range from 80 to 90 g - the volume increased, while beyond 90 g/100 g of the mixture the volume decreased. The volume of the bread with the addition of HPC baked with 100 g of water/100 g of the mixture was higher in comparison to the volume of the bread with 80 g of water addition in relation to the weight of the mixture.

The research confirmed the earlier observations on the favorable impact of soy protein or milk additions on the appearance of the loaf – particularly, the increase of its volume and the extension of its freshness time (GALLAGHER *et al.,* 2003; GALLAGHER *et al.,* 2004; GAMBUŚ *et al.* 2007). Similar observations were made by RANHORTA *et al.* (1975) in their studies. They found not only the increase of the value of nutrition, but also the improvement in the characteristics of the final properties of the gluten-free breads baked on the basis of gluten-free wheat starch and soy protein isolates. The usage of maltodextrin with different glucose equivalents – DE (3.6, 15.3, 18.3, and 21.8) in the gluten-free bread production process has been widely discussed. WITCZAK *et al.* (2010) demonstrated that the maltodextrins with high DE, especially equal to 18.0 and 21.8, positively affected the size of a loaf, while the maltodextrins with low DE reduced the volume of bread and caused the deterioration of its quality. The higher volume of the loaves prepared with the addition of the maltodextrin with higher DE derives probably from the increasing number of low molecule carbohydrates that can be used by yeast during fermentation.

The analysis of the results of the gluten-free bread crumb porosity (Fig. 1B) shows that the bread with the mixture of HPC and SPI with 100 g of the addition of water per 100 g of the mixture had the largest volume, and, moreover, the largest crumb porosity (47.7 %). Generally, the addition of SPI increases the porosity (only the porosity of the bread with 80 g of water addition per 100 g of the mixture did not change, which could have been caused by too small amount of water - soy protein isolates have good properties of water absorption). The addition of inulin had beneficial aspects for the bread with 80 and 90 g of water per 100 g of the mixture. However, a higher value of the porosity can be obtained by the use of maltodextrin instead of inulin. The addition of maltodextrin to the recipe proved to be effective in the case of the bread with 80 to 90 g of water addition/100 g of the mixture, because for the water addition of 100 g the porosity was slightly lower than in the bread with the largest value of this parameter among all analyzed samples.

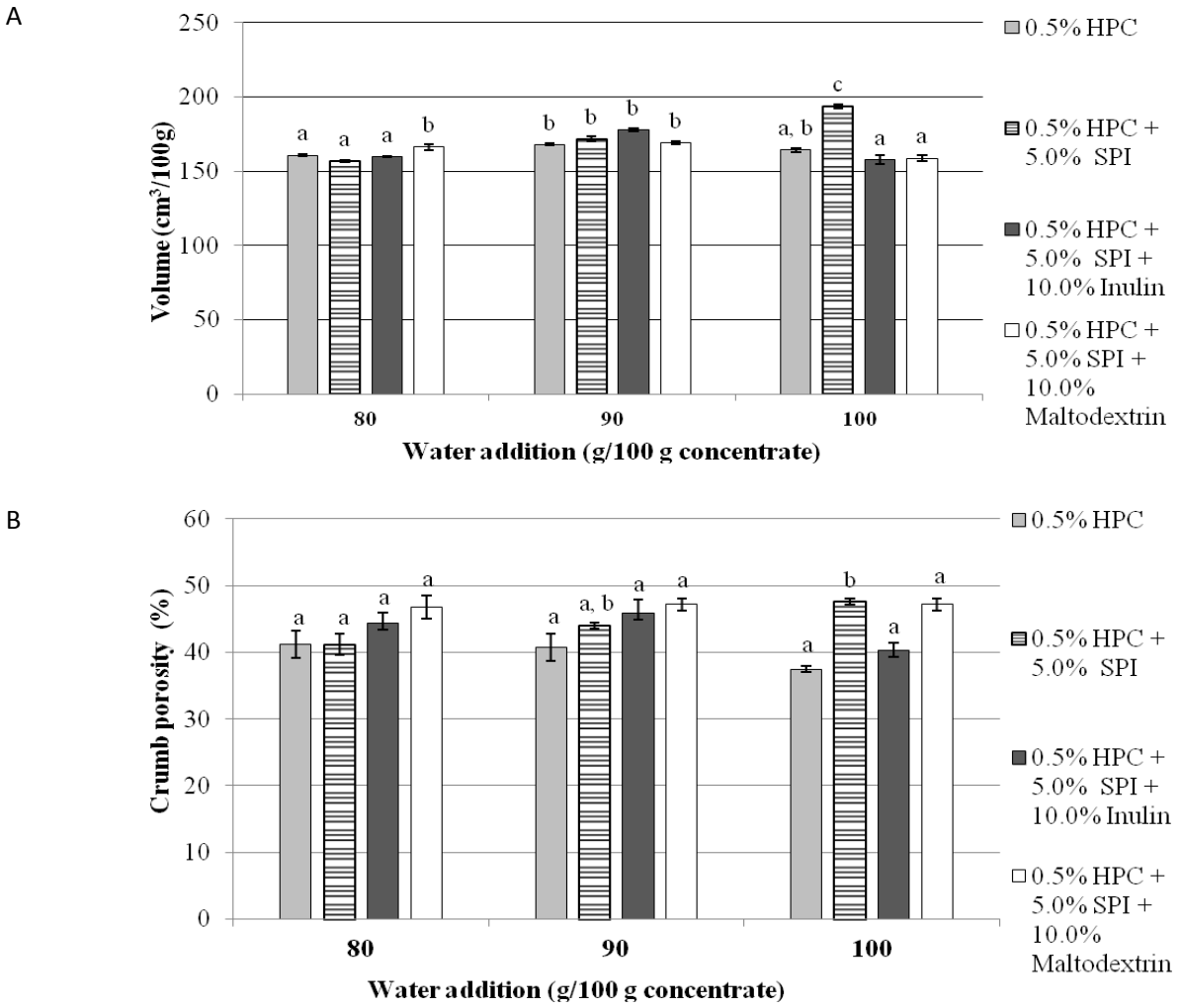

Figure 1. The physicochemical properties of gluten-free breads: A. Volume, B. Crumb porosity.
HPC - Hydroxypropyl cellulose, SPI - Soy protein isolate.
(a) to (c) – mean values denoted by different superscripts for the same addition differ significantly from each other ($\alpha = 0.05$).

The porosity value of the bread with only HPC decreased with the increase of the water content in the dough, while an inverse relationship was noted for the bread containing the mixture of HPC and SPI. For the bread with the addition of inulin and maltodextrin, the porosity increased together with the increase of water content in the range from 80 to 90 g per 100 g of the mixture.

In order to examine the process of ageing, the gluten-free breads were kept in open plastic bags in the room temperature for two following days. The moisture of crumb was measured after 24 and 48 hours of storage. The research showed that the moisture of the crumb, for all samples of the bread, decreased with the addition of water to the dough during the whole storage time (Table 3). The addition of SPI, on the one hand, alleviated the crumb moisture after 24 hours of baking, and, on the other hand, it caused the loss of moisture changes during its storage in comparison with the crumb moisture of the bread without the addition of SPI. Considering both the impact of the addition of inulin and maltodextrin, it was found out that more advantageous is the use of inulin is more advantageous. The addition of inulin for each level of water addition allowed to obtain the

largest crumb moisture after 24 h of storage for all the analyzed bread samples. Moreover, the bread with its addition preferably retained moisture during the whole period of storage.
The increase in the crumb moisture for the breads with the addition of HPC, the mixture of HPC and SPI, and the mixture of HPC, SPI and inulin was observed during its storage time, as opposed to the breads with the mixture of HPC, SPI and maltodextrin, for which the minimal decrease of moisture or its absence was observed.

Table 3. Physicochemical properties of gluten-free bread after 24 and 48 h storage.

No of sample	Type and amount of addition (g/ 100 g)		Amount of water addition (g/ 100 g mixture)	Crumb moisture (%) $\bar{x}$ ±SD		Crumb hardness (N) $\bar{x}$ ±SD	
				After 24 hours	After 48 hours	After 24 hours	After 48 hours
1	HPC	0.5	80	52.8±0.2^{c}	53.8±0.4$^{c'}$	22.78±6.93^{a}	30.48±8.72$^{a'}$
			90	50.4±0.2^{b}	51.1±0.1$^{b'}$	20.41±4.04^{a}	24.74±5.88$^{a'}$
			100	48.6±0.5^{a}	49.3±0.3$^{a'}$	19.88±2.25^{a}	22.39±1.81$^{a'}$
2	HPC SPI	0.5 5.0	80	51.8±0.0^{c}	52.0±0.2$^{c'}$	28.56±3.86^{b}	33.75±4.91$^{b'}$
			90	49.7±0.2^{b}	50.1±0.4$^{b'}$	15.40±4.16^{a}	20.76±6.44$^{a'}$
			100	47.2±0.1^{a}	47.6±0.1$^{a'}$	11.64±1.97^{a}	14.81±2.61$^{a'}$
3	HPC SPI Inulin	0.5 5.0 10.0	80	52.8±0.1^{c}	52.8±0.0$^{c'}$	30.34±4.07^{b}	36.09±4.81$^{b'}$
			90	50.6±0.3^{b}	50.7±0.2$^{b'}$	19.19±3.13^{a}	24.07±2.56$^{a'}$
			100	48.8±0.2^{a}	49.1±0.6$^{a'}$	22.70±6.97^{ab}	29.06±7.81$^{a'b'}$
4	HPC SPI Maltodextrin	0.5 5.0 10.0	80	52.6±0.2^{c}	52.2±0.2$^{c'}$	20.28±3.88^{a}	25.22±2.19$^{a'}$
			90	50.4±0.2^{b}	50.0±0.1$^{b'}$	19.63±5.02^{a}	23.07±5.04$^{a'}$
			100	47.9±0.4^{a}	47.9±0.2$^{a'}$	14.25±5.53^{a}	17.74±5.17$^{a'}$

HPC - Hydroxypropyl cellulose, SPI - Soy protein isolate.
$\bar{x}$ - mean value / SD – standard deviation.
mean values denoted by different superscripts (a) to (c) / (a′) to (c′) in the same column for the same addition differ significantly from each other ($\alpha = 0.05$).

3.3. Analysis of textural properties of gluten-free breads

The crumb hardness is one of the most obvious manifestations of bread staling. The crumb of the gluten-free breads hardens rapidly due to the starch content higher than in regular breads, as the starch components – amylose and amylopectin – retro gradate (RONDA and ROOS, 2011). The amount of water in the bread is also important in the process of bread staling. But this process is not caused by the loss of the water amount. It may be partly caused by the migration of moisture from the crumb to the crust. In addition, the water content and its activity can affect the degree of recrystallization of the starch (RONDA and ROOS, 2011). The crumb hardness in all examined breads increased during their storage. The addition of SPI to the bread containing HPC reduced the hardness of the bread with the 90 and 100 g of water per 100 g of the mixture after 24 h storage time (these breads were characterized by the lowest values of the hardness among all analyzed samples), but the addition of SPI increased the process of bread staling in all analyzed breads. Considering the addition of inulin and maltodextrin, it can be concluded that the addition of inulin proved to be disadvantageous (for the bread with 80 and 100 g of water addition to 100 g of the mixture largest values of the hardness 24 h after baking can be noticed). The

research has shown that better results were guaranteed by the addition of maltodextrin than inulin. This addition allows you to obtain not only smaller values of the crumb hardness during the whole period of storage, but also can reduce the rate of adverse changes. The crumb hardness of the breads containing only HPC, HPC and SPI and the breads containing maltodextrin decreased together with increasing the content of water added to the dough. Throughout the period of storage, the value of the crumb hardness for the breads with the addition of inulin decreased with the increase of water content in the range from 80 to 90 g/100 g of the mixture. However, the further growth of the amount of water resulted in the increase of the hardness value after 24 and 48 h of storage.
The desired effect of extension of the consumption freshness of breads associated with addition of soy protein, and different fractions of dietary fibre (including inulin) have been widely observed (CROCKETT *et al.*, 2011; Martinez *et al.*, 2014; TSATSARAGKOU *et al.*, 2016). HAGER *et al.* (2011) explored the impact of the addition of inulin and oat beta glucan on the quality of wheat and gluten-free bread. According to the authors, the inulin, although interesting from a nutritional point of view, has little use in the production of bread, both wheat and gluten-free, because its addition causes the increase in crumb hardness as well as the increase of staling. The use of a mixture of 5% to 8% inulin, fructo-oligosaccharides and flour from the chicory, for all applied doses, reduces the speed of the crumb hardness within three days of storage (KORUS *et al.*, 2006). This confirms the earlier observations concerning new functional properties of the mixtures of some biopolymers.
The changes of the physiochemical parameters of gluten-free breads due to different addition of water were investigated by a number of authors. For example, YAZAR *et al.* (2017) studied the nonlinear rheological properties of the gluten-free bread dough samples prepared using the optimum water absorption levels. Among others, they showed that the optimal addition of water depends on the ingredients included in the formulation of the gluten-free dough. 110%, 90%, 85%, and 160% water levels were found as optimal for rice, buckwheat, quinoa, and soy flour, respectively.
The evaluation of the panelist score concerning the gluten-free breads (Fig. 2) showed that they were accepted by consumers. The largest degree of consumer demand (49.4 j.u.) gained the bread with the mixture of HPC, SPI, and maltodextrin, with 100 g of water per 100 g of the mixture. The received samples of the gluten-free breads are shown in Fig. 3.

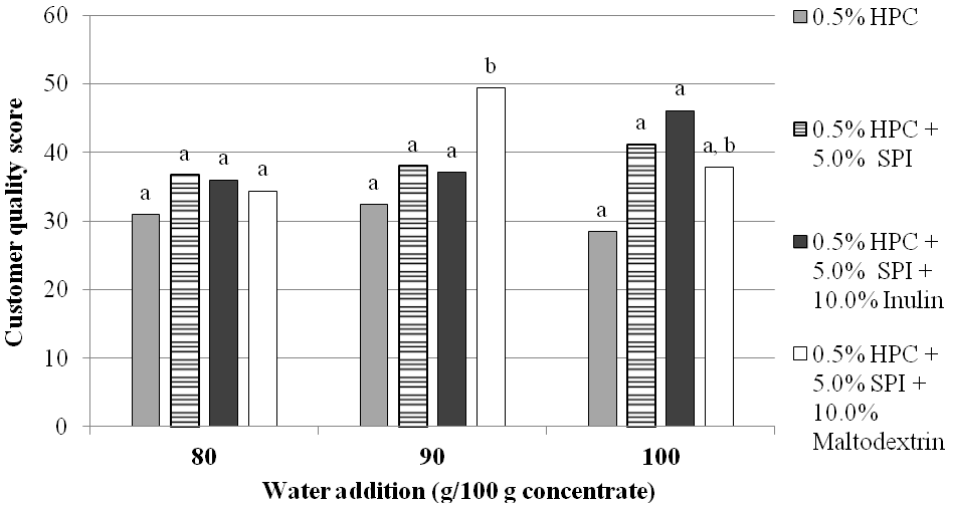

Figure 2. Quality assessment of consumer demand on gluten-free breads.
HPC - Hydroxypropyl cellulose, SPI - Soy protein isolate.
(a) to (b) – mean values denoted by different superscripts for the same addition differ significantly from each other ($\alpha = 0.05$).

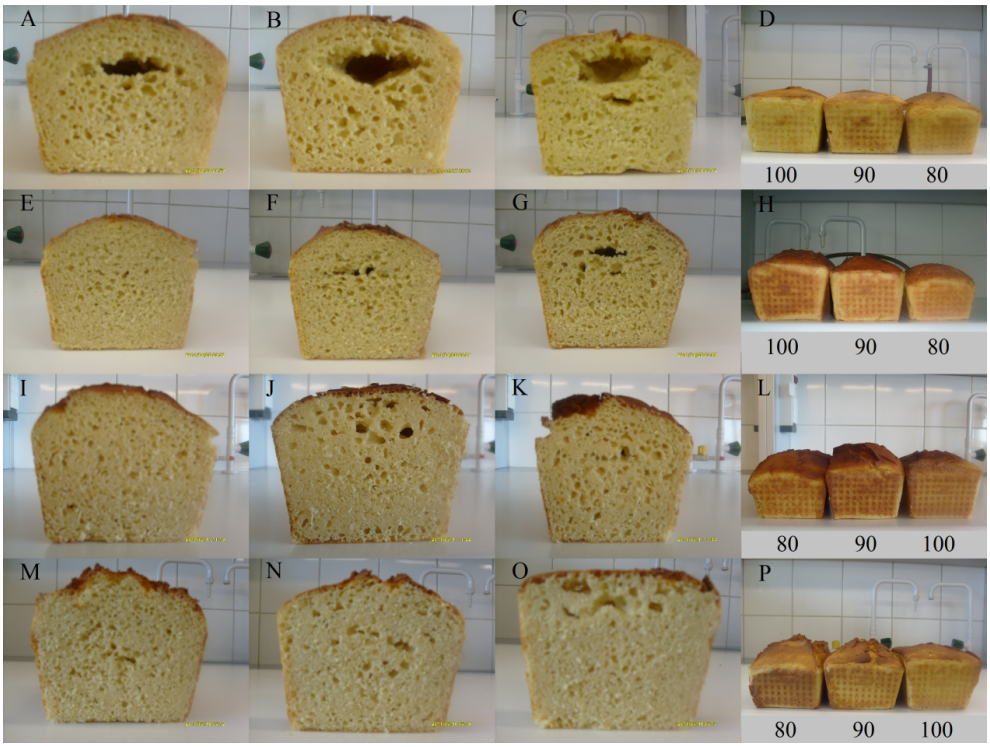

Figure 3. Gluten-free bread with addition: HPC and 80 (A), 90 (B), 100 (C) g W/100 g mixture, a general statement (D); HPC, SPI and 80 (E), 90 (F), 100 (G) g W/100 g mixture, a general statement (H); HPC, SPI, I and 80 (I), 90 (J), 100 (K) g W/100 g mixture, a general statement (L); HPC, SPI, M and 80 (M), 90 (N), 100 (O) g W/100 g mixture, a general statement (P). HPC - hydroxypropyl cellulose, SPI - soy protein isolate, I - inulin, M – maltodextrin, W – water.

The breads with the single addition of HPC (Fig. 3 A, B, C, D) have the lowest assessment due to the inadequate structure of their crumb, their specific aroma and yellow colour. The breads with the addition of inulin are darker and have roasted skins (Fig. 3 I, J, K, L), which is confirmed by the literature (GALLAGHER *et al.*, 2004). The browning of the skins of the gluten-free breads containing inulin is related to the partial hydrolysis of polysaccharides present in the powder of the inulin, which occurs during baking. It leads to a strong Maillard reaction, and, in consequence, the products are darker. This is a desired effect, because gluten-free breads are much lighter than wheat breads.

4. CONCLUSIONS

Summing up, firstly, the hardest and the gumminess gluten-free dough with the largest adhesiveness for each level of water addition was observed using the mixture of hydroxypropyl cellulose and soy protein isolate. The addition of maltodextrin had a positive impact on the textural properties of the gluten-free dough. In particular, it improved the springiness and reduced the hardness, gumminess and adhesiveness of the gluten-free dough. The textural properties of the gluten-free dough varied depending on the amount of water added to the dough.
Secondly, the most significant improvement of the quality of the gluten-free breads was observed when the mixture containing hydroxypropyl cellulose and soy protein isolate with the addition of 100 g of water was used. The sample had not only the largest volume,

the highest porosity and the smallest crumb hardness, but also the highest level of freshness during the whole storage test.
Thirdly, on the one hand, the addition of the mixture consisting of hydroxypropyl cellulose, soy protein isolate and inulin to the gluten-free bread increased and maintained the proper crumb moisture during its storage, but, on the other hand, it increased the crumb texture hardness.
Our results showed that the best formulation among the analyzed in this study is the one composed of 0.5 g of hydroxypropyl cellulose, 5.0 g of soy protein isolate, and 10.0 g of maltodextrin on 100 g of the mixture, with 100 g of water. The breads prepared from this formulation reached the highest level of consumer demand. The breads had the largest volume, and, moreover, the highest crumb porosity. Also the dough had the highest springiness and the lowest hardness, gumminess and adhesiveness. The research showed that better results were guaranteed by the addition of maltodextrin than inulin.
Considering the fact that commercial gluten-free breads have usually lower nutritional value than the traditional ones, it should be emphasized that the addition of soy proteins not only favorably affects the texture of gluten-free breads, but also increases their nutritional value. Also the research confirmed the earlier observations on the favorable impact of soy proteins on the appearance of a loaf – particularly, the increase of its volume and the extension of its freshness time.

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PAPER

INFLUENCING FACTORS ON CHINESE WINE CONSUMERS' BEHAVIOR UNDER DIFFERENT PURCHASING MOTIVATIONS BASED ON A MULTI-CLASSIFICATION METHOD

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ABSTRACT

This study investigates the importance rating of influencing factors in driving wine consumption under four specific situations, that is, gift, banquet, party, and self-drinking, and thus achieves consumer segmentation. The affecting factors containing wine quality and socio-demographic variables are measured on a national representative sample (N=609) in China. Lasso method is used to select the factors, and a binary classifier v-twin support vector machine (v-TSVM) is extended to a multi-classification case by using a "one-versus-one" approach, which predicts the purchasing behavior of consumers. The monthly income, occupation, and knowledge of a consumer toward wine, the origin of wine, the vintage, and advertisement, are critical factors in driving consumption. Wine color and packing emerge as leading factors when consumer purchase wine for gift and banquet. Promotion significantly contributes to wine price selection for banquet, party, and self-drinking. Results show that the importance ranking of determinants varies under different purchasing motivations. In addition, the recognition accuracy can be considerably increased with prior knowledge of the consumption purpose. The nonlinear classifier is recommended for application because this classifier performs better than the linear one. This paper offers a fresh perspective on wine consumption behavior in China by applying two machine learning methods to identify and quantify determinants in specific situations. The results significantly assist wine managers to provide informed decisions with regard to wine production and marketing.

Keywords: wine motives, personal traits, wine price, influential factor, consumers' purchasing behavior

1. INTRODUCTION

The Chinese wine market has been flourishing in recent years with the improvement of the living standards of people and influence of affluent western lifestyles. In 2015, China has produced and consumed 11.5 million and 16 million hectoliters of wine, respectively, and ranked sixth in the global wine production (OIV, 2016). The prediction of the purchase behavior of consumers has been recognized as a significant research topic over the past decades. The accurate prediction of purchasing behavior enables wine dealers to accurately locate consumers' demands, formulate appropriate marketing strategies, and achieve consumer segmentation.

A study on the influence of purchasing motivation will aid in understanding the progress of consumer decision-making. Previous studies have shown that a wine consumer exhibits different purchasing motivations under various consumption scenarios in a descriptive way (GERAGHTY and TORRES, 2009). The main motivations of Chinese wine consumers include health care, auxiliary dining, and social contact (LI, 2014). People perceive wine as a healthy and nutritional product that can be recommended for regular intake to prevent diseases because wine contains many kinds of organic acids, minerals, and vitamins (TANG, 2008; MU, *et al*., 2016). Consumers have different preferences for wine attributes, when they drink at home, drink with friends and give as gifts (QUESTER and SMART, 1998; LI, 2014; CHEN, 2014). Drinking with friends at parties is casual and relaxed, whereas the major function of wine is to please other people in a business banquet (HALL *et al*., 2001). Currently, an increasing number of Chinese aim to give red wine as a gift to display affection or enhance friendship, especially during festivals.

Wine consumption is influenced by many interrelating factors, such as wine product properties, lifestyle and situations of an individual, and psychological factors of consumers (PICKERING and HAYES, 2017; SCHMITT, 1997). Various attributes, such as taste, color, aroma, brand, production, and label information, are found to be important aspects that determine wine choice (THORPE, 2009). LOCKSHIN *et al*. (2017) summarized several methods used in marketing in combination with sensory science techniques to understand the changing consumer preferences in China. The consumption behavior of Chinese are highly related to the educational background of consumers, wine-related activities, wine taste, country of origin, quality, and price (BALESTRINI and GAMBLE, 2006; CAMILLO, 2012).

Most business models are based on a linear equation to estimate the weight of such factors when measuring the response of purchase intention to the contextual factors. The commonly used linear models are linear discriminant and logistic regression analyses (CULBERT *et al*., 2017; HONORÉ-CHEDOZEAU *et al*., 2017; LI, 2014; YORMIRZOEV, 2016). The prediction models for purchase behavior are over-concentrated and over-reliant on these linear models compared with other research fields. In addition, principal component analysis (PCA) is also combined with the linear models to reduce the dimensionality of factors (JOLLIFFE, 2002; CHANG, *et al*., 2015; TSOURGIANNIS *et al*., 2015). However, using PCA to extract the component feature may lose certain important information. The meaning of comprehensive evaluation function is unclear when the labels of load factor in the principal component are positive and negative; thus, this function is sensitive to the relative scaling of the original variables and has low variable interpretation. Moreover, we can collect additional consumer data information with the development of communication technologies. Analyses based on traditional linear models are insufficient in achieving the requirement of academics and practitioners (DAYKIN and MOFFATT, 2002; THONG and SOLGAARD, 2017).

In recent decades, increasing machine learning approaches have emerged. The least absolute shrinkage and selection operator (Lasso) is recognized for its capability to exploit

information from ordinary data and flexibility to capture different effects of explanatory variables (TIBSHIRANI, 1996). The Lasso method can continuously shrink certain coefficients to zero and automatically select a subset of variables. In addition, the Lasso method has better variable interpretability than other feature selection methods, such as principal component regression and least squares regression (TIAN *et al*., 2015). The support vector machine (SVM) has been considered an effective and promising binary classifier for its unique advantages (VAPNIK, 1995). The introduction of kernel function maps training variables into a high-dimensional space, thereby successfully solving the nonlinear SVM. Many variants of SVM have been proposed since then, and several binary SVMs have been successfully extended to multi-class scenarios by applying "one-versus-one" (OVO) and "one-versus-all" (OVA) strategies (TOMAR and AGARWAL, 2015; WANG and ZHOU, 2017). The SVMs have been widely applied in various aspects that range from disease diagnosis and bankruptcy prediction to consumption behavior prediction (e.g., electricity, health product, and building energy) (BAHAMONDE *et al*., 2007; GUO, 2013; KAVAKLIOGLU, 2011).
This study aims to use two representative machine learning methods, that is, Lasso and OVO v-TSVM, to investigate the determinants on the wine price selection under free and four purpose-based choices, that is, gift, banquet, party, and self-drinking, so as to predict the price of wine purchased by a consumer and estimate the effects of major factors selected through the Lasso method simultaneously.

2. MATERIALS AND METHODS

2.1. Conceptual framework

Numerous researches discipline including economics, marketing, psychology, and products, have a shared interest in consumers' behavior. More and more researchers have increasingly concentrated on consumers' attitudes, motivation, perceptions and preferences for wine. Previous studies show that the motivation for purchasing wine varies under different purchasing situations (BARREIRO *et al*., 2008). Moreover, GOODMAN (2009) found that previous tasting experience and opinion of other people significantly influence wine purchasing behavior. The knowledge of consumers toward wine positively and notably affects the wine purchasing behavior of these consumers (HUSSAIN *et al*., 2007). Consumers with higher production involvement are less sensitive to wine price, whereas consumers with lower production involvement focus more on price discounts (JAEGE *et al*., 2009). Furthermore, many researches have shown that consumers' purchase choices are well related with age and education in wine consumption. Based on the previous studies and combining with characteristics of wine consumption, the factors affecting wine consumption were summarizes in Fig. 1. It covers a range of purchasing motivations, reference group factor, marking factors, wine quality factors, the knowledge level towards wine and characteristics of consumers.

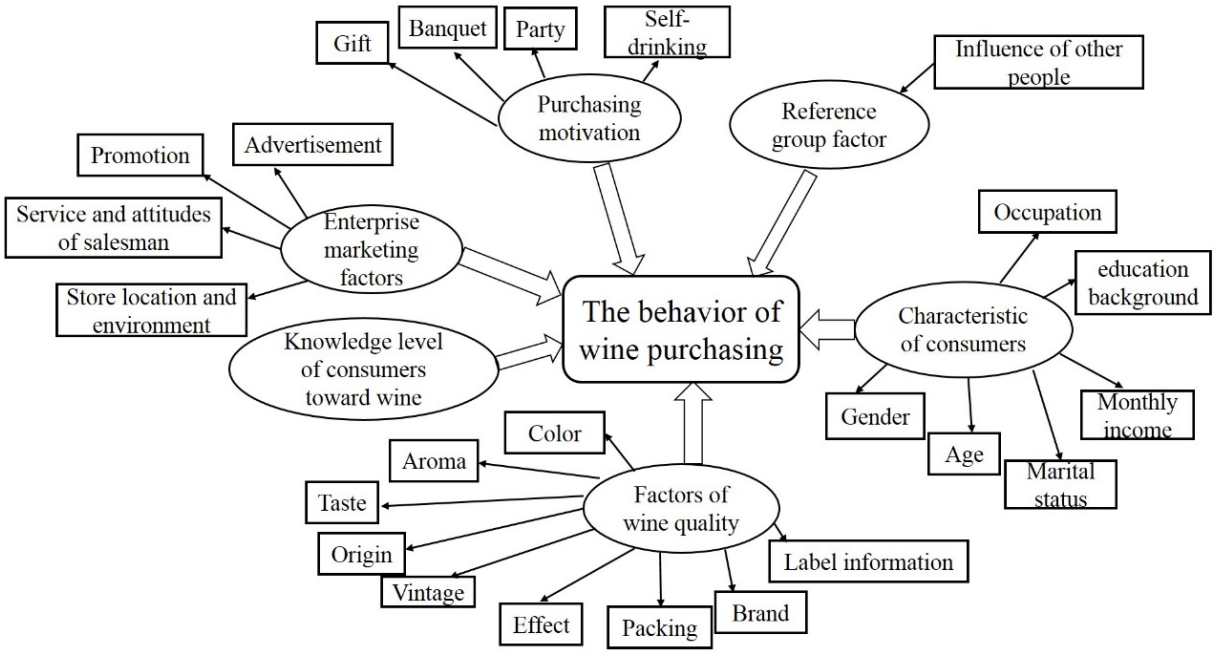


Figure 1. Conceptual framework of consumer's purchasing behavior for wine.

2.2. Questionnaire

The questionnaire of Chinese consumers' decision making behavior towards wine (It is shown in the appendix) was designed which consisted of 30 questions. This questionnaire includes the following contents:
(1) Questions regarding the purchasing behaviors of consumers (the frequency of purchasing and drinking).
(2) Questions investigating the price of wine that consumers frequently purchase. The consumers selected seven kinds of wine price, that is, 1="$0-7.5," 2="$7.6-15.1," 3="$15.2-22.6," 4="$ 22.7-30.1," 5="$ 30.2-45.2," 6="$ 45.3-75.3," and 7="$75.4 and above." Based on the literature review, four usually types of motivation (gift, banquet, party, and self-drinking) for wine consumption were extracted and described in the questionnaire. Besides, the consumers were asked to choose the price of wine that they purchase for the specific purpose;
(3) Questions that belong to multi-item scales, which measure factors that influence consumer purchasing, such as influence of others, quality of wine, enterprise marketing factors, knowledge of consumers. This study investigates the 10 items of wine quality factors, namely, the origin of wine and vintage, effects, packing, brand, label information, color, aroma, taste, and awards. The enterprise marketing factors contains 4 items, i.e. advertisement, promotion, service and attitude of the salesperson, and store location and environment. The 16-item scale was collected using a 5-point Likert scale from 1="Strongly disagree" to 5="Strongly agree."
(4) Consumers' socio-demographic characteristics: gender, age, marital status, monthly income, education background, and occupation. All the six features use the numbers "1, 2, 3, …" to assign the variable level from low to high.

2.3. Survey

Considering the sampling frame and economic development level in different regions, we hired and trained several undergraduate students from China Agricultural University to answer the survey. We realized that young people are the main force in wine consumption and many wine tasting groups are found on the Internet. The survey was conducted in 2016 and lasted for five months. A total of 1600 questionnaires were distributed in many provinces of China, and 995 questionnaires were returned. In the returned questionnaires, the respondents were instructed to evaluate the statement "In the past year, how often did you purchase wine?" The data were "cleaned" by removing responses of "Never bought wine." Therefore, the respondents in this study are consumers who, on one occasion, purchased wine. Finally, 609 questionnaires were used for final analysis.

2.4. Methods

The analysis of the data consisted of two steps. First, the Lasso method was conducted to select the determinants. In theory, the discrimination ability we can obtain is robust when we use considerable features. However, an excessive number of features may increase the learning speed and lead to "overfitting" problem. The accurate selection of features is a prerequisite for a high prediction accuracy. The Lasso method penalizes the regression coefficients with an L1 penalty, shrinking many of the features to zero. Any features with non-zero coefficients are "selected" through the Lasso method, which indicates that these selected features contribute most to the wine purchasing behavior of consumers. Second, the OVO Mv-TSVM method was used to predict the behavior of Chinese wine consumers. To the best of our knowledge, the v-TSVM (PENG, 2010) was initially proposed for binary problems. Owing to the K-class scenario, we use the ith class as the positive and jth as the negative to construct a binary v-TSVM classifier. The OVO Mv-TSVM method need to construct K(K−1)/2 binary v-TSVM classifiers. For a new testing point, we obtain the vote for each class and assign its label with a maximum vote.
For the nonlinear case, we used the Gaussian kernel function

$$Ker(\mathbf{x}_i, \mathbf{x}_j) = e^{-\left\|\mathbf{x}_i - \mathbf{x}_j\right\|^2 / 2r^2}$$

and grid research to find the optimal parameter. All algorithms were written and operated in MATLAB 2014a, and all statistical analyses were conducted using the SPSS version 20 and Microsoft Office Excel version 2013 software.

3. RESULTS

The whole Cronbach's of the questionnaire is 0.776, F=334.221, Sig=0.00, thereby indicating that the survey has a high internal consistency. The response rate of questionnaire is 62.19%. A majority of the respondents (63.71%) would purchase wine once or twice a year, and 81.94% would drink two or more bottles of wine in a year. The 609 samples were collected from 21 provinces, cities, and autonomous regions in China. We inquired the per capita monthly income of the above areas from the China Statistical Yearbook 2016, on which we calculated the global per capita monthly income as a standard, and the value is 780.06$. The provinces where the samples were collected are located in Eastern China, and most of these samples were relatively advanced in the

economic area. A total of 9.69% participants would purchase wine as a gift, 21.18% for banquet, 30.05% for parties, and 39.08% for self-drinking.
The results of wine price that the consumers purchased are listed in Table 1. Based on these samples, 65.51% would purchase wine in the price range of 7.6-30.1$ with free choice. The average price is 30.20$ (SD=0.83), with a 95% confidence interval of (28.58, 31.85). For the purpose of gift, 55.83% would select the wine price above 30.2$, and the average price is 40.64$ (SD=0.91). For the purpose of banquet, 64.20% would select the wine price in the range of 15.2-45.2$, and the average price is 30.71$ (SD=0.74). For the purpose of party, 64.86% would select the wine price in the range of 7.6-30.1$, and the average price is 27.97$ (SD=0.69). For the purpose of self-drinking, 65.19% would select the wine price in the range of 7.6-30.1$, and the average price is 28.11$ (SD=0.75).

Table 1. Statistical results of consumer's purchased wine price.

Wine price ($)	Free-choice (%)	Gift-based (%)	Banquet-based (%)	Party-based (%)	Self-drinking-based (%)
0-7.5	2.63	1.15	1.64	3.28	4.43
7.6-15.1	22.99	12.15	17.24	20.69	22.33
15.2-22.6	20.85	12.15	21.02	21.35	22.50
22.7-30.1	21.67	18.72	23.15	22.82	20.36
30.2-45.2	12.32	19.70	20.03	19.05	14.45
45.3-75.3	9.85	17.41	10.84	8.87	10.84
Above 75.3	9.69	18.72	6.08	3.94	5.09
Mean*	30.20	40.64	30.71	27.97	28.11
SD.*	0.83	0.91	0.74	0.69	0.75
95%Confidence interval*	(28.58, 31.85)	(38.89, 21.82)	(29.27, 32.17)	(26.65, 29.35)	(26.61, 29.59)

Note: *are the results of 10000 times Bootstrap resampling results.

The characteristics of the sample's demographics are detailed in Table 2. The average age is 35.18 years (SD=0.42). The average monthly income is 774.67$ in 10000 times Bootstrap estimation, which is nearly the same as the standard 780.06$. The respondents are 52.71% male and 47.29% female; a total of 32.35% are single, and 67.65% are married. A majority of the respondents who attained a college degree were 76.52%, 18.56% are senior high or in a special school, and only 4.93% are in primary or junior high school. The respondents vary in careers, 8.21% are students, 2.30% are peasantry, 25.94% are freelance, 2.96% are unemployed or retired, 11.99% are staffs of state-owned companies, 13.30% are staffs of foreign or private enterprises, 15.60% work as party and government officers, 9.36% work in education and scientific research units, and 10.34% work in other fields. Inspired by FORLEO *et al*. (2017), we lists the associations of wine consumption prices with demographics in Table 3. It is obvious that monthly income and occupation are significant no matter in what purpose-based. There are about 10% high-income and 3~4% low-income consumers choose high-priced wine. Male and female showed differences in the wine purchasing for free-choice, gift-giving and banquet-based purpose. There are 17.73% male and 14.12% female consumers choose wine price above 30.2$. The gender difference is not obvious in party-based and self-drinking based wine purchasing. There are only 8% elder people (above 46 years) choose high-priced wine (above 30.2$), and the percentage increased to 12% for gifted purpose. The single consumer and married consumer acted

different in wine price-choosing for party-based and self-drinking based purpose. Statistically significant differences between education and wine-price choosing for gifted and banquet-based purpose were identified. About 30% highly educated consumers choose high-priced wine, and only 1% consumers with Primary or Junior high school background chose high-priced wine. Fig. 2 illustrates the results of statistical affecting factors, where the mean of wine knowledge is the highest at 4.04, and the mean of advertisement is the lowest at 3.18.

Table 2. Statistical features of respondents.

Demographic characteristics	Category	Percentage	Sample population(n)
Gender	Male	52.71	321
	Female	47.29	288
Age	18-25	22.33	136
	26-35	31.86	194
	36-45	24.14	147
	46-55	17.24	105
	Above 55	4.43	27
	Mean/SD.*	35.18	0.42
	95%Confidence interval*	(34.35, 36.01)	
Marital status	Single	32.35	197
	Married	67.65	412
Per capita monthly income ($)	0-301.2	13.46	82
	301.3-451.8	14.29	87
	451.9-753.0	32.35	197
	753.1-1054.2	20.69	126
	1054.3-1506.0	9.52	58
	1506.1-2259.0	4.76	29
	Above 2259.0	4.93	30
	Mean/SD.*	774.67	21.81
	95%Confidence interval*	(732.09, 818.99)	
Educational background	Primary or Junior high school	4.93	30
	Senior high or Special school	18.56	113
	Junior college or Undergraduate	62.73	382
	Postgraduate and above	13.79	84
Job	Students	8.21	50
	Peasantry	2.30	14
	Freelance	25.94	158
	Unemployed/retired	2.96	18
	Staffs of state-owned companies	11.99	73
	Staffs of foreign or private enterprises	13.30	81
	Party and government officers	15.60	95
	Education and scientific research units	9.36	57
	Else	10.34	63

Note: *The Bootstrap estimate was calculated as the mid-value of the range.

Table 3. Association of wine consumption prices with demographics.

Items-prices	Gender	Age	Marital status	Monthly income	Education	Occupation
Free-choice	0.004*	0.111	0.097	0.000***	0.095	0.001***
Gift-based	0.014*	0.021*	0.369	0.000***	0.002**	0.000***
Banquet-based	0.017*	0.000***	0.215	0.000***	0.010**	0.000***
Party-based	0.160	0.000***	0.012*	0.000***	0.136	0.000***
Self-drinking based	0.230	0.001***	0.024*	0.000***	0.110	0.000***

Note: *0.01<p<=0.05; ** 0.001<p<=0.01; *** p<=0.001.

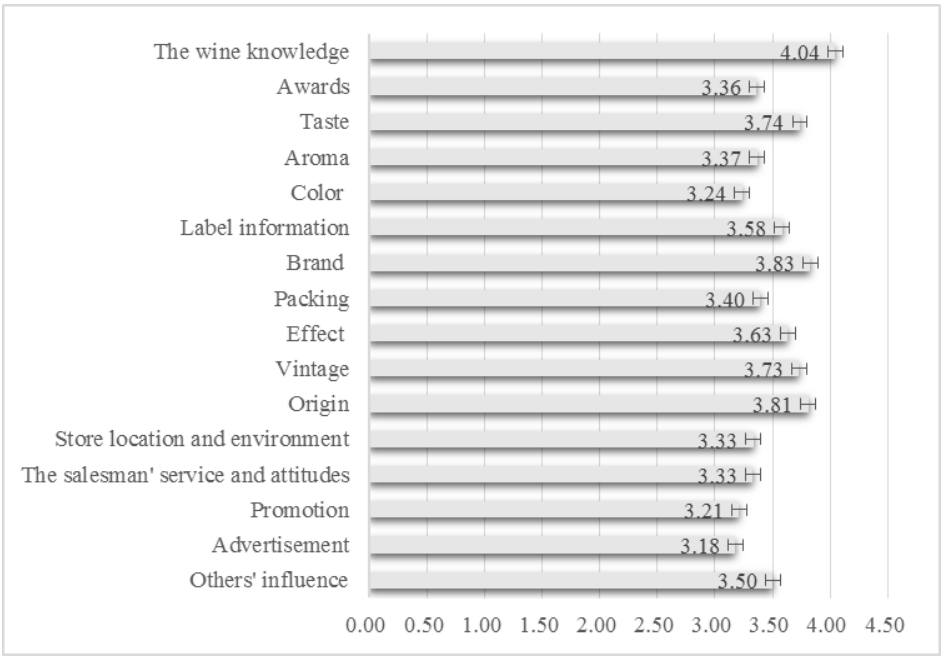
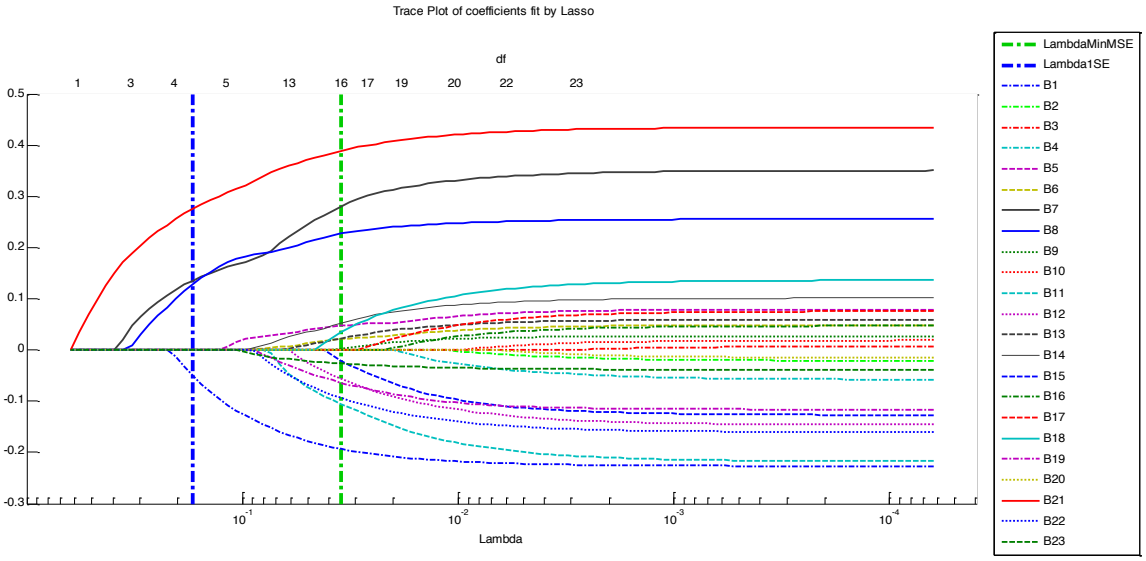


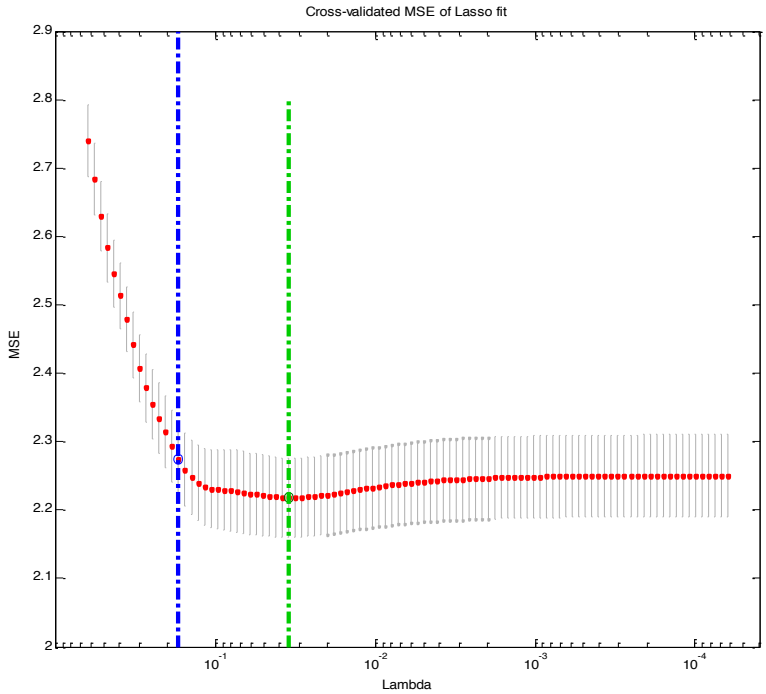
Figure 2. Statistical results of affecting factors.

The trace plot of coefficient through the Lasso method and the corresponding cross-validated MSE under free choice are depicted in Fig. 3, where different color lines represent various affecting factors (B1 to B23) in Fig. 3(a). The vertical bold blue and green dashed lines stand for the parameter under MinMSE and 1SE, respectively, and we only record the weights of factors under the minimum and 1SE in Table 4. From left to right in Fig. 3(a), the factors gradually shrink to zero, and the last factor that became zero represents the most important determinants. In the MinMSE position, 16 factors are selected. The factor selected through the Lasso method significantly affects the wine consumption behavior under free choice, which maintains a null hypothesis. In the index1SE position, only five factors are left; these factors are monthly income of consumers, the vintage, the origin of wine, purchasing motivation, and the service and attitudes of salesmen. We can achieve a prediction accuracy of 62.56% and 63.01% by using the first five important features for prediction in linear and nonlinear OVO Mv-TSVM, respectively. The algorithm relies considerably on the parameter, and the process of grid research is presented in Fig. 3(c).

(a)


Trace Plot of coefficients fit by Lasso
df
1 3 4 5 13 16 17 19 20 22 23
0.5 0.4 0.3 0.2 0.1 0 -0.1 -0.2 -0.3
10^-1 10^-2 10^-3 10^-4
Lambda
LambdaMinMSE
Lambda1SE
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 B13 B14 B15 B16 B17 B18 B19 B20 B21 B22 B23

(b)


Cross-validated MSE of Lasso fit
MSE
2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2
10^-1 10^-2 10^-3 10^-4
Lambda

(c)

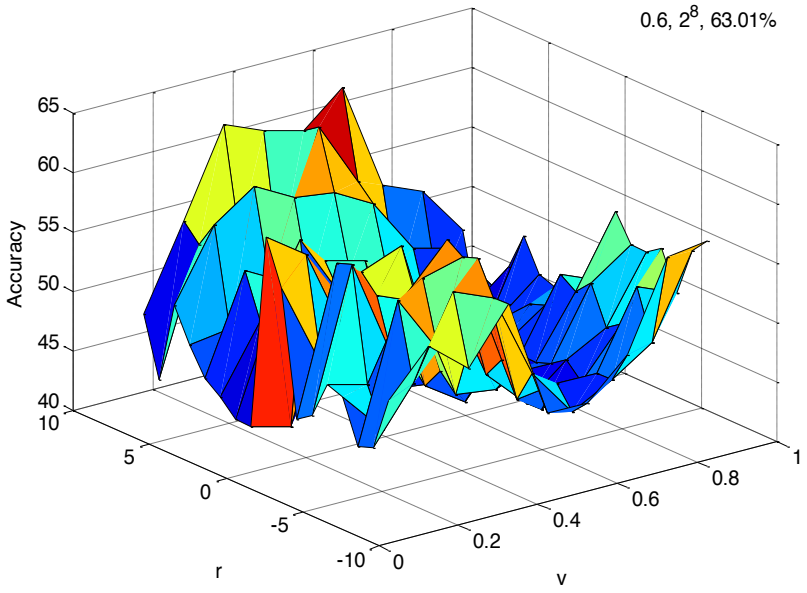


Figure 3. (a) The trace plot of coefficients by Lasso, (b) cross-validated MSE of Lasso fit under free-choice, (c) The influence of the parameter v, kernel parameter r and prediction accuracy for nonlinear OVO Mv-TSVM with free-choice.
#B1: Purchasing motivation, B2: Others' influence, B3: Advertisement, B4:Promotion, B5: Service and attitudes of salesman, B6:Store location and environment, B7: The origin of wine, B8: The vintage, B9:Effect, B10: Packing, B11:Brand, B12: Label information, B13: Color, B14: Aroma, B15: Taste, B16:Awards, B17: The wine knowledge, B18: Gender, B19: Age, B20: Marital status, B21:Monthly income, B22: Education, B23: Job.

Similarly, the parts of experimental results under four purposes, that is, gift, banquet, party, and self-drinking, are also summarized in Table 4. For the gift purpose, the first five important affecting factors are as follows: monthly income, occupation, wine knowledge of consumers, wine color, and production origin. For the banquet purpose, the first three determinants are monthly income, the origin of wine, the vintage. For the party purpose, the first five important affecting factors are as follows: monthly income, the origin of wine, wine knowledge of consumers, the vintage, and occupation. For the self-drinking purpose, the first five determinants are as follows: monthly income, the origin of wine, wine knowledge of consumers, occupation, and the vintage. The accuracy of nonlinear OVO Mv-TSVM can achieve 80.12%, 72.46%, 63.49%, and 74.69% by using the selected factors.

Table 4. Part of the Lasso results and prediction results using OVO M*v*-TSVM.

Metrics	Free-choice		Gift-based		Banquet-based		Party-based		Self-drinking based	
	Coef. Min λ	Coef Index1SE	Coef. Min λ	Coef Index1SE	Coef. Min λ	Coef Index1SE	Coef. Min λ	Coef Index1SE	Coef. Min λ	Coef Index1SE
Purchasing motivation	-0.227	0	0.067	0	0.050	0	0.078	0	0.064	0
Others' influence	-0.021	0	0.112	0	-0.017	0	-0.087	0	-0.134	0
Advertisement	0.007	0	-0.083	0	-0.119	0	-0.025	0	-0.100	0
Promotion	-0.058	0	0.008	0	-0.041	0	-0.102	0	-0.102	0
The salesman' service and attitudes	0.079	0	-0.105	0	0.005	0	0.002	0	0.015	0
Store location and environment	0.049	0	0.040	0	0.017	0	0.050	0	0.076	0
The origin of wine	0.350	0.014	0.098	0.014	0.195	0.070	0.174	0.088	0.231	0.140
The vintage	0.256	0	0.248	0	0.217	0.007	0.164	0.053	0.152	0.002
Effect	0.027	0	-0.043	0	0.049	0	0.092	0	0.045	0
Packing	0.019	0	-0.112	0	-0.022	0	-0.047	0	-0.035	0
Brand	-0.217	0	-0.111	0	-0.125	0	-0.132	0	-0.108	0
Label information	-0.146	0	-0.026	0	-0.009	0	-0.019	0	-0.015	0
Color	0.059	-0.059	-0.301	-0.059	-0.134	0	-0.058	0	0.036	0
Aroma	0.101	0	0.022	0	0.100	0	0.038	0	0.095	0
Taste	-0.127	0	0.070	0	0.016	0	-0.050	0	-0.093	0
Awards	0.047	0	-0.011	0	-0.006	0	0.065	0	-0.024	0.000
The wine knowledge	0.076	0.149	0.328	0.149	0.183	0	0.265	0.098	0.275	0.07
Gender	0.136	0	0.240	0	0.163	0	0.041	0	0.195	0
Age	-0.116	0	0.016	0	-0.020	0	-0.036	0	-0.026	0
Marital status	-0.015	0	0.025	0	-0.007	0	0.103	0	0.051	0
Monthly income	0.434	0.109	0.174	0.109	0.247	0.136	0.257	0.202	0.237	0.163
Education	-0.160	0	0.093	0	0.098	0	0.000	0	0.047	0
Occupation	-0.038	0.057	0.089	0.057	0.043	0	0.055	0.021	0.055	0.016
Intercept	2.172	3.578	1.918	3.578	1.041	3.248	0.897	2.049	0.692	2.240

df	23	5	23	5	23	3	23	5	23	5
MSE	2.250	2.573	2.542	2.573	2.035	2.068	1.938	1.938	2.233	2.249
SE	0.060	0.103	0.110	0.103	0.105	0.127	0.061	0.062	0.077	0.070
λ	6.26E-05	0.168	3.89E-05	0.168	4.62E-05	0.241	4.83E-05	0.131	4.51E-05	0.162
(I)Percentage correct prediction		62.56		79.22		65.22		49.21		61.45
(II)Percentage correct prediction		63.01		80.12		72.46		63.49		74.69

#I: Linear OVO Mv-TSVM; II: Nonlinear OVO Mv-TSVM.

4. **DISCUSSION**

This study provides a fresh perspective on the wine consumer segmentation based on importance ratings of factors by applying two popular machine learning methods. In addition, these segments are predictive in specific purchasing situations. The results provide valuable information for policymakers and marketing managers.

First, the consumers would pay a high wine price for gift giving, and a relatively low wine price for self-drinking. The importance rankings of determinants in wine consumption vary under different purchasing situations. These rankings will guide a salesperson in utilizing the key points when recommending wine products to customers. For example, a salesperson should primarily realize the monthly income, occupation, degree of wine knowledge of a consumer, wine color, the origin of wine, if the wine is purchased as a gift. The OVO Mv-TSVM can aid in predicting the type of consumers when the information is acquired. The retailer or salesman can then recommend wine with the corresponding price. If the purchasing motivation is for a banquet, then the retailer should primarily focus on the income of the consumer, the origin of wine, and the vintage. This result is our most important discovery. Furthermore, using the Lasso method can reduce the complexity before prediction because the relevant features can be selected, whereas the irrelevant features become zero under a fixed value.

Second, the nonlinear OVO Mv-TSVM behaves better than the linear OVO Mv-TSVM. These results indicate that the data we collected are linearly inseparable, thereby leading to poor testing accuracy with linear models. The kernel trick in the OVO Mv-TSVM maps the data into a high-dimensional space, hence successfully making the data linear separable in the projected space (SHAWE-TAYLOR and CRISTIANINI, 2004). Accordingly, the nonlinear model is suggested in wine consumption prediction area. Under the free choice, the nonlinear OVO Mv-TSVM can only achieve 63.01%, whereas this strategy obtains high-prediction accuracies under the four purchasing situations. The pre-knowledge of the purchasing situation of a consumer would help marketers provide an accurate recommendation to wine consumers. In terms of prediction accuracy, the case of purchasing under the gift purpose can explain the purchase behavior because its accuracy achieves 80.12%, followed by self-drinking, banquet, and party. In addition, the prediction accuracy of the OVO Mv-TSVM is significantly affected by the choice of parameters. The prediction accuracy must select the appropriate parameters beforehand in practical applications.

Third, most personal traits are the important determinants of wine consumption (PICKERING and HAYES, 2017). The monthly income and occupation of consumers are the leading positive influential factors in wine consumption regardless of the purchasing conditions, hence implying that consumers with high monthly income and a favorable job prefer wine with a high price. Occupational difference reflects the social status of consumers to a certain extent, LIU and MURPHY (2007) found that wine was seen as a symbol of one's social status and sophistication. Gender positively influence the high priced wine purchasing, especially in free choice, gift-giving and banquet purpose. Male consumer would prefer higher-priced of wine than female consumer. Education positively influences the high priced wine purchasing when the purposes are gift and banquet, whereas education is an insignificant factor for party. Age of consumers is found to be sensitive and negative to wine purchasing for banquet, party, and self-drinking purpose, thereby implying that young Chinese consumers prefer higher priced wine than elder persons who are

more thrifty. Marital status is not a leading factor for wine purchasing, while it emerges as a relatively significant factor for party-based wine purchasing.
Fourth, the knowledge of consumers about wine is a major positive driver for wine consumption (BARBER *et al*., 2008). In consumption decision-making, the consumers rely on their professional knowledge, especially on their subjective knowledge. Consumers will behave confidently and rely on their judgment when these consumers assume that they have professional knowledge, and vice versa. These results imply that consumers will spend more money on wine if they absorbed the wine culture.
Fifth, the vintage and origin of wine are found to be the more important determinants for prediction than wine taste and awards. The weights of the vintage and oration of wine are positive, which means consumers would pay more if they value the wine vintage and origin. For banquet, wine taste and aroma have a positive effect on wine price selection, which indicates that consumers would pay more money to receive a favorable quality of the wine. Wine color and packing are important factors when consumers purchase wine as a gift, whereas these factors are insignificant for self-drinking, which provides valuable suggestions for wine sellers and producers to sell or produce wine. For party, banquet, and self-drinking, the effect of wine emerges as a significant positive factor in high-priced wine selection. This result provides a hint to the winemakers to produce banquet and self-drinking wine with favorable efficacy to attract consumers, thus increasing the returns. Consumers attach wine aroma when purchasing wine for self-drinking and banquet compared with the purposes of gift and party. Furthermore, consumers are non-sensitive to wine brand and label information. Our findings are meaningful for and can be implemented by winemakers and suppliers to formulate appropriate strategies in accordance with the preference and purchasing purpose of consumers.
Sixth, this study demonstrates that purchasing motivation is a positive driver for wine consumption. Previous studies have shown that people trust their families, friends, colleagues, or acquaintances; recommendations of other people have a significant influence on the purchasing behavior of consumers (GOODMAN, 2009). Moreover, consumers are especially sensitive to opinions of other people when these consumers purchase wine for self-drinking and party, and the effect is negative to high-priced wine selection. By contrast, the influence is positive for gifts. The results suggest that wine dealers can invite wine critics to recommend gifting wine on TV or take measures for expanding the influence of friends.
Lastly, advertisement is a driver for purchasing wine regardless of the purpose, and the influence is negative for high-priced wine, which warns the wine dealers to invest reasonably in advertisements because excessive advertisement expenditure can conversely affect the sale of wine. The promotion activities of enterprises can inspire the latent purchasing behavior of consumers (POHJANHEIMO *et al*., 2010). Consumers are more influenced by the service and attitude of the salesperson than the promotion, store location, and environment when these consumers purchase wine as a gift. Managers are encouraged to improve the service and skills of their sales personnel to improve their sales of wine as a gift. Promotion becomes a significant factor when consumers purchase wine for banquet, party, or self-drinking, and the influence is negative because promotions result in an increased price of wine, thereby reminding managers to create suitable promotional activities. The store location and environment emerge as a driver when consumers purchase wine for self-drinking, and the influence is positive for selling high-priced wine, thereby indicating that consumers would pay a relatively high price for self-drinking if the store location is near their home or the environment is comfortable and clean.

5. CONCLUSION AND FUTURE WORK

The present study explored 23 factors that are associated with wine consumption under four specific purchasing situations based on a surveyed sample of the Chinese population. The Lasso method was used as a primary empirical tool for selecting the most affecting features, and the OVO Mv-TSVM method was used to predict the purchasing behavior of consumers. The findings can be applied in various commercial fields. Limitations should be noted that may aid in drawing avenues for future research, although our study exhibits interesting results in terms of using the machine learning methods.

First, the samples collected in the study were from 21 provinces in Central and Eastern China. Although the respondents who completed the survey were rewarded with a monetary deposit into their Alipay and WeChat accounts as an incentive, the recovery rate of the questionnaire is not high enough. Owing to the limited time and resources, we were unable to collect data from Western and Southwestern China, such as Xinjiang, Xizang, and Yunnan Provinces. The samples used in this study might not be representative of the whole county.

Second, information on consumer perception for a specific wine product, such as claret, white wine, or sweet red wine can be collected. CULBERT *et al*. (2017) investigated the sensory profiles and consumer acceptance of different styles of Australian Moscato and led us to focus on the determinants of a specific wine product in China. This area helped us predict the purchasing behavior of Chinese consumers, adjust models, and provide useful suggestions for marketers and companies to create reasonable and timely adjustments.

Third, the list of factors in this study was not intended to be exhaustive, and other factors could be incorporated. For example, we only set one item to investigate the knowledge of consumers toward wine consumption. To the best of our knowledge, familiarity with wine involves various aspects, such as grape varieties, viticulture, wine process, wine tasting, and wine storage management. Further, the questions on the consumption of wine and human health (TAMBURRO *et al*., 2017) can be considered in further research.

The emergence of machine learning methods leads to new research topics on wine consumption. The other multi-classification methods, such as directed acyclic graph (TOMAR and AGARWAL, 2015), can also yield favorable performance. Accordingly, future research may examine the method that performs optimally in the wine consumption area. The prospect for wine in China is promising, and we assume that future research will contribute to the existing literature with new and interesting findings.

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PAPER

ANGIOTENSIN I CONVERTING ENZYME INHIBITORY PEPTIDES FROM SWORD BEAN

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ABSTRACT

Sword bean is a healthy food and herbal medicine in China. In this study, the main components of sword bean were determined. Albumin, globulin, prolamin and glutelin were hydrolyzed by pepsin and then the angiotensin I converting enzyme (ACE) inhibitory activity was evaluated. Our results showed that glutelin peptides manifested the highest ACE inhibitory activity with inhibitory ratio of 22.10±1.57% followed by prolamin peptides and albumin peptides of 16.77±0.76% and 16.40±0.42%, respectively, at the final concentration of 0.01 mg/mL. Our results strongly suggest that sword bean at some extant have potential to lower blood pressure.

Keywords: sword bean, main component, angiotensin I converting enzyme, peptides

1. INTRODUCTION

Hypertension, a major risk factor for cardiovascular and renal diseases, has become the most common serious chronic health problem. The rennin-angiotensin system (RAS) is critically involved in the physiological regulation of blood pressure and pathogenesis of hypertension (CAT and TOUYZ, 2011). ACE, as an essential member of RAS, can catalyzes the conversion of angiotensin (ANG) I to ANG II by removing a carboxyterminal dipeptide (WYSOCKI *et al*., 2006). Meanwhile, ACE metabolizes bradykinin (BK), a vasodilator, to inactive BK-(1-7). Therefore, ACE inhibitors are effective first-line treatment against essential hypertension (THOMAS *et al*., 2004), such as captopril, enalapril and lisinopril. However, these synthetic drugs may also cause obvious side effects including cough, loss to taste, renal impairment, and angioneurotic oedema (ANTONIOS *et al*., 1995). Thus, peptides with potent ACE inhibitory activity derived from natural food provide an effectively alternative treatment (YU *et al*., 2006). In recent years, ACE inhibitory peptides from natural protein have been successfully isolated, such as corn (YANG *et al*., 2007), soybean (MALLIKARJUN *et al*., 2006) and Coix seed (YUAN *et al*., 2014). Recently, the antihypertensive peptides from traditional Chinese medicine proteins has drawn considerable attention.

Sword bean, the seed of the leguminous plant *Canavalia gladiate*, also has been treated as traditional medicine for containing canavanine, hemagglutinin, and concanavalin A (EKANAYAKE *et al*., 2006). It has been reported that sword bean may exhibit antioxidant activity of eliminating free radicals and against oxidative stress. In addition, it also has strong anti-inflammatory and anticarcinogenic effects. It is reported that soybean paste containing sword bean exhibits higher ACE inhibitory effects than other soybean pastes (HAN *et al*., 2015). In this study, this medicinal food was chose to prepare the ACE inhibitory peptides because of its ACE inhibitory activity and rich protein.

The aims of this study are: (1) to determine the main components and protein content of sword bean. (2) to obtain peptides with low molecular weight (≤ 3 KD) by hydrolyzing protein with pepsin, and estimate their ACE inhibitory activity. (3) to provide some reference for the clinical drug use of sword bean in traditional Chinese medicine.

2. MATERIALS AND METHODS

2.1. Material

Sword bean was purchased from Tongrentang (Beijing, China). The voucher specimen (No. 131121003) was deposited at -20°C. Pepsin, ACE and hippuryl-L-histidyl-L-leucine (HHL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trifluoroacetic acid (TFA, MS grade) and Acetonitrile (HPLC grade) were purchased from Merck KGaA (Darmstadt, Germany) and Fisher Scientific (Pittsburgh, PA, USA) respectively. All other chemicals and reagents were analytical grade.

2.2. Determination of the proximal compositions

Protein, fat, moisture and ash content of sword bean were determined according to the Chinese pharmacopoeia (Commission, 2015). The content of starch was determined by Ji (JI *et al*., 2016).

2.3. Sequential extraction of seed protein

The seeds were ground into powder by a universal high-speed smashing machine, and then defatted with cooled petroleum ether and dried at 40°C overnight. Albumin, globulin, prolamin and glutelin were then sequentially extracted with deionized water, 0.5 M NaCl, 70% ethanol (containing 0.5% NaAC, 5% β-mercaptoethanol) and 0.0125 M sodium borate buffer (containing 1% SDS, 2% β-mercaptoethanol), respectively. All of the protein solutions were dialysised against deionized water at 4°C for 24 h and then freeze-dried. The samples were stored at -80°C for further analysis.

2.4. Determination of protein molecular weight distribution

SDS-PAGE was conducted according to the method of Krizkova (KRIZKOVA *et al*., 2015) with some modifications to determine the molecular weight distribution of all the protein fractions. All the samples were run for approximately 100 min in 3% stacking gel with a electric current of 10 mA and then for another 100 min in 15% separating gel with 30 mA. After that, the gel was dyed with Coomassie brilliant blue overnight and then decolored with bleaching liquid until the strips were seen clearly.

2.5. Determination of the Amino acid content

For determination of amino acid composition, 100 mg samples were subjected to acid hydrolysis with 20 mL of 6 M HCl at 110°C for 24 h. Then the lyophilized hydrosate was dissolved in 0.02 M HCl and analyzed by a amino acid analyzer (L-8900; Hitachi, Tokyo, Japan) (WANG *et al*., 2008).

2.6. Preparation of enzymatic hydrolysates

To produce bioactive peptides, enzymatic hydrolysis method was applied. The protein (2%, w/v) was dissolved in 0.01 M HCl, and pepsin was added with enzyme/substrate ratio of 1/10 (w/w). The mixture was incubated at the temperature of 37°C for 48 h. To terminate the reaction, the mixture was heated 95°C for 5 min. The hydrolysates supernatant was collected after the centrifugation (at 10,000 rpm, 10 min, 4°C).

2.7. Ultrafiltration (UF) of protein hydrolysates

To produce low molecular weight peptides, the hydrolysates were passed through ultrafiltration membrane(MWCO, 3 KD). The peptide concentration of each collected fractions was estimated by the Lowry method (SAPAN, and LUNDBLAD, 2015).

2.8. The assay of ACE inhibitory activity

The ACE inhibitory activity was determined according to the method reported by Yuan (YUAN *et al*., 2014). Briefly, the reaction system contained 10 μL sample, 20 μL ACE (2 mU) and 20 μL HHL (2 mM). Sample and ACE were incubated at 37°C for 10 min prior to adding substrate HHL, and then for an additional incubation for 80 min at the same temperature. To terminate the reaction, 100 μL acetonitrile was added. Captopril and borate buffer solution was used as positive and blank control, respectively. ACE inhibitory activity was confirmed by monitoring the formation of HA which was generated by HHL under enzymatic hydrolysis. HA was detected by RP-HPLC on a C_{18} column (250 × 4.6 mm, 5 μm, Tianhe). The column was eluted by a mobile phase of acetonitrile/water (0.05%

TFA) at a volume ratio of 25 : 75 with the flow rate of 1 mL/min. The elution was monitored at 228 nm. The ACE inhibitory ratio of each sample was calculated as follows:

$$\text{Inhibitory activity (\%)} = [\,(A-B)/A] \times 100\%$$

where A was the HA peak area of blank control; B was the HA peak area in the presence of the sample.

2.9. Statistical analysis

All data were conducted in triplicate and expressed as the mean±SD. The SAS 9.3 program was used for Multiple comparison, and $P < 0.05$ were considered to be significant.

3. RESULTS

3.1. Proximal compositions of sword bean

The proximal compositions of sword bean were presented in Table 1. The starch content of sword bean ranked first (36.59±2.93%). As the member of Leguminosae, the protein content of sword bean accounted for 31.95±0.24%. The moisture and ash contents of this medicinal food all conformed to the requirements of the Chinese pharmacopoeia.

Table 1. Proximal compositions (%) of sword bean.

	Protein	Crude fat	Moisture	Ash	Starch
Sword bean	31.95 (±0.24)	0.71 (±0.04)	8.33 (±0.01)	3.16 (±0.04)	36.59 (±2.93)

Results were expressed as the mean±SD (n = 3).

3.2. Protein fractions distribution of sword bean

Protein patterns of sword bean were shown in Table 2. Considerable variability among albumin, globulin, prolamin and glutelin was observed. Albumin had the highest percentage of 70.93±0.25% followed by globulin of 16.75±0.51%. The prolamin and glutelin contents of this leguminous seeds were rather low. Insoluble protein in the residue only accounted for 5.83±0.04% indicating effective extraction of protein.

Table 2. Protein pattern of sword bean (% of total protein).

	Albumin	Globulin	prolamin	Glutelin	Residual
Sword bean	70.93^{a} (±0.25)	16.75^{b} (±0.51)	1.48^{e} (±0.02)	7.02^{c} (±0.26)	5.83^{d} (±0.04)

Results were expressed as the mean±SD (n = 3). Different letters of indicated having significantly different ($P < 0.05$).

3.3. SDS-PAGE pattern of protein fractions of sword bean

The molecular weight (MW) distributions of different protein fractions for sword bean were detected by SDS-PAGE, which was shown in Fig. 1. Albumin and globulin resolved into similar subunits ranging from 97 KD to 19 KD, with the major subunit of 50 KD. The bands of prolamin and glutelin were heterogeneous ranging from 57 to 14.4 KD and 97 to 19 KD, respectively.

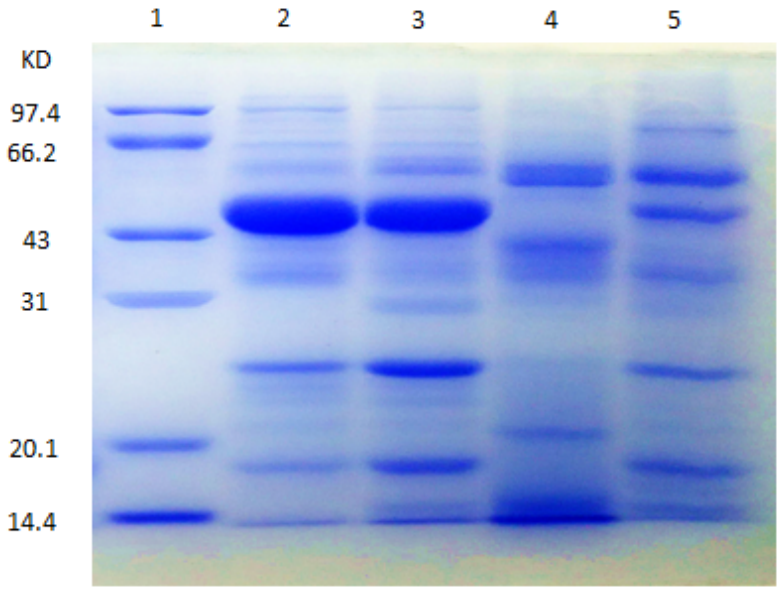


Figure 1. The molecular weight distribution of proteins extracted from sword bean.
Lane 1, marker; Lane 2, albumin; Lane 3, globulin; Lane 4, prolamin; Lane 5, glutelin.

3.4. Amino acid composition of seeds flour

For sword bean, a total of 13 kinds of amino acid were detected. Including almost all the essential amino acids and semi-essential amino acids for human beings. From Table 3, it could be seen that Phe had the highest percentage of 4.55±0.11 mg/100 mg, while His recorded the lowest (0.50±0.01 mg/100 mg) in sword bean.

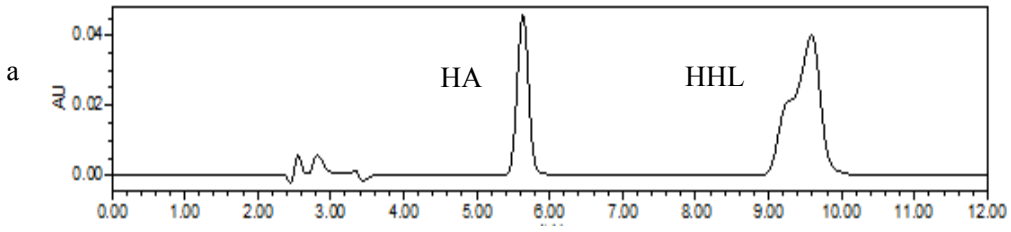
3.5. ACE inhibitory activity assay

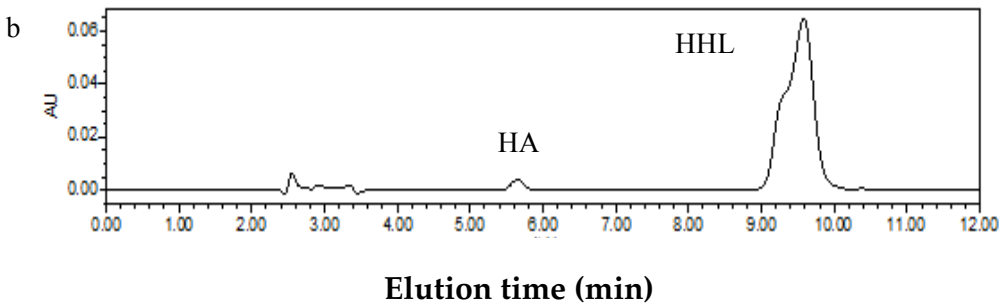
The RP-HPLC method was utilized to estimate ACE inhibitory activity of peptide mixtures (≤ 3 KD). The blank control displayed a strong peak area of HA (Fig. 2a), while the positive control (captopril, final concentration of 2×10^{-9} mol/L) manifested a strong ACE inhibition ratio of 91.64±0.07% (Fig. 2b). The glutelin peptides (≤ 3 KD) revealed the highest ACE inhibitory activity at the final concentration of 0.01 mg/mL with 22.10±1.57 (Fig. 2c). All results were showed in Table 4.

Table 3. The amino acid content of sword bean (mg/100 mg).

Amino acid	Sword bean (mg/100 mg)
Asp	2.84±0.03
Thr[a]	1.32±0.03
Ser	1.49±0.02
Glu	3.26±0.07
Gly	0.88±0.02
Ala	0.68±0.01
Cys	-
Val[a]	2.74±0.06
Met[a]	-
Ile[a]	1.07±0.02
Leu[a]	1.69±0.05
Tyr	-
Phe[a]	4.55±0.11
Lys[a]	1.47±0.02
His[a]	0.50±0.01
Trp[a]	-
Arg[a]	1.56±0.02
Pro	-

[a](semi-) essential amino acid for human, - not detected. The data was expressed as the mean±SD (n = 3).





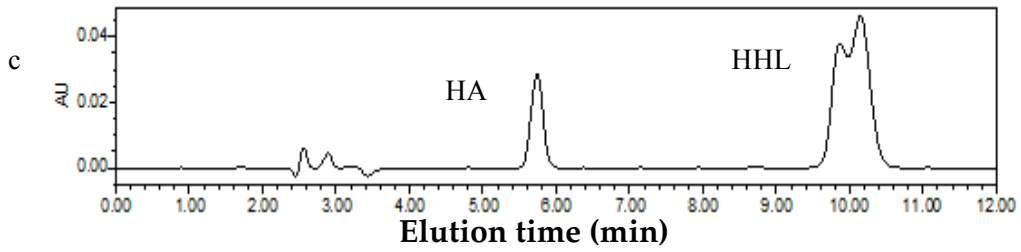


Figure 2. RP-HPLC chromatograms of (a) blank control, (b) positive control (captopril, final concentration of 2×10^{-9} mol/L), (c) glutelin peptides (≤ 3 KD) of 0.01 mg/mL. The mobile phase consisted of 25% acetonitrile (containing 0.05% TFA), eluting at a flow rate of 1.0 mL/min and the absorbance of eluent was detected at 228 nm.

Table 4. ACE inhibition rate (%) of peptides (≤ 3 KD) from different protein fractions.

Peptide	ACE inhibition rate (%)
Albumin peptides	16.40±0.42^{b}
Globulin peptides	12.72±0.29^{c}
Prolamin peptides	16.77±0.76^{b}
Glutelin peptides	22.10±1.57^{a}

Results were expressed as the mean±SD (n = 3). The samples were measured at the final concentration of 0.01 mg/mL. Different letters of indicated having significantly different ($P < 0.05$).

4. DISCUSSION

Food-derived ACE inhibitory peptides can provide an effectively alternative treatment for hypertension. There are different methods to produce ACE inhitory peptides from precursor proteins, such as enzymatic hydrolysis (CHEN *et al*., 2007), microbial fermentation (YAMAMOTO *et al*., 1994) and chemical synthesis. Among these methods, enzymatic hydrolysis is the most commonly used method (YUAN *et al*., 2014). There are a great number of studies have proved that food-derived protein hydrolysates and peptides possess ACE inhibitory activity (BALTI *et al*., 2010; LASSISSI *et al*., 2014; LEE *et al*., 2010). Soybean paste containing sword beans exhibits higher ACE inhibitory effects (HAN *et al*., 2015). Research has shown that the presence of hydrophobic amino acids can increase ACE inhibitory activity (LI *et al*., 2004). Our study showed that sword bean included all the essential amino acids (except Met) and semi-essential amino acids for human beings. Moreover, sword bean protein may become effective sources for preparation of ACE inhibitory peptides because of its high proportion of hydrophobic amino acid and proline, with 44.58% of total amino acid. Albumin, globulin, prolamin and glutelin were sequentially extracted, which is of benefit to study different proteins activity. The high levels of protein and starch make sword bean good sources of these nutrients.

5. CONCLUSION

Our study mainly focused on the ACE inhibitory activity of protein hydrolysates. The result showed that glutelin peptides manifested the highest ACE inhibitory activity with

inhibitory ratio of 22.10±1.57% followed by prolamin peptides and albumin peptides of 16.77±0.76% and 16.40±0.42%, respectively, at the final concentration of 0.01 mg/mL. After further separation, purification and structural identification of hydrolysates (≤ 3 KD), bioactive peptides with better antihypertensive activity might be obtained. Our data might contribute to further research into food derived antihypertensive compounds, meanwhile it also provides some reference for the clinical drug use of sword bean in traditional Chinese medicine.

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SHORT COMMUNICATION

EFFECTS OF REGULAR AND MODIFIED POTATO AND CORN STARCHES ON FRANKFURTER TYPE PRODUCTS PREPARED WITH VEGETABLE OIL

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ABSTRACT

The effects of regular and modified potato and corn starches on improving meat batters prepared with 20% canola oil were investigated. Five of the six modified starches significantly reduced cooking loss as compared to control. The native corn did not, and its microstructure revealed incomplete gelatinization during cooking. One of the modified corn starches was totally incompatible with the meat matrix, resulting in low hardness, yield and incoherent microstructure. Fat loss was low, but reduced/eliminated by starch. Color was slightly affected by starch, but no major trend was observed. Overall, processors should carefully evaluate the functionality of starch they employ.

Keywords: frankfurters, meat, microstructure, starch, texture

1. INTRODUCTION

Meat processors use different non-meat ingredients for reasons such as improving water/fat binding, enhancing texture, sliceability, flavor, appearance, and controlling cost (BARANOWSKA *et al*., 2004; BREWER, 2012). The functional non-meat ingredients range from proteins (e.g., soy, milk) to carbohydrates (e.g., wheat flour, carrageenan), and spices (BARBUT, 2015). Starches, derived from various sources (e.g., corn, potato, tapioca), represent one of the most diverse groups of non-meat ingredients used to bind water, enhance freeze-thaw stability, and improve sliceability. In general, starch is a naturally occurring substance which is also used as a thickening agent in sauces, yogurt, and emulsion type products (BARANOWSKA *et al*., 2004). Different starches are available for industrial application in their natural form or as modified starches; the former type usually have more limited use in meat products. Overall, starch functionality may vary due to different botanical origins (e.g., potato, corn, tapioca, rice, pea) and modifications (e.g., enzyme, acid, heat) applied by the food industry (AKTAŞ and GENÇCELEP, 2006). Some meat products such as fish surimi have traditionally been produced with starch to increase firmness and water binding especially in such a highly minced, high water added product (VERREZ-BAGNIS *et al*., 1993; FOGAÇA *et al*., 2013). In other red meat/poultry products, starches are used to enhance gel strength and water holding, to replace fat, and control formulation cost (LI and YEH, 2003a, b; BREWER, 2012). DEXTER *et al*. (1993) reported that starch added to turkey bologna was very effective in decreasing purge while not increasing hardness. They also reported that starch effect depended on the type of starch used, water-to-starch ratio, processing factors, and presence of ingredients such as fat. Modified potato starch was reported to improve the texture of low fat bologna (CLAUS and HUNT, 1991) and comminuted scaled sausage (PIETRASIK, 1999). CARBALLO *et al*. (1995) indicated that adding starch to meat emulsions resulted in more compact and stronger heat induced meat protein network. RESCONI *et al*. (2015) reported that using rice starch (0.3-1.5%) helped increase yield and hardness of whole muscle cooked hams.

Replacing animal fat with vegetable oil is another significant trend seen today, and additives such as starch are important in stabilizing the high moisture added to reduced fat meat products (BREWER, 2012; BARBUT *et al*., 2016). Currently there are some starch suppliers that claim that their products can also enhance fat stabilization in meat products. Therefore, the objectives of this study were to compare the effects of potato and corn starches in their native and modified forms (total 8 starches) on cooking losses (moisture and fat), texture, microstructure, and color of emulsified meat products prepared with vegetable oil.

2. MATERIALS AND METHODS

2.1. Preparation of meat batters

Lean shoulder blade beef meat was obtained from the University of Guelph abattoir. All visible connective tissue and fat were removed from the lean meat. Meat was comminuted in a bowl chopper (SMK 40; Schneidmeister, Berlin, Germany) at the low speed setting for 1 min to obtain a homogenous mass. The meat (73.6% moisture, 19.6% protein, and 5.9% fat. AOAC, 1996) was vacuum-packed and frozen (-18ºC) in polyethylene bags (750g/package) for up to 1 month prior to use. Nine different formulas were prepared in 3 independent trials. The starches used include native potato (NP) starch (Herman Laue Spice Co., Uxbridge, ON, Canada), modified potato starch-1 (MP-1, PenCling 530; Penford

Food Ingredients Co., Denver, CO, USA), modified potato starch-2 (MP-2, Farinex VA 15; Avebe Foods, Veendam, Netherlands), modified potato starch-3 (MP 3, Eliane VE 420; Avebe Foods, Veendam, Netherlands), native corn starch (NC, Amioca; National Starch, Westchester, IL, USA), modified corn starch-1 (MC-1, Em-Cap; Cargill Inc., Minneapolis, MN, USA), modified corn starch-2 (MC-2, PenCling 570; Penford Food Ingredients Co., Denver, CO, USA), modified corn starch-3 (MC-3, Firm-Tex; National Starch, Westchester, IL, USA), and a control with no starch (CONT). Meat was thawed at 5ºC overnight. Pure canola oil (No Name®; Sunfresh Limited, Toronto, ON, Canada), was used as the main fat source. The batters were formulated to contain 25% fat/oil (5.9% as beef fat within the lean meat, and the rest added as canola oil), 13.5% protein and 2% starch in all treatments except no starch in the control. Meat was initially chopped at the low speed setting for 30 s. Later, while chopping at the high speed setting for 30 s, 2.0% salt and 0.25% sodium tripolyphosphate were added. This was followed by a 2 min break (for protein extraction to occur). Next, canola oil and the appropriate starch (prepared by dissolving the starch in the 2% water added to the product) were added to the batter while chopping at the high speed setting for 1 min, followed by ice addition and further chopping for 4 min. The temperature of the batter did not exceed 12ºC in any of the treatments. Each batter was vacuum-packed (Multivac model A300/16; Wolfertschwenden, Germany) to remove trapped air. For each batter, 35g samples were stuffed into three separate 50 ml polypropylene tubes, which were centrifuged (Model 224; Fisher Scientific, Pittsburgh, PA, USA) at the low speed setting for 30 s to remove any remaining small air bubbles. The batters were cooked in a water-bath (W-26; Haake, Berlin, Germany) from 25 to 70ºC within 1.5 h. A thermocouple unit was used to monitor the core temperature of the samples (Model 52 KJ1, Fluke, Everett, WA, USA).

2.2. Cooking loss

Test tubes were cooled in ice water for 5 min, and then liquid separated during the cooking cycle was collected and expressed as % cooking loss (liquid expelled (g)/raw batter weight (g) x 100). The next day the volume of the fat (floated to the top overnight) separating out was determined and expressed as fat loss.

2.3. Texture profile analysis (TPA)

After an overnight storage (5°C), TPA parameters were determined using nine cooked cores (each 16 mm diameter and 10 mm high) per treatment. Cores were compressed twice to 75% of their original height by a texture analyzer (Stable Micro Systems, Model TA.XT2; Texture Technologies Corp., Scarsdale, NY, USA) at a crosshead speed of 1.5 mm/s. The following parameters were determined: hardness, springiness, cohesiveness, chewiness, and gumminess (ROSENTHAL, 2010).

2.4. Color

The color of fresh cut cross-sections of the cooked meat batters (9 per treatment) was determined (Mini Scan MS/S; Hunter Laboratories, Reston, VA, USA) using the D65 illuminant setting, and 10-degree standard observer. Color is expressed according to the Commission International de l'Eclairage (CIE) system and reported as Hunter L* (lightness), a* (redness), and b* (yellowness) (WIEGAND and WALOSZEK, 2003).

2.5. Microstructure

Samples (2.0×2.0×0.5 cm) were cut from the centers of cooked meat batters, fixed in 10% neutral buffered formalin for 10 h at room temperature, dehydrated in 70% isopropanol for 2 h, 95% for 1 h, and 100% for 4 h and embedded in paraffin. Samples were cut into 4-6 μm sections, stained with hematoxylin-eosin for 4 min, and observed using a light microscope (Model BX60F5; Olympus Optical Company, Tokyo, Japan). Black and white pictures were taken (Image-Pro Plus, Version 5.1; Media Cybernetics Inc., Silver Spring, MD, USA).

2.6. Statistical analysis

The experiment was designed as a complete randomized block, with three separate replications. Statistical analysis was performed using a software package (SAS version 8.02; SAS Institute, Cary, NC, USA). The SAS General Linear Model procedure was used for analysis of variance. Tukey's multiple comparison analysis was performed to separate the means ($p < 0.05$).

3. RESULTS AND DISCUSSION

The addition of all four potato starches resulted in a significant reduction in cooking loss compared to the control (Table 1).

Table 1. Effects of native and modified starches on overall cooking loss, fat loss, moisture loss and color parameters (L*=lightness, a*=redness, b*=yellowness) of meat batters prepared with canola oil.

Treatment* (#)	Cooking loss (%)	Fat loss (%)	Moisture loss (%)	Color coordinates (Lightness)	(Redness)	(Yellowness)
1 CONT	2.26±0.29^{b}	0.57±0.18^{a}	1.69±0.26^{b}	65.4±0.30^{b}	3.60±0.03^{c}	13.2±0.08^{b}
2 NP	0.67±0.09^{d}	0.14±0.05^{b}	0.53±0.10^{c}	63.9±0.16^{c}	3.93±0.03^{a}	13.3±0.05^{ab}
3 MP-1	0.44±0.12^{d}	0.12±0.06^{b}	0.32±0.06^{c}	62.9±0.28^{d}	4.04±0.03^{a}	13.2±0.09^{b}
4 MP-2	0.19±0.07^{d}	0.00±0.00^{b}	0.19±0.07^{c}	63.1±0.22^{d}	3.91±0.04^{a}	13.2±0.07^{b}
5 MP-3	0.29±0.07^{d}	0.00±0.00^{b}	0.29±0.07^{c}	62.4±0.27^{d}	4.00±0.03^{a}	13.2±0.09^{b}
6 NC	1.86±0.20^{bc}	0.19±0.09^{b}	1.67±0.11^{b}	65.0±0.27^{b}	3.69±0.04^{bc}	13.3±0.10^{ab}
7 MC-1	5.89±0.44^{a}	0.00±0.00^{b}	5.89±0.44^{a}	66.5±0.34^{a}	3.35±0.05^{d}	13.5±0.11^{a}
8 MC-2	1.10±0.68^{cd}	0.00±0.00^{b}	1.10±0.68^{bc}	62.4±0.29^{d}	4.04±0.05^{a}	13.2±0.10^{b}
9 MC-3	0.25±0.06^{d}	0.06±0.04^{b}	0.19±0.03^{c}	63.1±0.26^{d}	3.78±0.02^{b}	13.1±0.08^{b}

$^{a-d}$means, ± standard error, with no common superscript are significantly different ($p < 0.05$).
*all formulated with 2.0% salt and 0.25% sodium tri-polyphosphate. CONT = Control; NP = Native Potato starch; MP = Modified Potato starch; NC = Native Corn starch, MC = Modified Corn starch.

This can be correlated to starch molecules opening up, during heating, and absorbing moisture; a process known as gelatinization (BARANOWSKA *et al*., 2004). In the case of the added corn starches (Treatments 6-9), the native corn starch did not show a significant improvement over the control. Examination of the micrographs shows that this treatment did not reach the gelatinization point (i.e., granules shown as compact non-open structures; Fig. 1F).

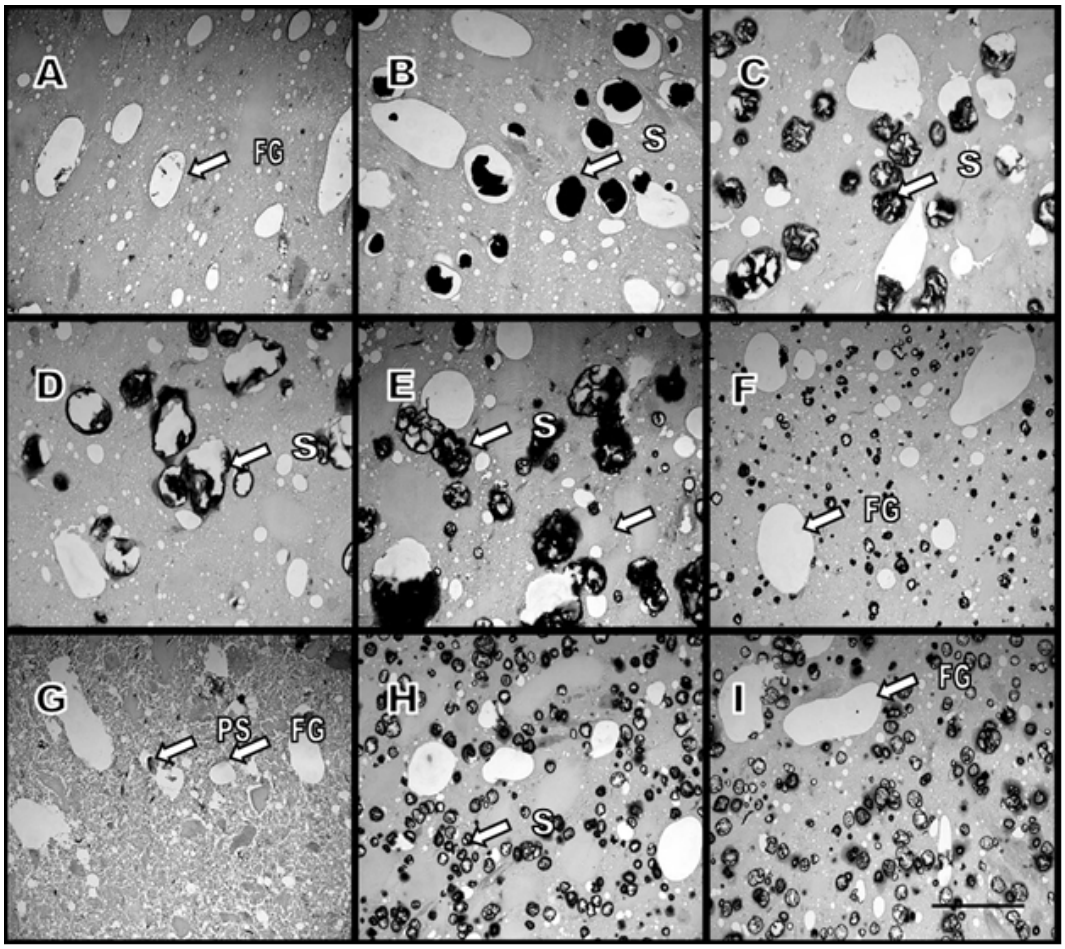

Figure 1. Light micrograph of cooked meat batters (13.5% protein) prepared with canola oil and different starches: (A) control; (B) native potato starch (C) modified potato-1 (MP-1); (D) MP-2; (E) MP-3; (F) native corn; (G) modified corn-1 (MC-1); (H) MC-2; and (I) MC-3. FG-fat globules (i.e., fat removed during sample preparation; prior to paraffin embedding); S-starch; PS-probably starch. Bar = 200μm.

This is the reason that quite a few of the starches used by the meat industry are pre-gelatinized, meaning that they are pre-exposed to a certain heat treatment; i.e., to open or partially open their structure and make them capable of absorbing water even at a low temperature (BARANOWSKA *et al*., 2004; RESCONI *et al*., 2015). A certain degree of unopened structures is also seen in the MC-2 treatment (Fig. 1H) where a number of very dense non-gelatinized starch granules can still be seen after heating the meat batter to 70°C. The MC-2 and MC-3 treatments resulted in a significant reduction in cooking loss compared to the control (2.26 vs. 1.10, a 50% reduction and 0.25, a 90% reduction, respectively). The MC-1 treatment showed the highest cooking loss value (Table 1) and revealed extremely small starch granules. However, the high cooking loss seems to be related to the disruption of the entire microstructure (Fig. 1G) as seen by the many open channels (discontinuities) and gaps within the matrix. This kind of microstructure has also negatively affected the texture (e.g., hardness, springiness; see below). It should be mentioned that describing the nature of the starches' modifications (provided by the manufacturers) is very vague. As a result, it is difficult to relate the modifications to specific effects in the meat system.

In terms of fat loss, although relatively low in the control (0.5%), all starches helped to lower or eliminate it. This is most likely due to the starch increasing the viscosity of the

meat batters rather than actually binding the fat. Overall, the fat/oil was held very well within the meat matrix of the control (Fig. 1A). The micrograph shows small, stable, and well distributed fat globules, and is in agreement with previously published micrographs (BARBUT *et al*., 2016). The addition of starch did not show any interference with the stability of the fat globules and/or any direct interaction with the fat phase. The slight improvement in fat retention seen here is also in agreement with AKTAS and GENÇCELEP (2006), who noted that some modified potato and corn starches can reduce fat loss from bologna type sausage produced with sheep tail fat.

Table 2. Effects of native and modified starches on texture profile parameters of cooked beef meat batters prepared with canola oil.

Treatment* (#)	Hardness (N)	Springiness (cm)	Cohesiveness (ratio)	Chewiness (N x cm)	Gumminess (N)
1 CONT	67.6±1.5^{c}	0.81±0.01^{a}	0.39±0.01^{a}	21.5±0.8^{a}	26.5±0.7^{ab}
2 NP	79.4±2.2^{a}	0.75±0.01^{b}	0.35±0.01^{b}	21.3±0.9^{a}	28.3±1.1^{a}
3 MP-1	68.1±1.7^{bc}	0.73±0.02^{bcd}	0.30±0.01^{d}	15.1±0.6^{cd}	20.4±0.5^{cd}
4 MP-2	64.9±1.2^{cde}	0.74±0.01^{bc}	0.29±0.01^{d}	14.1±0.4^{d}	19.0±0.4^{d}
5 MP-3	62.9±1.4^{def}	0.69±0.01^{d}	0.26±0.01^{e}	11.6±0.43^{e}	16.6±0.5^{e}
6 NC	72.2±1.6^{b}	0.76±0.01^{b}	0.34±0.01^{b}	19.3±0.7^{b}	25.2±0.7^{b}
7 MC-1	59.5±1.4^{f}	0.59±0.02^{e}	0.29±0.01^{d}	10.1±0.2^{e}	17.1±0.3^{e}
8 MC-2	66.3±1.1^{cd}	0.76±0.01^{b}	0.31±0.01^{c}	16.1±0.4^{c}	21.0±0.5^{c}
9 MC-3	61.0±1.2^{ef}	0.70±0.01^{cd}	0.27±0.01^{e}	11.7±0.5^{e}	16.5±0.5^{e}

$^{a-f}$means, ± standard error, with no common superscript are significantly different ($p < 0.05$).
*all formulated with 2.0% salt and 0.25% sodium tripolyphosphate. CONT = Control; NP = Native Potato starch; MP = Modified Potato starch; NC = Native Corn starch, MC = Modified Corn starch.

Texture profile analysis results (Table 2) show that using the two native starches (potato and corn) significantly increased hardness values above the ones seen in the control. VERREZ-BAGNIS *et al*. (1993) and LI and YEH (2003a) also reported that adding native starch to meat products increased hardness/storage modulus. The other modified starches either did not influence hardness or caused a reduction. SANJEEWA *et al*. (2010) reported that in some of the Canadian varieties of chickpea flours (contain 36-41% starch) they added to low-fat bologna, they observed increased TPA hardness values, while in others they did not. In the present study, the lowest hardness value was seen in the MC-1 treatment, which also lost the highest amount of water. As indicated earlier, this is probably due to formation of channels/disruptions within the meat matrix (Fig. 1G), and resulted in a weaker physical structure. A similar result was also observed for the springiness value, which was the lowest for this treatment (Table 2). Overall, the control (no starch) showed the highest springiness value. All starches caused the formation of less elastic cooked meat structures as evidenced by the lower springiness value (Table 2). This might be due to some discontinuities imparted by the starch (gelatinized or still granular) within the meat matrix. The same was observed for cohesiveness values. However, it should be mentioned that differences between the starches exist and the two native starches (potato and corn) resulted in higher values than the modified starches. Chewiness and gumminess followed the same trend in which the native potato starch was actually not significantly different from the control, but both native starches (potato and corn) resulted in higher values compared to the modified starches. This could be due to more

interactions of the modified starches with meat proteins (i.e., because some are pre-gelatinized and can interact with the proteins before they become heat denatured; BARBUT, 2015). However, this point needs further investigation.
The color of the potato starch added treatments was not as light as the one in the control (lower L* values; Table 1). However, the difference of about 2 L* units (scale: 0=black, and 100=pure white) should not be expected to cause a major obstacle in terms of consumer acceptance. In the case of corn starch, the MC-1 ended up lighter than the control, and MC-2 and MC-3 were darker. Again these differences are not expected to be a problem in terms of marketing. Red color (a*) did not show a major change except for the MC-1 treatment which had the highest cooking loss values (i.e., twice as high as the control; Table 1). This resulted in more of the water soluble red pigment (myoglobin) leaching out of the product. Yellowness values were basically unchanged by starch addition.

4. CONCLUSIONS

The study demonstrates the positive effect of using modified potato and corn starches in an emulsified meat product. The MP-2 showed the best performance in terms of minimum cooking loss and hardness compared to the control. The study also highlights the fact that attention should be given to starch selection for a specific application. In addition, it should be mentioned that some ingredient suppliers sell blends of 2-3 starches to cover various aspects within the same formula, and the meat processor should be aware of the composition, cost, and added value of each component.

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CONSUMER PERSPECTIVE REGARDING DRIED TROPICAL FRUITS IN TURKEY

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ABSTRACT

The purposes of this study are to evaluate the tropical fruit (banana, kiwi, pineapple) preferences of consumers in Turkey and their willingness to pay and to assess the factors that affect this willingness to pay. In this context, tropical fruit products were presented in packages of 50 grams to 386 individuals who had never tasted these products before, and after the products had been tasted, surveys were administered. The findings revealed that dried banana has sensory issues related to hardness and taste and that dried kiwi has sensory issues related to taste and odour. The results show that improving the taste characteristics and increasing the emphasis on health while promoting the products could have a positive impact on increasing the demand for these products in Turkey.

Keywords: consumers, dried fruits, sensory analysis, willingness to pay

1. INTRODUCTION

In Turkey, fruit is a widely cultivated product. However, demand for banana, pineapple and kiwi, which are tropical fruits, is met through imports because of climatic conditions and land constraints in Turkey. In 2015, Turkey had a total of 5,896,156 USD of pineapple imports, 108,334,990 USD of banana imports and 2,945,173 USD of kiwi imports. Many of these imports are from countries such as Costa Rica, Ecuador, Chile, Guatemala and Panama (ANONYMOUS, 2016). Importing from other countries increases the risk that these products will be decayed. The protections applied to remove this risk, however, make it difficult to maintain quality standards that differ from country to country.

These issues can be resolved by drying the products. Drying essentially means removing the moisture from the product with the help of the sun or mechanical devices (CHANG *et al*., 2016). This process reduces packaging, shipping and transportation costs for the product due to the decrease in its weight and mass and reduces the measures needed for product protection (OMOLOLA *et al*., 2017). On the other hand, dried fruits contain more antioxidants, fibre and vitamins than do fresh fruits (BENNETT *et al*., 2011). This information shows that dried fruit consumed regularly and in the correct amount can reduce risk for various health factors such as glycaemia and cardiovascular diseases (JESZKA-SKOWRON *et al*., 2017).

Although dried fruits have advantages related to both consumer health and commercial risk, few studies have focused on consumer preferences for these products. Accordingly, JESIONKOWSKA *et al*. (2009) determined the factors affecting the consumption preferences of Dutch, French and Polish consumers who expressed that they consumed dried fruit and fruit products at least once a month. Similar studies have been conducted on Dutch, French (SIJTSEMA *et al*., 2012) and Chinese (WU, 2017) consumers. In these studies, it was argued that emphasis on health can play an especially important role in increasing the consumption of these products. ALPHONCE *et al*., (2015) assessed consumer preference in Europe and the willingness of consumers to pay extra for tropical dried fruit produced in Africa. The organic status of dried fruit and fair trade factors have been shown to influence consumer willingness to pay extra for dried fruit.

Various fruits such as dried apricots, figs and grapes, which are extensively cultivated in Turkey, are consumed frequently and are well recognized by consumers. Therefore, the Turkish consumer is familiar with dried fruit products and the process of their production. However, in Turkey, dried kiwi, banana and tropical fruits such as pineapple have recently started to be sold. Currently, these products are occasionally sold in private stores, in a limited number of supermarkets and online. Therefore, consumers in Turkey do not know much about these tropical dried fruits, and many of them have never tasted these products.

Specifically, approximately 75-90% of the food products that are newly introduced into a market may fail in the first few years after being launched (TALAVERA *et al*., 2017). To understand and improve market success for new products, consumer analysis is needed (CHEN *et al*., 2013; DE ANDRADE *et al*., 2017).

However, consumers' decision to eat fruits and derivative products is the result of the interaction between a large variety of factors (SABBE *et al*., 2009). Studies show that the foremost factor governing consumer preferences is the fruit's internal features such as taste, odour, colour, and firmness (POLLARD *et al*., 2002; HARKER *et al*., 2008). Another important factor is the importance that the consumer gives to health and their lifestyle (POLLARD *et al*., 2001). Considering health, it is indicated that the probability of purchasing fruit is affected positively when health-related information or the possible benefits of the product are noted on the label (SILVESTRI *et al*., 2018). According to SABBE *et al*., (2008), consumers' socio-demographic structures such as gender and habitat are

related to their willingness to purchase tropical fruits. In addition, while consumers can be affected by a wide range of external product features such as brand, price, convenience, availability, and packaging in food selection, they pay more attention to price, especially when buying fruit (SABBE *et al*., 2009). Overall, apart from consumers' socio-demographic features, the price of the product and the health-consciousness and lifestyle of the consumer are important considerations in fruit consumption. For this reason, these matters were the primary focus of this study.

Realizing consumers' sentimental expectations, leads to consumer satisfaction and increases the marketing of the product (GRUNERT, 2002). HALAGARDA and SUVALA (2018) argue that referring to consumers' experiences on the current products increases the level of significance of the research outcomes. MIGLIORE *et al*., (2017) has emphasized that not recognizing and being unfamiliar with tropical fruits is one obstacle to purchasing. A product overview was written and a taste test administered in order to overcome these problems as well as to appraise these products, which are new to the market from the consumers' point of view.

The fundamental hypothesis underlying this study is that the willingness to buy a new fruit product can change depending on an individual's sensitivity towards health and the internal features of the product, as well as the demographic and socio-economic features of consumers. That information contributes to the choice of the target market. In addition, another hypothesis formed claims that sensory evaluation tests presented to the consumers helps them to evaluate the characteristics of the products. That information, if exists, contributes to the development of the sensual features of the product. The last hypothesis indicates that a new product has a perceptual position compared to the various derivative products. That information helps to determining possible competitor products.

In this context, the main purpose of this study is to determine the willingness of consumers who have never tasted dried tropical fruits (kiwi, banana, pineapple) to pay for these products.

In addition to this objective, other objectives of my study are as follows:

- Determining the amount of money that consumers, who want to buy dried tropical fruits, are willing to pay;
- Revealing the factors that affect willingness to pay for dried tropical fruits;
- Analysing the degree of preference for and deficiencies of dried tropical fruits depending on their sensory properties; and
- Determining the consumption preference hierarchy for dried tropical fruits compared to their other derivative products.

Given the assumption that dried tropical fruit products are quite new for many consumers, it is important to understand the different factors that affect consumers' willingness to purchase and consumption behaviour in terms of developing promotion policies. Few studies have examined consumers' preferences for dried fruits, and none of these studies focused on the Turkish market. Through an extensive literature search and application of the different methods used in this study, better marketing strategies can be developed for the decision makers and operators in the dried tropical fruit industry.

2. MATERIALS AND METHODS

2.1. Overview

This study was conducted in Izmir, which is located in the western part of Turkey and is the biggest city in the area. Participants who had not tasted dried tropical fruits before were given banana, kiwi, and pineapple in packs of 50 grams to taste free of charge. The participants were then asked to rate the taste, colour, appearance, hardness, and odour of these products in points. Then, a questionnaire was administered to the participants, and the consumers' willingness to purchase these products and the factors that influence this willingness were determined. Finally, the participants' preference for different types of these products (fresh fruit, mineral water, fruit milk, fruit yogurt) and the ranking of the derivative products in the consumer mind were determined based on the information gathered from these same questionnaires.
SPSS 21 (Statistical Package for Social Sciences) and R package programs were used for the statistical analysis in this study. The materials and methods used in this study at presented in detail below.

2.2. Sample selection

The location of the survey was İzmir Province, as it contains the largest city in the Aegean region and the third largest city in Turkey. The total population of İzmir Province is 4,223,545. This province, according to code TR31, represents a region of its own. In the study, the number of consumers surveyed was determined by the following sample formula (NEWBOLD, 1995):

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

where n is the sample size, N is the population size (4,223,545), and p is the prediction rate (0.5 for the maximum sample size) and the probability level confidence interval (95% confidence interval, σ_p: 0.02551 for 0.05 margin of error from the equation of $1.96\sigma_p$: 0.05). Accordingly, the number of consumers randomly selected for the face-to-face survey was 385. Questionnaires were administered to a total of 386 consumers, of which there were 193 females and 193 males; thus, the numbers of male and female consumers were equal.
The main constraint of this study is about the sample collected from a limited part of Turkish society. The sample in this study may not be a representative of all segments of the Turkey population. The research can be expanded with sample size acquired from different regions and cultures.

2.3. Sensory evaluations

In this study, dried tropical fruits (kiwi, banana, pineapple) were evaluated by consumers who had not previously tasted such products before. Participants were selected on a volunteer basis. The products were presented to the participants free of charge as dried kiwi, banana, and pineapple in packs of 50 grams. A little time was given, and water was provided in order to minimize residual effects between tasting the different products. The participants were then asked to rate the taste, odour, appearance, firmness, and colour of each product objectively on a 9-point Likert-type scale (1. Dislike extremely, 5. Neither like

nor dislike, 9. Like extremely). On this scale, for example, for firmness, 1 defines the expression “the product is extremely firm”, while 5 defines the expression “the product is neither firm nor soft”, and 9 defines the expression “the product is an ideal firmness”. Although this evaluation method, which makes use of previous studies (ZHAO *et al*., 2007; BARRETT *et al*., 2010), is generally used by expert panellists, it can also be used by consumers (WORCH *et al*., 2010).

2.4. Conditional valuation method and lower bound meaning for payment

In this study, the conditional evaluation method was applied to determine consumer willingness to pay for dried tropical fruits. For this method, a hypothetical market is created for any goods or services that cannot be sold on the market, the benefits that can be gained from such goods or services are explained, and how much consumers are willing to pay for the benefits that they receive in consuming these goods or services is determined (CARSON, 2000; UZMAY and CINAR, 2017). In this research, dried kiwi, banana and pineapple were first tasted by consumers in complementary packages of 50 grams. These consumers had not previously tasted these products before. After this step, consumers were informed about the products, specifically, the products' health advantages. Then, consumers were asked separately about kiwi, banana and pineapple and whether they would like to buy these products. If the response was positive, the final price that they were willing to pay was determined. The prices obtained from each consumer were converted to the consumer's general willingness to pay based on the lower bound method presented below (BLAINE *et al*., 2003).

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

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.

2.5. Logistic regression

In this study, the dual logistic regression method is used to determine the factors that affect consumer willingness to pay for dried fruit. In the logistic regression model, the dependent variable is discrete, and the estimated probability values range from 0 to 1. A general logistic regression model is expressed below (GUJARATI, 1995).

$$P_i = F(z_i) = F(\alpha + \beta X_i) = \frac{1}{1 + e^{-(zi)}} = \frac{1}{1 + e^{-(\alpha + \beta X_i)}}$$

where P_i=i is the probability that the i^{th} individual chooses a specific option, F is the cumulative probability function, α is the constant coefficient, β is the estimation parameter for each independent variable, and X is the independent variable. In this study, consumer dried fruit consumption status (no/yes) was the dependent variable in the logistic regression model. Models are designed separately for the three separate products. In the models, the dependent variables are consumers who are not willing to pay for the dried products. The independent variables are education level of the consumers, understanding of a healthy life, gender, income, age and body mass index. The response obtained from the expression "I think I am a consumer who understands a healthy life" represented the consumers' understanding that a healthy life is included in the response model. For this,

before receiving their answer, it was expressed to the consumers that health-consciousness involves living a healthy life, eating healthy, exercising, sleeping well, and being in touch with nature frequently. Accordingly, the consumers were asked to evaluate themselves objectively. In addition to this process, the study also used hypothesis tests such as Mann-Whitney U, Friedman, and Kendall's W.

2.6. Fuzzy pairwise comparison method and multidimensional scaling analysis

In this study, after the products were tasted by the consumers, questionnaires were administered. The sections included in the questionnaire were in the order of the consumption preferences among fresh fruit, fruit yoghurt, plain fruit, fruit milk, fruit soda and dried tropical fruits. In this section, the fuzzy pairwise comparison method was used. The steps of the method can be summarized as follows (TANAKA, 1997). Pairwise comparisons were presented to indicate individual preferences. The total distance in a comparison was equal to 1. If G_{KH}=0.5, then K≈H; if G_{KH}>0.5, then K>H; and if G_{KH}<0.5, then K<H. The number of paired comparisons of the objectives (C) was determined as C=[(Z.(Z-1))/2]. Z referred to the preferred number of objectives in the formula. In this study, 10 comparisons of five different products were presented to everyone. For each pairwise comparison, the g_{cr} preference was obtained. Measurement of the preference degree of r according to c can be expressed as g_{cr}=1-g_{rc}. Then, the fuzzy preference matrix was generated.

$$G_{cr} = \begin{cases} 0 & if \;\; c = r \;\; \forall\, c, r = 1, \ldots . n \\ g_{cr} & if \;\; c \neq r \;\forall\, c, r = 1, \ldots . . n \end{cases}$$

In this study, a 5×5 fuzzy preference matrix was created for each individual as follows:

$$G = \begin{bmatrix} 0 & g_{12} & g_{13} & . & g_{1r} \\ g_{21} & 0 & . & . & . \\ g_{31} & g_{32} & 0 & . & . \\ . & . & . & 0 & . \\ g_{c1} & . & . & . & 0 \end{bmatrix}$$

The separately preferred density of each objective (μ_j) was obtained using the following equation:

$$\mu_j = 1 - \left(\sum_{c=1}^{n} G_{cr}^2 /(n-1)\right)^{1/2}$$

The value of μj ranged between 0 and 1. This information provides a more efficient structure than traditional sorting methods (GUNDEN and TERRENCE, 2012).
The objective hierarchy obtained by fuzzy pairwise comparisons was converted to perceptual maps using multidimensional scaling. This analysis obtained the projection of the objects in a k-dimensional space based on the determined distance between n objects according to p argument. This projection used the distance between units. This analysis used Kruskal's stress to determine concordance with distances between estimated distances.

$$\text{Stress} = \sqrt{\frac{\sum\sum\left(d_{ij} - k_{ij}\right)^2}{\sum\left(d_{ij}^2\right)}}$$

3. RESULTS

3.1. Consumer profile

In Table 1, various demographics such as gender, age and educational level of the participants are presented. According to the regional results of the Turkish Statistical Institute's research on income and living conditions for 2014, the average annual household income was 32,639 TL. The monthly average annual income is 2719 TL. In this study, the average household income was 2904 TL. According to TURKSTAT, the average height of Turkish citizens is 167.2 centimetres, and the average weight is 71.5 kilograms (ANONYMOUS, 2003a). The weight and height ratios of the participants were obtained with the aim of establishing the body mass index variable to use in the model at a subsequent stage. In the present study, the average weight of the participants was 70 kilograms, and the average height was 168 centimetres. Participants with a primary education accounted for 51.3% of the total participants, 32.4% of the participants were high school graduates, and 16.4% of the participants were university graduates. This information agrees with the educational characterization of İzmir province. Approximately 49.6% of the Izmir population is female, and 51.4% is male. Based on these data, the numbers of male and female participants selected for the sample group were equal. The household size of the participants was 4. In general, the demographic findings corresponded to the Turkish consumer profile.

Table 1. Participants' socioeconomic characteristics*.

Demographic variable	Minimum	Maximum	Mean
Weight (kilograms)	50	130	70.292
Size (centimetres)	150	190	168.450
Age (year)	18	75	41.233
Number of people in the household	1	10	4.256
Income level of the household (Turkish lira)	1400	11000	2904.330
Educational status			
Primary/secondary school		198	51.3%
High school		125	32.4%
College/Faculty/Postgraduate		63	16.4%
Gender			
Female		193	50%
Male		193	50%

*at the time of the survey, 1 USD=2.72 TL.

As previously mentioned, consumers that had not consumed dried tropical fruits (banana, kiwi, pineapple) prior to the study were selected. In addition, consumers with no chronic illnesses caused by fruits and who generally consumed fruit fresh, especially banana, kiwi and pineapple, were also selected. The consumers were asked open-ended questions about the most consumed, the most liked and the most disliked fruit types that they had consumed in the previous year. The responses obtained are presented in Table 2. According to the responses, the most consumed fruits in the last year were apples, followed by oranges. These findings are consistent with the Turkish Statistical Institute's data on the consumption of food items. Accordingly, apple is the most consumed fresh

fruit in Turkey, with an annual consumption of 67,634,642 kilograms (ANONYMOUS, 2003b). In general, it can be stated that the fruit consumption characteristics of the consumers participating in the survey were well matched with the Turkish consumer.
Specifically, the fact that these fruits can be produced in Turkey, can easily be accessed, and are cheaper are the most basic reasons for them being consumed the most. In addition, some products (watermelon, melon) are consumed by consumers as fruit although they are regarded as plants in the literature. On the other hand, the most popular fruit is banana, and the least popular fruit is grapefruit. Additionally, 62.2% of the consumers preferred to buy fresh fruits from the bazaar, 20.2% from the supermarkets and 10.6% from grocery stores. For these selections, product type, cost and availability are the primary factors affecting their selection.

3.2. Sensory data analysis

In this research, sensory evaluation by consumers was performed on dried kiwi, banana and pineapple. The evaluated sensory criteria were hardness, smell, taste, appearance and colour. The highest score for these criteria was 9, while the lowest score was 1. When evaluated in terms of overall score, the highest score was for dried pineapple (7.14). This score was described as a "good at a moderate level" on the sensory evaluations scale. In addition, the second-best score was for kiwi (5.96). This score was described as "neither good nor bad". The lowest score belonged to dried banana, with an average of 4.54. This score was described as "slightly bad".
Figure 1 presents the sensory evaluation data for the products. According to these data, the average hardness scores were 1.6 for banana, 7.4 for kiwi and 7.5 for pineapple. The taste score averages were 2.1 for banana, 5.1 for kiwi and 6.7 for pineapple. The colour score averages were 6.8 for banana, 7.5 for kiwi and 7.2 for pineapple. The average point of appearance was 5.8 for banana, 6.6 for kiwi and 7.1 for pineapple. The average scores for smell were 6.4 for banana, 3.2 for kiwi and 7.2 for pineapple. In summary, banana had a low score in terms of hardness and taste, and kiwi had a low score in terms of taste and smell. Pineapple also had a higher value than the other products in five criteria.

3.3. Willingness to pay

Table 3 presents consumer willingness to pay for dried products. According to the table, 62.2% of consumers were reluctant to buy dried banana. Of these consumers, 78.3% attributed the reason for not buying this product to the problems with taste and hardness, and 92.1% attributed the reason to other sensory attributes. On the other hand, the lowest price given for this product was 0.10 TL and the highest price was 10 TL by consumers who wanted to buy the product. However, findings from the lower bound of the payment methods showed that the general consumer group tended to be willing to pay 0.91 TL for a 50 gram package of dried banana.
When the dried kiwi findings were examined, it could be observed that 52.2% of the consumers wanted to buy dried kiwi, while 47.8% did not want to buy it. Among the consumers who were reluctant to purchase this product, 78.7% had reasons relating to product odour and taste, and 86.4% had reasons relating to other sensory properties.
On the other hand, for the consumers who wanted to buy the product, the lowest price for this product was 0.10 TL, and the highest price was 13 TL. However, the findings from the lower bound method show that the general consumer group tended to be willing to pay 1.30 TL for a 50 gram package of this product.

Table 2. Consumer preferences for fresh fruit consumption.

The fruit that I consume the most			My favourite fruit			The fruit that I never eat		
	Frequency	%		Frequency	%		Frequency	%
Apple	118	30.570	Banana	119	30.829	None	100	25.907
Orange	77	19.948	Strawberry	46	11.917	Grapefruit	51	13.212
Banana	61	15.803	Plum	45	11.658	Medlar	27	6.995
Tangerines	34	8.808	Apple	41	10.622	Quince	25	6.477
Plum	28	7.254	Kiwi	29	7.513	Grape	23	5.959
Strawberry	15	3.886	Peach	13	3.368	Alligator Pear	18	4.663
Watermelon	13	3.368	Watermelon	12	3.109	Apple	16	4.145
Kiwi	11	2.850	Cherry	12	3.109	Plum	11	2.850
Pear	5	1.295	Tangerines	12	3.109	Orange	11	2.850
Melon	4	1.036	Pineapple	10	2.591	Diospyros Kaki	10	2.591
Pomegranate	4	1.036	Orange	9	2.332	Eriobotrya Japonica	10	2.591
Pineapple	3	0.777	Grape	7	1.813	Apricot	10	2.591
Apricot	3	0.777	Unripe Almond	6	1.554	Pear	9	2.332
Peach	3	0.777	Pear	5	1.295	Pomegranate	9	2.332
Grape	3	0.777	Melon	4	1.036	Peach	8	2.073
Ages	2	0.518	Apricot	4	1.036	Strawberry	7	1.813
Quince	1	0.259	Pomegranate	3	0.777	Mulberry	5	1.295
Cherry	1	0.259	Other	9	2.331	Other	36	9.324
Total	386	100		386	100		386	100

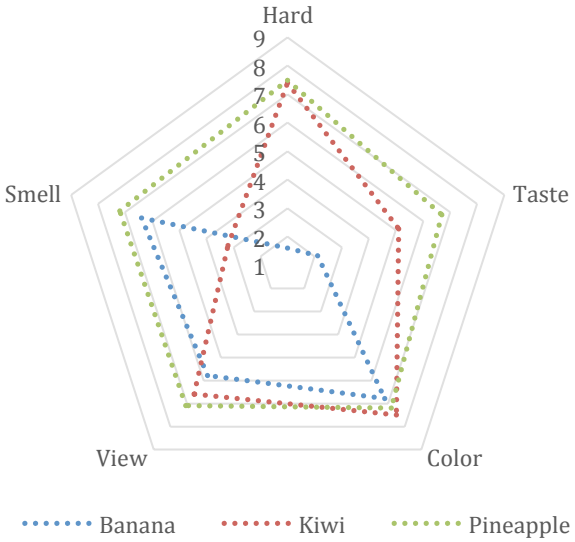

Figure 1. Sensory evaluation for dried banana, pineapple and kiwi.

While 60.1% of the consumers were willing to pay for the dried pineapple, 39.1% did not want to buy this product. Accordingly, dried pineapple was the product with the highest willingness to pay. Of the consumers, 71.2% that were reluctant to purchase had issues relating to taste, and 89.8% of the consumers indicated that they had issues with other sensory characteristics. However, among the consumers who wanted to buy the product, the lowest price given for this product was 0.25 TL, and the highest price was 10 TL. However, the findings from the lower bound method showed that the general consumer group tended to be willing to pay 1.97 TL for a 50 gram package of this product. In general, the highest willingness to pay was for dried pineapple. The second highest willingness to pay was for dried kiwi, followed by dried banana.

3.4. Factors influencing consumer preferences

In this part of the study, factors affecting consumer willingness to buy dried banana, kiwi and pineapple have been revealed. For this, a logistic regression model was used, and each product was analysed separately (Table 4). Consumers who wanted to purchase products were the dependent variables in the model, and the independent variables were their socio-demographic characteristics. The data obtained indicated that the models were significant ($p<0.05$). The correct estimation rate of the dependent variable was 71.2 for dried banana, 70.7 for dried kiwi and 69.7 for dried pineapple. The explanatory power of the dependent variables (R^2) for the independent variables was 16.3% for dried banana, 24.7% for dried kiwi and 24.0% for dried pineapple. In Table 4, the direction of the B coefficient represented the odds ratio (probability of occurring) of the Exp (B) coefficient.
The findings showed that gender affected the consumption of dried fruit. Female participants wanted to pay more for dried products than did the male participants. While this willingness to pay was significant for dried pineapple and kiwi, it was not statistically significant for banana. According to these results, females tended to buy 6.99 (1/0.143) times more dried pineapple than males and 4.83 (1/0.207) times more dried kiwi than males (Table 4).

Table 3. Willingness of consumers to pay for dried banana, pineapple, and kiwi.

Dried kiwi (TL/package)				Dried pineapple (TL/package)				Dried banana (TL/package)			
WTP(TL)	**Frequency**	**%**	**C%**	**WTP(TL)**	**Frequency**	**%**	**C%**	**WTP(TL)**	**Frequency**	**%**	**C%**
13	1	0.26	0.26	10	12	3.11	3.11	10	3	0.78	0.78
10	7	1.81	2.07	8	1	0.26	3.37	5	21	5.44	6.22
7	1	0.26	2.33	7	4	1.04	4.40	4	3	0.78	7.00
6	1	0.26	2.59	6	3	0.78	5.18	3	22	5.70	12.70
5	30	7.77	10.36	5	51	13.21	18.39	2.5	4	1.04	13.73
4	8	2.07	12.44	4	11	2.85	21.24	2	40	10.36	24.10
3.5	1	0.26	12.69	3	43	11.14	32.38	1.5	8	2.07	26.17
3	28	7.25	19.95	2.5	5	1.30	33.68	1	33	8.55	34.72
2.75	2	0.52	20.47	2	47	12.18	45.85	0.75	2	0.52	35.24
2.5	2	0.52	20.98	1.5	6	1.55	47.41	0.5	8	2.07	37.31
2.25	1	0.26	21.24	1	38	9.84	57.25	0.25	1	0.26	37.57
2	38	9.84	31.09	0.75	1	0.26	57.51	0.1	1	0.26	37.83
1.5	7	1.81	32.90	0.5	12	3.11	60.62	0	240	62.18	100.00
1	35	9.07	41.97	0.25	1	0.26	60.88	Total	386	100.00	
0.75	1	0.26	42.23	0	151	39.12	100.00				
0.5	8	2.07	44.30	Total	386	100.00					
0.25	1	0.26	44.56								
0.1	1	0.26	44.82								
0	213	55.18	100.00								
Total	386	100.00									
LBM=1.30 TL/package (26.0 TL/kg)				LBM=1.97 TL/package (39.4 TL/kg)				LBM=0.91 TL/package (18.2 TL/kg)			

Table 4. Results of the logistic regression model.

Independent variable	Description	WTP dried banana			WTP dried kiwi			WTP dried pineapple		
		B	Exp(B)	S.E.	B	Exp(B)	S.E.	B	Exp(B)	S.E.
Educational status	College/Faculty									
	High	0.025	1.025	0.256	-0.281	0.326	0.745	-0.617	0.539	0.329
	Primary/Secondary	-0.189	0.828	0.324	-0.580	0.560*	0.260	-0.671	0.511*	0.258
PHLA	No	1.270	3.262*	0.233	1.662	5.271*	0.238	1.635	5.130*	0.267
	Yes									
Gender	Women	-0.552	0.576	0.621	-1.575	0.207*	0.752	-1.946	0.143*	0.687
	Male									
Income	Scale	0.001	1.001*	0.001	0.001	1.001*	0.001	0.001	1.001*	0.001
Age	Scale	-0.007	1.007	0.020	0.006	0.994	0.021	0.015	0.985	0.022
BMI	Scale	0.019	1.020	0.011	-0.016	0.984	0.011	-0.017	0.983	0.011
Constant		-4.296	0.014*	0.951	0.186	1.205	0.870	1.396	4.037	0.880
Model details										
Model Coefficients		-0.497	0.608*	0.105	-0.208	0.812*	0.102	0.442	1.556*	0.104
X^2			49.230*			78.612*			75.257*	
R^2			16.3			24.7			24.0	
Log likelihood			462.757			452.345			441.426	
Predicted			71.2			70.7			69.7	

*$p<0.05$.

On the other hand, the tendency to consume products was not statistically significant in terms of education, except for pineapple. Consumers with a primary/secondary school level of education were willing to buy fewer products than were those with a college/faculty level of education. For dried kiwi and pineapple, this information was statistically significant. Those with education at the college/faculty level tended to purchase 1.95 (1/0.511) times more dried pineapple and 1.78 (1/0.560) times more dried kiwi than did those with a primary/secondary level of education. In addition, an increase in income significantly increased the willingness to pay for all three products ($p<0.05$). However, there was no significant relationship of consumer age or body mass index with the desire to consume these products. Finally, a positive relationship was found between consumers being health-conscious consumers and their willingness to purchase products (PHLA). According to this result, those who expressed that they were health-conscious consumers tended to buy 3.26 times more dried banana, 5.27 times more dried kiwi and 5.13 times more pineapple than did those who did not pay attention to health ($p<0.05$).
In general, income, health consciousness and gender were influential factors on willingness to purchase.

3.5. The hierarchical selection of consumers and perceptual mapping of products

In Table 5, the fuzzy comparison results are presented. This method has been applied to determine the hierarchy of consumer preference for banana, pineapple and kiwi products as fresh, in mineral water, in fruit milk, in fruit yoghurt and as dried fruit. The validity of the fuzzy comparison method was tested by the Friedman test and Kendall's W test. The Friedman test determines whether consumers are behaving differently in at least one product selection. Accordingly, the H_0 hypothesis was rejected, and at least one ranking was found to be different from the others ($p<0.01$).
This method was carried out after consumers tasted the dried tropical fruit products. The findings indicated that fresh banana, kiwi and pineapple were most preferred by participants (0.651). Specifically, the preference to consume fresh product was statistically significantly higher in males than in females ($p<0.05$). The second highest preference for consumers was to consume these products in mineral water (0.438). There was no significant difference between males and females in the consumption of the product in mineral water ($p>0.05$). The third highest preference for consumers was to consume the products as fruit milk (0.401). The preference for consuming the product as fruit milk was statistically significantly higher in females than in males ($p<0.05$). The fourth highest preference for consumers was to consume the products as fruit yoghurt (0.366). The preference for consuming the product as fruit yoghurt was statistically significantly higher in females than in males ($p<0.05$). The lowest preference for consumers was to consume the products dried (0.203). The preference for consuming the product as dried was statistically significantly higher for females than for males ($p<0.05$).
However, in Table 5, consumption preference order by gender is also presented. When the table is examined, the preference order of the male's products was as follows: fresh (0.715), in mineral water (0.467), in fruit milk (0.350), in fruit yoghurt (0.310) and dried (0.178). For females, the order of preferences was as follows: fresh (0.587), in fruit milk (0.452), in fruit yoghurt (0.423), in mineral water (0.410) and dried (0.228). On the other hand, males preferred fruit yoghurt and fruit milk, while females were more indifferent about making similar selections as mineral water, fruit yoghurt and fruit milk.

Table 5. Fuzzy pairwise comparison findings.

Variable	Gender	Mean	Preference	Mann-Whitney U	Asymp. Sig.	General mean**	Std. Deviation	General preference
Fresh fruits	Woman	0.587	1	13924.00	0.000	0.651	0.285	1
	Male	0.715	1					
Mineral water	Woman	0.410	4	12943.00	0.080	0.438	0.235	2
	Male	0.467	2					
Fruit milk	Woman	0.452	2	14235.00	0.000	0.401	0.253	3
	Male	0.350	3					
Fruit yoghurt	Woman	0.423	3	13502.00	0.000	0.366	0.211	4
	Male	0.310	4					
Dried fruits	Woman	0.228	5	16046.00	0.018	0.203	0.200	5
	Male	0.178	5					

**Significant by Friedman test for $p<0.01$; Kendall's $W = 0.267$.

In this research, multi-dimensional scaling analysis was used to create a perception map for consumers for different types of tropical fruits (kiwi, banana, pineapple). This analysis included products seen as substitutes in the market and their differentiation from one another (KINNEAR and TAYLOR, 1996). According to multidimensional scale findings, the stress value was 0.01786, and the R^2 value was 0.99863. These values indicated that the findings could be interpreted. Figure 2 presents a consumer perception map generated from fuzzy comparison data. Accordingly, consumer perceptions differed according to the different selling processes of the fruits. Only fruit milk and fruit yoghurt were given the same weight by the consumers, while fresh fruit was placed in another dimension with dried fruit and with mineral water. According to the findings of the positioning matrix, the most distant perceived products were fruit soda and dried fruit, with a distance of 3.970, and the closest perceived products were fruit yoghurt and fruit milk, with a distance of 0.787. Dried fruit was the closest product to the fresh fruit, with a distance of 2.206. However, the distance between dried fruit and fruit milk was 2.841, and the distance between dried fruit and fruit yoghurt was 2.844. Hence, since fresh fruit was the closest product to dried fruit, it may be a rival for it. However, dimensional distinctions and overall distance values indicated that consumers perceived dried tropical fruits in a different position compared to other products.

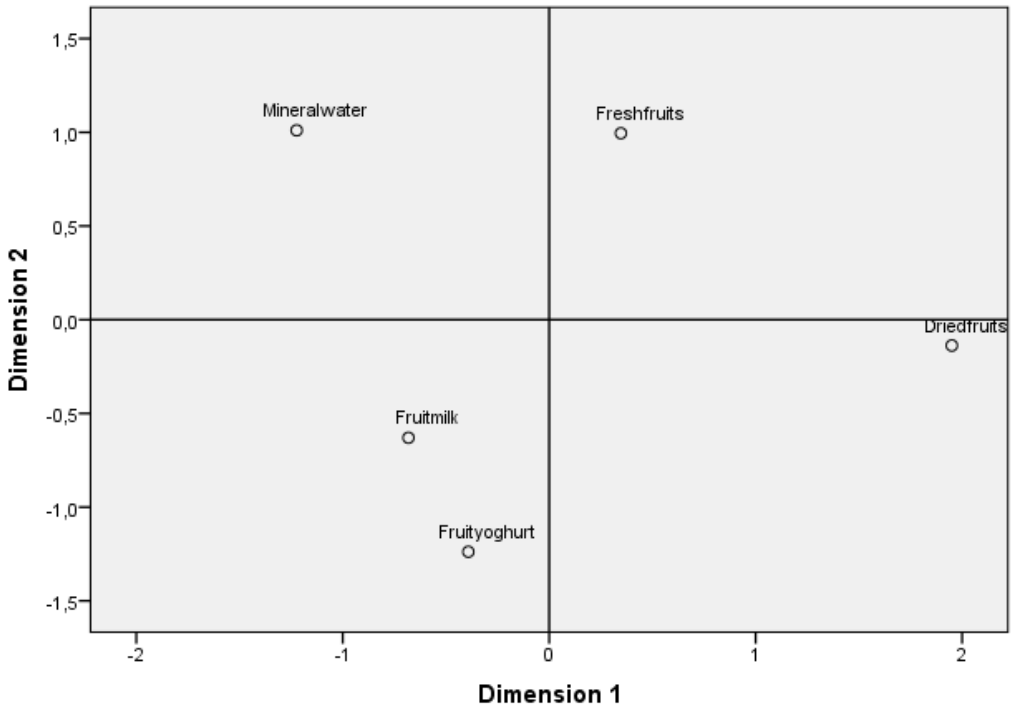

Figure 2. Two-dimensional positioning of the general perception for the products.

4. DISCUSSION AND CONCLUSIONS

This study was conducted to evaluate the factors affecting Turkish consumer preferences for dried tropical fruits (banana, kiwi, pineapple) and the willingness of these consumers to pay for these products. Significant results have emerged from this research.

First, the results of the sensory evaluation method showed that there were issues with dried bananas related to taste and hardness and that the issues for dried kiwi were related to odour and taste. The sensory properties of pineapple were better than those of the other products. In ALPHONCE *et al*., (2015)'s study of European consumers, the average firmness of dried banana was defined as being below average, with 4 points, while its taste was defined as being average, with approximately 5 points. Both the firmness and taste scores of pineapple were just above average (5 points). In this study of Turkish consumers, however, the firmness and taste scores of dried banana were less than those in ALPHONCE *et al*., (2015)'s study, with 1.6 and 2.1 points, respectively. The average taste score given by Turkish consumers to pineapple was 6.7, and the average firmness score was 7.5. These scores were above average. European consumers gave the highest scores to dried mango, pineapple, and banana, in order. In this study, Turkish consumers preferred dried pineapple, kiwi, and banana, in order. In previous studies, dried kiwi was not evaluated sensually by consumers. Generally, the dried banana taste and hardness issues and the average scores for dried pineapple agree with the study of European consumers (ALPHONCE *et al*., 2015). The maturity of the fruit before drying is the most important factor affecting fruit taste and flavour because the maturity level of the fruit is related to the sugar level. Very mature fruits contain high levels of concentrated sugar. During the drying process, this sugar becomes more apparent, and the dried fruit can be very sweet. Conversely, this highly concentrated sugar will lead to a worse taste after drying. On the other hand, excessive drying can harden the fruit. The presence of protections against decay may cause odour problems. Therefore, it is important to determine the level of moisture that does not disturb the drying style or the product taste (MANZUNGU and MACHIRIDZA, 2001). The products had equal and acceptable features in terms of appearance (shape and size) and colour. This information suggests that suppliers should focus on improving poor sensory features rather than the appearance of the products.

The second important result of the survey is related to the willingness to pay for the products. It has been emphasized in previous studies that sensory attributes of food products are effective in procurement decisions (TOPCU *et al*., 2015). Specifically, taste is one of the most important factors affecting the purchase preferences for both dried products (ENDIYANI and SALIMA, 2017) and fruits (KAMENIDOU *et al*., 2002, PANICO *et al*., 2011). The results of this study also confirmed these data. In this study, 37.8% of the consumers did not want to buy dried banana, 44.8% did not want to buy dried kiwi, and 38.8% did not want to buy dried pineapple. Many of the consumers who did not want to buy these products cited sensory characteristics, especially taste. In addition, the research results showed that the willingness to pay was 18.2 kg/TL for dried banana, 26.0 kg/TL for dried kiwi and 39.4 kg/TL for dried pineapple. The lowest willingness to pay was for banana. The market sales price for these products in Turkey is 36 kg/TL. Accordingly, consumers were willing to purchase only dried pineapple for a price above market value. Therefore, the market prices and sensory characteristics of dried banana and kiwi show that they are very unlikely to be consumed frequently by consumers.

The third important result of the study relates to the socio-demographic characteristics of the consumers, which affect the willingness to pay for products. The results showed that income had a positive effect on the willingness to pay for these products. These data overlap with a study conducted on consumers in Zimbabwe relating their preferences for dried fruit (MANZUNGU and MACHIRIDZA, 2001). In addition, health consciousness has a significant impact on the willingness to consume a product. This result agrees with research on Chinese consumer preferences for dried mango (WU, 2017). Additionally, female consumers were more willing to purchase these products than were males. The education level was particularly influential on the consumption of kiwi and pineapple. Studies examining consumption habits of Turkish consumers have indicated that gender,

education level and income level are influential in food purchasing decisions. In addition, high-income, highly educated female consumers are more sensitive to food safety (GOKTOLGA *et al*, 2006). Recently, increasing income levels resulting from economic development in Turkey and increasing education levels, combined with the need for a balanced diet, have created a new consumer market. In this context, the socio-demographic characteristics of the consumer group that wants to purchase dried tropical fruits may facilitate the desire of the target consumer for these products.

The fourth important result of the study is the creation of a hierarchy of preferences for different states of the products. Different studies on dried products have revealed that products are preferred in their fresh state as opposed to their dried state (SIJTSEMA *et al*, 2012, OWUREKU-ASARE *et al*, 2017). The belief that fresh fruit has more vitamins causes French consumers to consume more of the product when it is fresh (JESIONKOWSKA *et al*, 2007). However, this situation may differ from country to country (JESIONKOWSKA *et al*, 2008). The results of this study support the idea that consumers prefer fresh fruit, as Turkish consumers preferred fresh products from among all the different types of fruit products. However, consumers primarily preferred products in mineral water, then fruit milk and then fruit yoghurt. The differences in the consumption preferences and rankings of these products according to gender have been determined. Specifically, females were more indifferent about whether the product was in mineral water, fruit yoghurt or fruit milk. The averages for dried tropical fruits differed from the other products. In addition, consumers positioned dried tropical fruits differently than other forms of the same fruits. This result suggests that tropical dried fruit is unique. Therefore, the consumer may not make comparisons with other products while purchasing these products. This information can be used for developing advertising strategies.

In general, the results indicate that sensory issues related to these products need to be addressed. In addition, when determining the target market, more attention needs to be given to consumers with higher income and education levels and to consumers that pay more attention to health; in addition, the presentation of dried tropical fruit products specifically to women may increase the likelihood of their consumption. Moreover, it may be necessary to emphasize a new product rather than identifying a competitive market for the promotion of products. When the results of the research are holistically assessed, they can help to develop better tropical dried fruit products and more effective marketing tools. Finally, this research was limited to a specific region. Thus, future researchers may be advised to focus on examining whether consumers of different cultures have an impact on the willingness to pay for dried tropical fruit products.

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DETERMINATION OF MICROBIAL CONTAMINATION, PH AND TEMPERATURE CHANGES IN SHEEP AND CATTLE CARCASSES DURING THE SLAUGHTER AND PRE-COOLING PROCESSES IN KONYA, TURKEY

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ABSTRACT

This study was conducted to determine microbial contamination, pH and internal temperature changes in sheep and cattle carcasses during slaughter and chilling stages. Samples were analysed for the presence of *Salmonella* spp. and Enterobacteriaceae and aerobic colony counts (ACC) were performed. Air sampling was also performed in slaughtering areas and chilling rooms.

Mean values of ACCs were between 2.57±0.61 and 4.71±0.24 log CFU/cm^2 and between 3.51±0.48 and 5.19±0.28 log CFU/cm^2, whereas Enterobacteriaceae counts were between 0.89±0.46 and 2.61±0.10 log CFU/cm^2 and between 0.55±0.37 and 3.63±0.39 log CFU/cm^2 in cattle and sheep carcasses, respectively. Enterobacteriaceae contamination in the shoulder region of cattle carcasses after washing, Enterobacteriaceae contamination in all regions in sheep carcasses after chilling and ACC in the shoulder region of sheep carcasses after chilling all exceeded the limits of EC regulation (EC No 2073/2005).

Keywords: air sampling, Enterobacteriaceae, *Salmonella* spp., slaughtering stages, ACC

1. INTRODUCTION

It is desirable to keep the initial microorganism load in meat as low as possible and to observe hygiene rules during slaughtering. Therefore, it is very crucial that slaughtered animals are cut according to hygiene rules in slaughterhouses. Carcasses naturally have a low level of microbial flora and can be regarded to be sterile immediately after slaughtering. Microbial contamination can occur in slaughtered animals in most of the stages throughout the slaughtering process. The slaughtering steps are basically followed by bleeding, dressing, evisceration, washing and finally storage. The hygienic bleeding and dressing system described by FAO is to allow the animal to move up from the back leg to an upright position to allow the bleeding to continue until the blood flow reaches negligible level. Besides, inadequate hygiene conditions along the dressing cause the bacteria to spread from the carcass to the knives and to the hands of the operators. Contamination of carcasses may also occur through direct contact with equipment or hands of the personnel or may also occur indirectly through microorganisms in the air of the slaughterhouse following slaughter (UNTERMAN *et al*., 1997, GILL and BAKER, 1998, BURFOOT *et al*., 2006).

The European Union legislation has declared that the Enterobacteriaceae content and Aerobic colony count (ACC) should be used as hygiene criteria throughout the slaughtering process and that measures should be taken if the values increase above the criteria for slaughtered animals during the slaughtering process (Barco *et al*., 2015). The legislation required monitoring of the above bacterial groups as process hygiene criteria for cattle, sheep and other slaughtered animals and were declared to be Hazard Analysis and Critical Control Point (HACCP) indicators for an acceptable food processing system (EC No 2073/2005; Barco *et al*., 2015). According to the legislation, the ACC and Enterobacteriaceae limits for carcasses of cattle and sheep were declared as minimum (m) and maximum (M) values were 3.5 log CFU/cm^2 - 5.0 log CFU/cm^2 and 1.5 log CFU/cm^2 - 2.5 log CFU/cm^2, respectively.

The European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ) has also introduced the current requirement that the interior temperature of the carcass should not higher than 7 °C immediately after the post-mortem examination, before transporting. A panel of researchers has stated that temperature-time profiles can be applied to obtain similar or reduced levels of carcass contamination and that contamination levels at this temperature range are typically related to the initial level of contamination.

In most studies on carcass surface microbiology, non-invasive methods are used, such as the swab method (MCEVOYA, 2004). The swab method is the preferred method for carcass sampling according to HACCP requirements for European Union slaughterhouses (Pepperell *et al*., 2005). There are a number of published studies in which swab sampling methods have been utilised (ANDERSON *et al*., 2005; BLAGOJEVIC, 2012; BARCO *et al*., 2015; PETRUZZELLI *et al*., 2016; ALONSO-CALLEJA *et al*., 2017).

Carcass samples used in this study were slaughtered using procedures based on 'Good Hygiene Practices' and 'HACCP' principles, related to European Union Regulation 852/2004. The main purpose of the present study was to determine whether the Turkish Food Codex Hygiene Criteria and Commission Regulation (EC) No 2073/2005, are met in cattle and sheep slaughterhouses in the Konya province, which is the biggest producer of red meat in Turkey. In addition, we aimed to detect the airborne contamination in slaughtering area and cold storage rooms, to identify the sources of contamination during slaughtering and to detect the incidence (presence or absence) of *Salmonella* spp. contamination in sheep and cattle carcasses.

2. MATERIALS AND METHODS

2.1. Sample collection

In this study, changes in microbial flora, pH and temperature in cattle and sheep carcasses during different slaughter stages were investigated in three different large-scale slaughterhouses (with a daily cutting capacity of at least 40 cattle, according to the classification of Turkish slaughterhouses) between December 2013 and April 2016 in Konya, Turkey. Swab samples moistened with sterile buffered peptone water (BPW) were collected using the swab technique consisting of 5 vertical and horizontal passes described by USDA with slight modification. We swabbed an area of 10 × 10 cm^2 from five randomly chosen regions of the carcasses, including two shoulders, two rumps and briskets, after three different cutting stages (dressing, evisceration and washing) and after storage of the same carcass in chilling rooms for 24 h. A total of 480 samples from sheep (n = 240) and cattle (n = 240) were collected from the carcasses. Samples were cold chain transported to the laboratory, and microbiological analyses were performed within 3 h of sampling. Samples were cold chain transported to the laboratory

2.2. Microbiological analysis

ACC were performed as follows: 1 ml from a 1:10 diluted swab sample was poured onto plate count agar (PCA, Merck 105463) plates. Incubation was performed under aerobic conditions at 37°C for 24 h. The total number of Enterobacteriaceae was determined according to the International Organization for Standardization (ISO) 21528–2:2004. The procedure was as follows: 1 ml of serial dilutions in Buffered Peptone Water (BPW, Merck, Germany), were poured onto violet red bile glucose agar (VRBG, Merck, Germany) and incubated at 37°C for 24 h. Typical colonies grown on plates were quantified after incubation. Isolation and identification of *Salmonella* spp. was performed using the method recommended by ISO 6579:2002 + A1:2007 with slight modifications. Accordingly, swab samples in BPW were incubated overnight at 37°C for pre-enrichment. For selective enrichment, 0.1 ml of the pre-enriched culture was added to 10 ml of modified Rappaport-Vassiliadis broth (MRVB, Merck, Germany) and incubated at 41.5°C for 24-48 h. Subsequently, 0.1 ml from the enriched culture was streaked onto Xylose Lactose Tergitol 4 (XLT4, Merck, Germany) agar supplemented with XLT4 Selective Supplement (Merck, Germany). These plates were incubated at 37°C for 24 h. DNA isolation was performed from five selected black or black-centred colonies on each plate which were considered to be 'presumptive *Salmonella* colonies' grown on XLT4 agar.

2.3. Conventional m-PCR for detecting *Salmonella* spp.

DNA isolation from suspicious *Salmonella* colonies was performed using the boiling method. Following optimisation of PCR conditions, conventional multiplex-PCR (m-PCR) was performed. Gene primers used for *Salmonella* spp. are shown on Table 1.
The m-PCR master mix comprised 1 U *Taq* Polymerase and *Taq* buffer (5 mM KCl and 0 mM Tris-HCl), 1.5 mM $MgCl_2$, 0.025 mM of each primer, 0.9 μM Inv-A primers and 0.4 μM IE1 and Flic-C primers in a 20-μl reaction volume. The m-PCR protocol comprised an initial denaturation step for 5 min at 95°C followed by 30 cycles of 1 min at 95°C, 1 min at 58°C and 30 s at 72°C, with a final extension step of 7 min at 72°C (PAIAO *et al*., 2013).

Table 1. The primer pairs used in this study.

	Primers	Product length	Reference
Salmonella spp. (Inv-A)	F:GTGAAATTATCGCCACGTTCGGGCAA R:TCATCGCACCGTCAAAGGAACC	284 bp	Rahn *et al.*, 1992
S. enteritidis (IE-1)	F:AGTGCCATACTT TTAATGAC R:ACTATGTCGATACGGTGGG	316 bp	Wang and Yeh, 2002
S. typhimurium (Flic-C)	F:CCCGCTTACAGGTGGACTAC R:AGCGGGTTTTCGGTGGTTGT	432 bp	Paiao *et al.*, 2013

2.4. Air sampling

Air sampling was employed to determine the number of ACC and fungal counts in slaughter operation and cold storage rooms during processing. PCA (Merck, Germmany) was used for ACC and potato dextrose agar (Merck, Germany) supplemented with 10% tartaric acid solution was used for fungal counts in the air sampler (Air Ideal 3P, Biomerieux, France). The air sampler device was placed 1-1,5 m above the floor along the slaughter line and chilling rooms. In the sampling areas, 190 l of air was vacuumed by placing the Petri dishes on the vacuum surface of the device. The plates were incubated before determining microbial counts. The samples were taken six independent times on different sampling days.

2.5. Determination of pH and temperature

pH and temperature values of the carcass were measured during different sampling stages using a portable pH and temperature probe (Testo 205, Germany). Changes in pH and temperature of sheep and cattle carcasses were also determined during the following slaughtering steps: dressing, evisceration, washing and chilling.

2.6. Statistical analysis

Data obtained from the study was analysed using SPSS software package 21.00. Data was subjected to variance analysis (one-way ANOVA) and two sample t-tests in accordance with the experimental design. Significant differences ($p < 0.05$) were identified using multicomparisons of the means, with Duncan's test, within the variance analysis. Means and standard errors of the means were reported.

3. RESULTS AND DISCUSSION

In our study, ACC in cattle carcasses were observed between 2.57±0.61 and 4.71±0.24 log CFU/cm^2 and Enterobacteriaceae counts were observed between 0.89±0.46 and 2.61±0.10 log CFU/cm^2. Further, contamination with Enterobacteriaceae in the shoulder region after washing was found to exceed the limits of EC regulation for cattle carcasses. Our highest ACC in cattle carcass were observed in shoulders and rumps after dressing and in briskets after washing (Fig. 1). Contamination levels were not found to be statistically different ($p > 0.05$) for ACC in shoulders, rumps and brisket regions at different stages of cattle slaughtering.

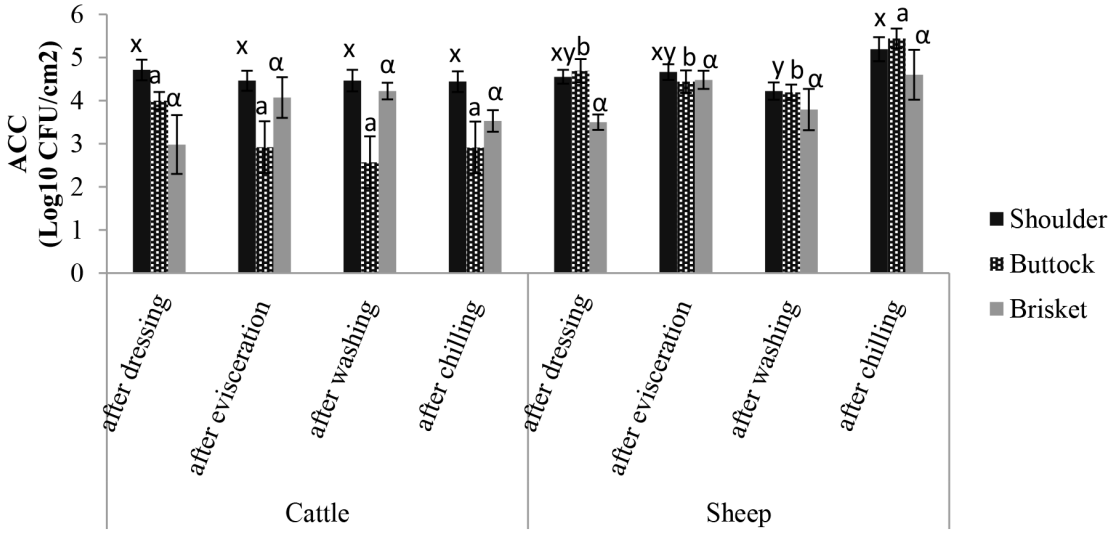

Figure 1. ACC at different slaughtering stages of cattle and sheep carcasses ($log_{10}CFU/cm^2$) Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) indicate significant difference ($p<0.05$).

Although there were no statistically significant differences in the total number of Enterobacteriaceae in cattle carcasses ($p > 0.05$) in shoulders, rumps and brisket regions at all stages of slaughtering ($p > 0.05$; Fig. 2), we observed that Enterobacteriaceae contamination was the highest after the washing stage. This can be explained by partial contamination originating from faeces or internal organs that are spread to other regions during washing. In sheep carcass samples, the highest level of Enterobacteriaceae contamination was observed at the chilling stage, and this was statistically significant ($p < 0.05$; Fig 2). In sheep carcasses, mean values of ACC were between 3.51±0.48 and 5.19±0.28 log CFU/cm^2, whereas Enterobacteriaceae counts were between 0.55±0.37 and 3.63±0.39 log CFU/cm^2. Further, ACC in the shoulder region and Enterobacteriaceae contamination in all regions after the chilling stage exceeded the limits of EC regulation. The highest ACC were found in all sampled parts after the chilling stage (Fig 1) and this was statistically significant ($p < 0.05$). In a similar study, ZWEIFEL *et al*. (2014) obtained similar results in cattle carcasses and they determined that chilling was the most important stage for preventing contamination.

Cattle and sheep carcasses were compared in terms of slaughtering steps and contamination levels in different sampling regions. Statistical graphs and a comparison table of ACC and Enterobacteriaceae count from both sheep and cattle carcasses at different slaughtering stages are given below ($p < 0.05$, Table 2). ACC were higher in the shoulder region of cattle carcasses than in that of sheep carcasses ($p < 0.05$, Table 2). Similar differences were observed in ACC in rump area after washing and that in brisket after the dressing process in the two carcasses ($p < 0.05$).

Further, ACC in the rump area of sheep carcasses had higher contamination than that in the rump area of cattle carcasses during the dressing stage. After evisceration, contamination in the brisket of sheep carcasses was higher than that in the brisket of cattle carcasses ($p < 0.05$, Fig. 3.).

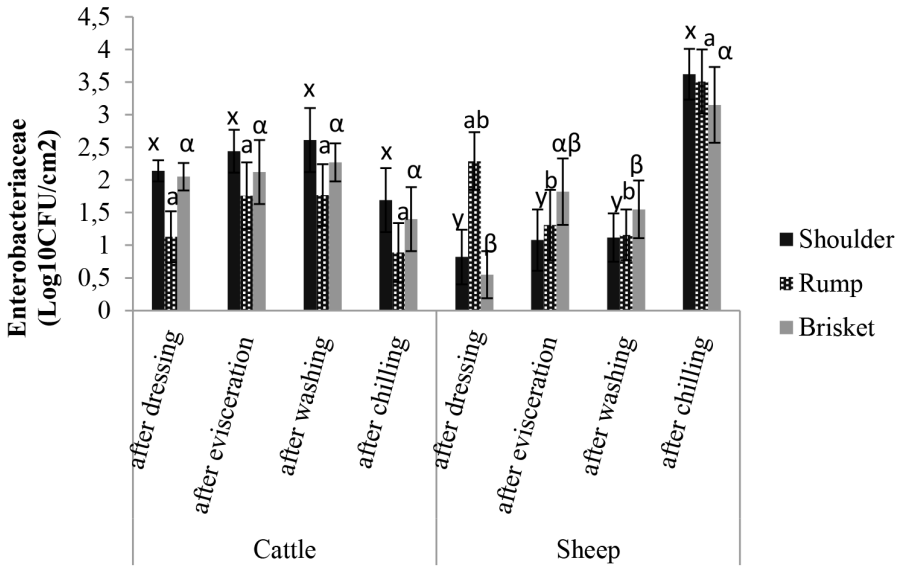

Figure 2. *Enterobacteriaceae* counts at different slaughtering stages of cattle and sheep carcasses ($log_{10}CFU/cm^2$). Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) above each bar indicate significant difference ($p<0.05$).

Table 2. Comparison the ACC and *Enterobacteriaceae* contamination levels of cattle and sheep carcasses.

	Processing steps		Shoulder x± Sx	Rump x± Sx	Brisket x± Sx	P
ACC (Log CFU/cm^2)	after dressing	cattle	4,71±0,24^a	2,99±0,69^b	4,01±0,21^{ab}	*
		sheep	4,55±0,17	4,70±0,28	3,51±0,48	
	after evisceration	cattle	4,46±0,23	4,08±0,47	2,93±0,68b	
		sheep	4,66±0,18	4,45±0,26	4,48±0,21	
	after washing	cattle	4,46±0,26^a	4,22±0,20^a	2,57±0,61^b	**
		sheep	4,22±0,21	4,19±0,19	3,79±0,48	
	after chilling	cattle	4,44±0,24	3,54±0,26	2,92±0,68	
		sheep	5,19±0,28	5,44±0,24	4,60±0,59	
Enterobacteriaceae (Log CFU/cm^2)	after dressing	cattle	2,14±0,16^a	2,06±0,22^a	1,14±0,40^b	*
		sheep	0,83±0,42^b	2,30±0,45^a	0,55±0,37^b	*
	after evisceration	cattle	2,45±0,33	2,13±0,50	1,77±0,52	
		sheep	1,09±0,47	1,31±0,54	1,82±0,51	
	after washing	cattle	2,61±0,10^a	2,28±0,30^a	1,77±0,48^b	*
		sheep	1,12±0,38	1,16±0,39	1,55±0,44	
	after chilling	cattle	1,69±0,50	1,41±0,50	0,89±0,46	
		sheep	3,63±0,39	3,52±0,49	3,15±0,59	

x,y: values within a row with different letters are significantly different ($p<0.05$), x: mean, Sx: standard error of mean *: $p<0.05$;**: $p<0.01$.

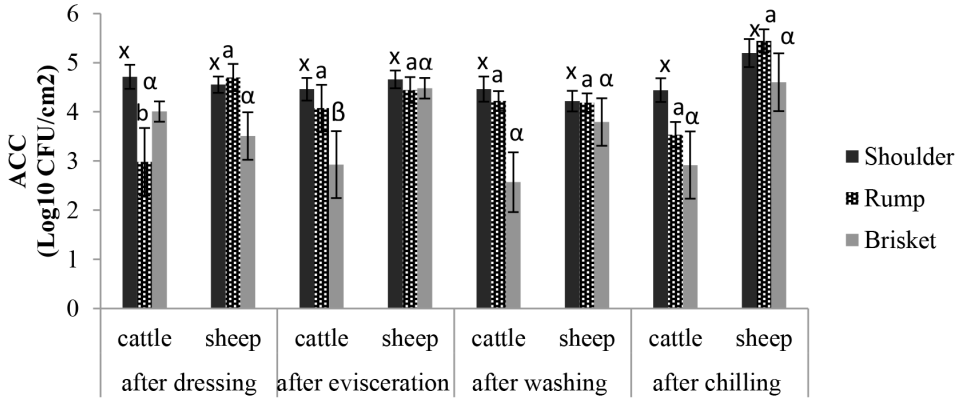

Figure 3. Comparison the ACC Levels of Cattle and Sheep Carcasses. Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) above each bar indicate significant difference ($p<0.05$).

The shoulder region of cattle carcasses after dressing and in the shoulder and rump regions after washing had higher Enterobacteriaceae contamination values than those of sheep carcasses ($p < 0.05$, Fig 4.), this implied that the washing process in cattle was inadequate.

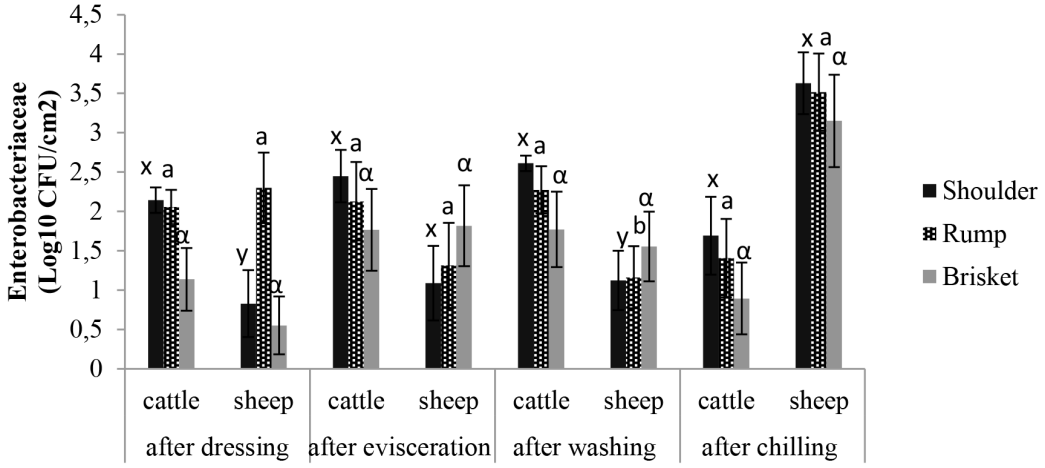

Figure 4. Comparison *Enterobacteriaceae* spp. Contamination Levels of Cattle and Sheep Carcasses.Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) above each bar indicate significant difference ($p<0.05$).

Other previous studies (BARBOZA DE MARTINEZ *et al*., 2002; MIES *et al*., 2004) have supported these findings and stated that the washing process during slaughtering may be ineffective in decreasing the rate of bacterial contamination. Therefore, many investigators suggest that washing should be done with effective disinfectants and that more strict preventive measures should be taken. A similar study (BARBOZA de MARTINEZ *et al*., 2002) investigated the effect of nisin and lactic acid against bacterial loads, including ACC, total coliforms and *E. coli* in cattle carcasses. Researchers stated that washing alone did not reduce the bacterial load and that spraying with a mixture of lactic acid and nisin provided the highest reduction in all bacterial groups tested. In another study (MIES *et al*., 2004), researchers assessed the effect of washing cattle carcasses with or without

disinfectant solutions prior to slaughtering on aerobic plate, coliform, *E. coli* and *Salmonella* counts. The greatest reductions were observed in mean logs of bacterial counts in groups sprayed with 4%-6% ethanol and lactic acid.

Our microbial counts are comparable with those obtained in other surveys at the national and international level using similar techniques (swabbing or sponging) (GILL and BAKER, 1998; DUFFY *et al*., 2001; YALÇıN *et al*., 2004; PEARCE *et al*., 2005; SALMELA *et al*., 2013; PETRUZELLI *et al*., 2016). GILL and BAKER (1998) examined aerobic counts and coliform and *E. coli* contamination rates on randomly selected sheep carcass surfaces using the swab method in Canada. The highest *E. coli* load was detected on shoulder and rump regions after the dressing stage and that the ACC load was the highest after the evisceration stage. Duffy *et al*. in 2001 investigated lamb carcasses in the United States using sponge sampling to detect the presence of *Salmonella* spp and *E. coli* and determine ACC and total coliform loads after the chilling process. *Salmonella* spp. was detected in 1.5% samples, whereas ACC, total coliform and *E. coli* counts were observed at 4.42, 1.18 and 0.70 log CFU/ cm^2, respectively. A similar study in Turkey by YALÇıN *et al*. (2004) stated that ACC in sheep carcasses were 2.96, 3.10, 2.81 and 1.69 log CFU/ cm^2 after dressing, evisceration, washing and chilling steps, respectively. Moreover, in Ireland PEARCE *et al*. (2005) reported that ACC in sheep carcasses, as tested by the excision method, in the thorax, shoulder-neck, chest-brisket, and flank areas were 3.4, 3.6, 3.5 and 2.4 log CFU/cm^2, respectively, whereas measurements using the polyurethane sponge method indicated 2.9, 2.8, 3.3 and 2.5 log CFU/cm^2 and that using the cellulose acetate sponge method indicated 2.7, 2.5, 2.7 and 2.3 log CFU/cm^2 in the same stages, respectively. SALMELA *et al*. (2013) also assayed ACC of carcasses sampled by excision and swab methods in Finland; results were 3.77 and 3.16 log CFU/cm^2, respectively. Researchers reported that ACC and Enterobacteriaceae and *E. coli* counts were higher in the excision method than the swab method. The authors reported that Enterobacteriaceae was detected using the excision and swab methods in 72% and 76% carcasses, respectively, whereas *E. coli* was detected in 48% and 61% carcasses, respectively. Similar to our findings, PETRUZZELLI *et al*. (2016) investigated ACC and Enterobacteriaceae contaminations in ovine, bovine and swine carcasses in Italy using the sponge method following EC regulations. They found that contamination levels in bovine, ovine and swine carcasses, when measuring ACC were 1.96, 2.27 and 2.27 log cfu/cm^2, respectively, whereas Enterobacteriaceae counts were 0.01, 0.27 and 0.20, respectively. They also stated that cattle carcasses had significantly lower levels of ACC and Enterobacteriaceae counts than swine and ovine carcasses. ALONSO-CALLEJA *et al*. (2017) also studied lamb carcasses in two slaughterhouses in Spain. They stated that total viable counts (TVC, 2.74 log CFU/cm^2) were higher than Enterobacteriaceae (2.21 log CFU/cm^2) counts and that there was a high correlation between them. They also stated that 0% and 30.8% of the samples in abattoir A and 10% and 40% of the samples in abattoir B exceeded EC regulations for TVC and *Enterobacteriaceae* counts, respectively.

An overall evaluation demonstrated that as the samples progressed through the steps of the slaughtering process, an increase in the total number of microorganisms in the shoulders, rump and brisket regions was observed after dressing in both cattle and sheep carcasses. In this context, GILL *et al*. (2003) argued that despite the use of decontamination methods, such as pasteurisation, hot-water washing or lactic acid spraying, after the evisceration step, the increase in bacterial counts after chilling of carcasses was related to the proliferation of initial microorganisms rather than subsequent contaminations. However, it has been determined that ACCs, as one of the determinative criteria of slaughtering hygiene, are also in conformity with the Turkish Food Codex Regulations on Microbiological Criteria of Meat and Meat Products and Commission, except in the shoulder region of sheep carcasses after the chilling stage. In addition, Enterobacteriaceae

limits were exceeded in shoulder, brisket and rump regions of sheep carcasses after the chilling process and in the shoulder region of cattle carcasses after washing. Thus, it is very important to take necessary precautions to prevent contamination, particularly during the process of washing and evisceration during slaughtering. Likewise, it is thought that the chilling process should be performed at maximum performance and speed and that carcass decontamination methods should be applied, if necessary, to minimise the growth of pathogenic and spoilage microorganisms.

The present study aimed to determine the presence of *Salmonella* spp. in sheep and cattle slaughtering process. However, no *Salmonella* spp. were detected in the 480 samples analysed, which is highly satisfactory in terms of slaughtering hygiene and public health. SALMELA *et al*. (2013) did not detect any *Salmonella* spp. by swab and excision methods in carcasses in any of the samples, similar to that observed our present study. Nevertheless, MADDEN *et al*. (2001) found that three of the 200 samples from cattle carcasses were contaminated with *Salmonella* spp. CHAVEZ *et al*. (2015) used the sponge sampling technique to collect samples (n = 142) after the washing step and before the chilling step and determined that 18% cattle carcasses were contaminated with *Salmonella* spp. at the three inspected abattoirs. Similarly, HALD *et al.* (2003), collected pig carcass samples from 12 slaughterhouses in five countries of Europe. The researchers stated no *Salmonella* was found in one country while 5.3 % of 3485 samples found to be positive in the other four countries. In a recent study in Ethiopia, MULUNEH and KIBRET (2015) found 7.6 % of the beef carcasses collected from an abattoir positive for *Salmonella* spp.

We observed that as the sheep and cattle slaughtering process progressed, the temperature progressively decreased in all carcass regions and that this was statistically significant ($p < 0.05$; Table 3). The temperature observed after storage did not reach the desired low temperature (4°C-6°C), indicating that cooling is insufficient. This demonstrates the necessity for systematically controlling the size of carcasses, internal temperature, air flow and humidity of the chilling rooms. It was observed that inner temperatures of sheep carcasses after chilling storage were lower than cattle. Nevertheless, it has been observed that this decrease in temperature does not provide any advantage in reducing the microbial load. As a matter of fact, according to the EFSA panel on BIOHAZ (2014a,b), it has been declared that it is important to go to the transport stage while the chilling process is performed on the carcasses so that the number of spoilage bacteria cannot reach an unsatisfactory level.

Table 3. pH and temperature (°C) values of the shoulder and rump regions at different slaughtering stages.

		Sheep		Cattle	
		Shoulder	Rump	Shoulder	Rump
	Processing Steps	x±Sx	x±Sx	x±Sx	x±Sx
pH	after dressing	6.40±0.09^{a}	6.35±0.05^{a}	6,40^{a}±0,09	6,35^{a}±0,05
	after evisceration	6.48±0.12^{a}	6.14±0.05^{ab}	6,48^{a}±0,12	6,14^{ab}±0,05
	after washing	6.33±0.23^{a}	6.15±0.01^{ab}	6,33^{a}±0,23	6,15^{ab}±0,01
	after chilling	5.61±0.28^{b}	5.28±0.04^{c}	5,61^{b}±0,28	5,28^{c}±0,04
	P*	***	***	***	***
°C	after dressing	38.66±0.22^{a}	38.48±0.38^{a}	32,77^{ab}±1,54	34,47^{b}±1,93
	after evisceration	38.12±0.26^{a}	37.46±0.47^{a}	33,85^{a}±2,82	37,50^{a}±0,35
	after washing	36.27±0.68^{b}	37.22±0.92^{a}	28,23^{b}±3,95	37,05^{ab}±0,85
	after chilling	7.72±0.77^{c}	8.13±0.85^{b}	11,43^{c}±1,18	13,36^{c}±1,24
	P*	***	***	***	***

Air sampling results for ACC and fungal counts were found to be lower in the chilling rooms than in the slaughtering area (Table 4; $p < 0.05$). These values indicated that the hygiene in chilling rooms is satisfactory. This can explain why microbial counts were reduced in the chilling rooms compared with those in the slaughtering area and increasing general hygienic conditions is critical for reduction of carcass microbial growth. In a similar study, PRENDERGAST *et al.* (2004) also investigated the relation between airborne bacterial counts and carcass contamination in two abattoirs in Ireland. The ACC were found to be between 1.79-3.49 $\log_{10}$CFU/m^3 at different stages of slaughtering. They also stated the clean areas of slaughtering process had a lower microbial load than the dirty areas. Researchers found the correlations between carcass contamination and aerial load was low. Similarly, BURFOOT *et al*. (2006) noted that airborne contamination of cattle and sheep carcasses during the evisceration phase is less of a concern than other contamination sources.

Table 4. AC, Yeast and Molds Counts in the Slaughtering Room and Cold Storage Room ($\log_{10}$cfu/m^3).

Areas	ACC (x±Sx)	Yeast and Molds (x±Sx)
Slaughtering room	2.54±0.01^a	1.23±0.12
Cold storage room	1.98±0.03^b	1.04±0.01
P	***	

a, b, c: The differences between different letter values in the same column are significant ($p < 0.05$). N: Number of samples. x: Mean value. Sx: Standard error of mean, *: p<0,05;**: p<0,01;***: p<0,001.

4. CONCLUSION

In the present study, Enterobacteriaceae limits were exceeded in shoulder, brisket and rump regions of sheep carcasses after the chilling process and in the shoulder region of cattle carcasses after washing. Further, ACC in the shoulder region and Enterobacteriaceae contamination in all regions after the chilling stage exceeded the limits of EC regulation. The highest ACC were found in all sampled parts after the chilling stage. This is thought to be due to the provision of a suitable environment as a result of poorly cooled chilled rooms to gradually increase microbial load, which is relatively low in cutting stages. Furthermore, it is satisfactory that *Salmonella* spp. is not detected in the present study, but it should be considered that this result may be due to possible insufficiency of the swabbing method compared with the excision.

In conclusion, the microbial quality of meat for consumption is closely related to public health. Thus, it is critical to understand specific microbial risks of each work step, from slaughtering to chilling of carcasses. Microbiological analyzes which are carried out only at the end of the process, may not provide realistic information on the main causes of the microbial contamination. Therefore, the microbiological criteria which are 'Process-based' related to measurements including different methods at various stages of the process should be preferred and more detailed risk assessments should be undertaken to assess and develop preventive measures that can reduce sources of contamination during the most critical stages of slaughtering.

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