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RELATION BETWEEN INNOVATION AND SUSTAINABILITY IN THE AGRO-FOOD SYSTEM

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ABSTRACT

This review paper explores the complexity of relation between innovation and sustainability and relates it to the agro-food arena. Many scholars argue that meeting the Sustainable Development Goals (SDGs) requires major transformation in modes of innovation. As for the agro-food system, relationship between innovation and sustainability is far from straightforward. Innovation (especially technical one) provides a fertile ground for alternative agro-food movements to criticize the over-industrialization of the food system. However, it seems that it is not about questioning innovation *tout court*, but about what type of innovation (see, sustainable innovation) should be promoted to foster transition towards sustainable food systems.

Keywords: social innovation, sustainability transitions, sustainable agriculture, sustainable food systems, sustainable innovation, technical innovation

1. INTRODUCTION

Innovation is rather an ambivalent term and that may explain the existence of different understandings of what innovation means. In fact, innovation has been defined in many different ways to the point that there is somehow a 'lack of definitional clarity' (SHAVER, 2016). OECD and EUROSTAT (2005) describe innovation as "*the implementation of a new or significantly improved product (good or service), or process, a new marketing method, or a new organisational method in business practices, workplace organisation or external relations*". Innovation refers to a complex phenomenon, involving the production, diffusion and translation of scientific or technical knowledge into new products, techniques, services (MENRAD and FEIGL, 2007). According to STERRENBERG *et al*. (2013:62), "*Innovation is the creation of better or more effective products, processes, services, technologies, or ideas that are accepted by markets, governments, and society. Innovation differs from* invention *in that innovation refers to the use of a new idea or method, whereas invention refers more directly to the creation of the idea or method itself*". This definition clearly shows that there are different types of innovation, with different degrees of application in the agro-food sector.

There are also broader understandings of innovation. According to STEPS CENTRE (2010:1), innovation means "*new ways of doing things. This includes not only science and technology, but – crucially – the related array of new ideas, institutions, practices, behaviours and social relations that shape scientific and technological patterns, purposes, applications and outcomes*". Innovation concept is strongly linked to that of knowledge, which is fundamental to move towards sustainable practices (GRIN *et al*., 2010). Knowledge plays an important role in transitions to sustainable food systems. Therefore, it is important to pay attention to the different types of knowledge (information, skills, judgment and wisdom) that are needed in different situations (LOCONTO, 2016). However, knowledge, as well as innovation, needed to make transition is often contested and inconclusive (BATIE, 2008; LEVIN *et al*., 2012; PETERS and PIERRE, 2014).

The literature contains many categorisations of innovation along many different dimensions. According to STUMMER *et al*. (2010), innovations can be categorized according to innovation type (product, service, process, market), dimension (objective or subjective), scope of change (radical, incremental, reapplied), or how innovation was created (closed or open). The OECD and EUROSTAT (2005) distinguish product, process, marketing and organisational innovations. Agricultural innovation as well as innovation in agri-food can be classified using the same categories (AVERMAETE *et al*., 2004; AVOLIO *et al*., 2014).

SCHUMPETER (1934, 1942) is often identified as the first to feature innovation as a central driver of the economy and to reject neoclassical economics' idea of a static equilibrium. His idea that the process of innovation "*incessantly revolutionises the economic structure from within, incessantly destroying the old one, incessantly creating a new one*" (SCHUMPETER, 1942:83) continues to be influential to this day. However, Schumpeter and his followers employed the so-called 'linear model' in which innovation begins with an invention, is developed into a commercially viable technology in a firm, and is then diffused into the market place (TWOMEY and GAZIULUSOY, 2014). A consequence of this model was a strong prioritisation of research and development (R&D) and the entrepreneur as the driver of innovation. This is sometimes referred to as the technology- or supply-push perspective of innovation. An alternative perspective put forward in the 1950s and 60s, but still within the linear model approach, was that demand for products and services is more important in stimulating innovation activity and is known as the demand-pull perspective (SCHMOOKLER, 1966).

In the last decades there has been a shift from an innovation concept centred on research to innovation as a result of interactions among several actors that establish diverse networks and linkages (WORLD BANK, 2006) in an innovation system. In fact, over the last fifty years, a more nuanced and richer picture of the innovation has emerged, with a wider set of implications for those hoping to assist, shape or direct innovation process. Modern innovation theory has moved towards the recognition that innovation is a joint activity involving a large number of actors with different interests, perceptions, capabilities and roles (TWOMEY and GAZIULUSOY, 2014). It also reshaped the relation between innovation and science (TYFIELD, 2011). An interesting further development was the recognition of the importance of users (firms and individual consumers) in the innovation process (VON HIPPEL, 2005; BOGERS *et al*., 2010).

According to OSBURG (2013), innovation theory has seen constant change of its focus over the last decades: concept of newness (1950s), management theory (1960s), demand side (1970s), process innovation (1980s), service innovations (1990s), and, more recently, open innovation (CHESBROUGH, 2003a) and social innovation (VAN DE VEN *et al*., 2008).

Appreciation of the importance of actor networks is a key idea in modern innovation field. In the mid-1980s the concept of 'innovation systems' (FREEMAN, 1995; HEKKERT *et al*., 2007; JACOBSSON and BERGEK, 2010) was introduced. Innovation systems (IS) theory is a heuristic framework that starts from the basis that innovation occurs in the context of an entire system. Furthermore, there have been efforts towards integrating innovation systems approach and the socio-technical transitions (MARKARD and TRUFFER, 2008). The socio-technical transition approach (KEMP, 1994; GEELS, 2005; ROTMANS *et al*., 2000) is an umbrella term that includes, among others, the Multi-Level Perspective on socio-technical transitions (MLP). The MLP approach differs in focus and scope from the IS approach; MLP is conceived in a societal context that is broader than the innovation systems approach (GEELS, 2005). A central theme of MLP is the recognition of the co-evolutionary development of technologies, institutions and social and economic subsystems. MLP is particularly powerful in understanding the complex interplay of different forces at the macro-, meso- and micro-level in creating disruptive change (GEELS, 2010; GEELS, 2011).

The modern theory of innovation provides a number of concepts and insights similar to that of transition (TWOMEY and GAZIULUSOY, 2014; TYFIELD, 2011). The common term 'transition' is often used interchangeably with the term 'systems innovation', either at the technology system or society-wide level. KEMP and ROTMANS (2005), however, argue that "*For the purposes of managing change processes to sustainability it is useful to use the concept of a 'transition' rather than system innovation*" since it brings into focus the new state, the path towards the end state, the transition problems and the wide range of internal and external developments which shape the outcome.

The concept of "transitions" was first coined by Alex de Tocqueville in the 19th century (COENEN-HUTHER, 1996). The term was also utilized in other research areas, such as evolutionary biology, demography, and studies on power relations (MARODY, 1996). In the 1990s, the 'transition' concept was introduced within socio–technical research. In the latter, 'transitions' initially referred to large-scale transformations within society or important subsystems, during which the structure of the societal system fundamentally changes (ROTMANS *et al*., 2001). More recently, the definition has been refined in such a way by LOORBACH and ROTMANS (2010) that the concept now stands for "*a fundamental change in structure (e.g. organizations, institutions), culture (e.g. norms, behaviour) and practices (e.g. routines, skills)*". According to STERRENBERG *et al.* (2013:9), radical systems innovations or transitions involve "*innovations that are directed to redesigning entire systems of practices and provisions, instead of individual products or processes*". Transitions efforts have often borrowed from different strands of research and disciplines, resulting in a myriad of approaches towards understanding and exercising transitions (LACHMAN, 2013). Overviews of transition theories and approaches can be found in GEELS (2005), OLSTHOORN and WIECZOREK (2006), GRIN *et al*. (2010), and MARKARD *et al*. (2012).

The momentum generated by the diffusion of the term 'sustainable development' (WCED, 1987) spurred an interest in research on transitions towards sustainable futures (MARKARD *et al*., 2012; LACHMAN, 2013; FALCONE, 2014). Ambiguity, complexity, interconnectedness and multidimensionality of sustainability problems imply that incremental changes are no more sufficient and there is a need for transformative change at the systems level (STRN, 2010). Embracing the goal of transition towards sustainable systems, the notion of 'sustainability transition' was coined (GEELS, 2011; KEMP and VAN LENTE, 2011; LACHMAN, 2013). MARKARD *et al.* (2012:956) defined sustainability transitions as *"long-term, multi-dimensional and fundamental transformation processes through which established socio-technical systems shift to more sustainable modes of production and consumption"*. Sustainability transitions are needed also in the agro-food arena to move towards sustainable food systems.

According to the High Level Panel of Experts on Food Security and Nutrition (HLPE, 2014), *"A sustainable food system (SFS) is a food system that delivers food security and nutrition for all in such a way that the economic, social and environmental bases to generate food security and nutrition for future generations are not compromised"*. This definition clearly shows the strong linkage between food system sustainability and long-term food security. The International Panel of Experts on Sustainable Food Systems (IPES-FOOD, 2015) pointed out that a multi-directional flow of knowledge between the worlds of science, policy and practice is needed to foster a genuine transformation of food systems, which is necessary to make transition towards sustainability. This is urgently required, among others, because food systems are complex 'social-ecological' systems that require different sources of knowledge to be combined. Therefore, there is a need for a real food-related knowledge revolution to overcome persistent lock-ins and path dependencies. Transition will most likely not depend on one or even a small number of technological innovations, but is likely to arise from a constellation of mutually interacting systems of innovations (TWOMEY and GAZIULUSOY, 2014). This is particularly true in the case of food system where social innovations seem also important. According to HINRICHS (2014), social and organizational innovations are as central to sustainability transitions in food systems as any particular innovative technology. Social innovation will likely play an important role in transitions to sustainability in agriculture and food sector that may not be primarily technology-driven (DARNHOFER, 2015) and the transition to sustainable food systems requires complex and holistic change processes in which social innovation plays as big a role as technological innovation (IPES-FOOD, 2015).

The review paper aims to analyse innovation narratives in sustainability literature. In particular, the paper explores the complexity of relation between innovation and sustainability with a particular focus on agro-food systems.

The paper is structured as follows. In section 2, I explore the complex relation between innovation and sustainability (cf. sustainable development) by analysing, among others, references to innovation in the outcomes of the main conferences on sustainable development as well as in the Sustainable Development Goals (SDGs). This section also introduces the concept of Sustainability-Oriented Innovation (SOI) with particular reference to sustainable innovation and eco-innovation as a way to combine innovation and sustainability. In section 3, I analyse the relation between innovation and sustainability in the agro-food arena and I highlight harmony and conflict areas. Sustainable intensification is taken as an example to show diversity of perspectives, agendas and visions regarding sustainable agriculture. The section also analyses attitude of some alternative agro-food movements (e.g. organic agriculture, agro-ecology, food sovereignty, Slow Food) towards innovation.

2. INNOVATION AND SUSTAINABILITY: EXPLORING MULTIFACETED LINKAGES

The relation between innovation and sustainability can be analysed at least in two different ways: innovation as a driver of sustainability (role of innovation in achieving sustainable development) or sustainability as a driver of innovation (sustainability as a new paradigm and guiding concept for innovation).

Innovation, science and technology have essential roles to play in meeting the interlinked global environmental, social, and economic challenges of environmental sustainability, poverty reduction, social justice and climate change (STEPS CENTRE, 2010; UN, 2012). In fact, innovation is seen as a route to economic growth and competitive economy as well as to propose effective solutions to real problems such as poverty and environmental challenges (STEPS CENTRE, 2010). The International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD, 2009) highlights that knowledge, science and technology (AKST) is crucial to address different sustainable development issues such as food insecurity and poverty. It sheds also light on the fact that the scope of agricultural knowledge goes beyond the narrow confines of science and technology (S&T) and encompasses other types of relevant knowledge that is held by agricultural producers, consumers and end-users.

The contribution of innovation to sustainability is highlighted in many strategic and policy documents dealing with sustainable development such as the outcomes of the recent world conferences of Rio, Johannesburg and Rio+20 (Box 1).

Innovation was also addressed recently in the context of the 2030 Agenda for Sustainable Development and Sustainable Development Goals (SDGs) (Box 2). According to LEACH *et al*. (2012:2), delivering SDGs requires a radically new approach to innovation. They add that "*What is now needed is nothing short of major transformation—not only in our policies and technologies, but in our modes of innovation themselves—to enable us to navigate turbulence and meet SDGs that respect the safe operating space*".

Sustainability is considered nowadays a driver for innovation especially in the private sector (e.g. NIDUMOLU *et al*., 2009). Many companies are seizing the strategic opportunities in innovation for sustainability. In fact, sustainability, environmentalism, and corporate social responsibility (CSR) have become during the last decades buzzwords among multinational corporations, agribusinesses included, with a risk of 'green-washing' (e.g. MUNSHI and KURIAN, 2005). Many private firms see nowadays sustainability as a key factor for their competitiveness and understand that innovation will be crucial factor in developing sustainability. Sustainability is often understood as the voluntary integration of environmental, economic and social concerns in firm operations (VILANOVA and DETTONI, 2011). KASKINEN *et al*. (2013) suggest that there are fundamentally three ways of incorporating sustainability into companies; risk aversion strategy (use of certificates and standards as a reaction to an external critique), cost-effectiveness strategy (cost savings through a smart and effective use of resources), and differentiation strategy (using sustainability to distinguish company's offering on the market). As put by DEARING (2000:2), the operational and commercial challenges for companies are "[…] *to learn to treat sustainable development as a framework for innovation and to use and extend established management principles to make this framework operational and effective*".

Box 1. Innovation in the outcome documents of world conferences on sustainable development.

In '*Our Common Future*', the final report of the World Commission on Environment and Development (WCED, 1987) – that, among others, mainstreamed the concept of sustainable development – there is quite a number of references to innovation. The document is rather critical towards innovation and calls for reorienting technology and managing risk by enhancing capacity for technological innovation in developing countries and adapting recent innovations to their needs. It also calls for broadening the scope of innovation beyond product and process innovations. *Our Common Future* emphasizes the need to blend traditional and modern technologies and to promote collaborative learning in agriculture: "*Researchers must learn from and develop the innovations of farmers and not just the reverse*" (p. 116). This is a clear stance against linear innovation model. It should be highlighted that much of the focus of *Our Common Future* is on technological innovation, whereas there is no reference to social innovation; the document even reports that traditional social systems and community control over agricultural practices "*may have limited the acceptance and diffusion of technical innovations*" (p. 44).

Interestingly, the first reference to innovation in *Agenda 21*, the Rio Declaration on Environment and Development (UN, 1992), was in its chapter on *Changing Consumption Patterns* where it highlighted the multiple sources of innovation "*Peoples' organizations, women's groups and non-governmental organizations are important sources of innovation*" (p. 15). This broader scope of innovation is confirmed in chapter on conservation of biological diversity that calls for promoting "*the wider application of the knowledge, innovations and practices of indigenous and local communities*" (p. 150). Besides 'technological innovation', there is also a clear reference to 'social innovation' (p. 129) as well as 'informal innovations' (p. 156), and 'indigenous innovations' (p. 319). Innovation is considered as important to prevent pollution and control environmental degradation. However, *Agenda 21* also highlighted the importance of assessing the relationship between innovation and development as well as effects of innovation. There is also an entire chapter dedicated to science for sustainable development.

In the Plan of Implementation of the World Summit on Sustainable Development 2002 (UN, 2002) as well as in the report of the Summit (UN, 2002a) there is only one reference to innovation in relation to recognition of the rights of local and indigenous communities as holders of traditional knowledge, innovations and practices regarding biodiversity.

The *Future We Want*, outcome document of the United Nations Conference on Sustainable Development 2012 (UN, 2012a), includes also a few references to innovation. In its preamble, it calls for continued and strengthened international cooperation in the area of innovation to achieve sustainable development. It also recognizes the critical role of promoting innovation especially in developing countries and invites governments to create enabling frameworks that foster environmentally sound innovation, including in support of green economy. In its *Framework for action*, it emphasizes the importance of investments in scientific and technological innovation in job creation. Once again, the traditional knowledge and innovations of indigenous peoples in relation to biodiversity are recognized. Further references to innovation are in the context of education and finance.

Box 2. Innovation in the 2030 Agenda for Development Sustainable and SDGs.

The General Assembly of the United Nations adopted on September 25th, 2015, a set of 17 goals (and 169 targets) as part of the 2030 Agenda for Sustainable Development (UN, 2015). The only SDG where innovation is explicitly mentioned is SDG9 '*Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation*'. There are only few references to innovation in the adopted Agenda. Innovation is considered in the Agenda preamble especially regarding its potential in medicine and energy sectors as well as in sustainable urban development; interestingly no reference to agriculture here. Moreover, there was no reference to innovation either in relation to the targets of SDG2 '*End hunger, achieve food security and improved nutrition, and promote sustainable agriculture*'. Only in the case of SDG8 '*Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all*', technological upgrading and innovation are considered essential to achieve higher levels of economic productivity and there is a call to promote development-oriented policies that support, *inter alia*, creativity and innovation. Innovation is further mentioned two times in targets of SDG9 in reference to upgrading technological capabilities of industrial sectors by, *inter alia*, encouraging innovation especially in developing countries. Nevertheless, the part of the document that abounds with references to innovation is that related to SDG17 "*Strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development*" especially in sections dealing with technology (see, access to science, technology and innovation; innovation capacity-building mechanism for least developed countries), and means of implementation and the global partnership (see, Addis Ababa Action Agenda, which addresses also systemic issues in science, technology and innovation, and established a Technology Facilitation Mechanism - TFM; private business activity, investment and innovation). It seems, anyway, that there is a recognition of, and maybe also concerns about, the role of technology and innovation in sustainable development and that may explain the establishment of many mechanisms for follow-up of this issue during the implementation of the 2030 Agenda. In fact, TFM - that involves representatives of Member States, civil society, the private sector and the scientific community – is composed of a United Nations inter-agency task team on science, technology and innovation for the SDGs; a collaborative multi-stakeholder forum on science, technology and innovation for the SDGs; and an online platform to share information on existing science, technology and innovation initiatives.

According to SZEKELY and STREBEL (2012), 'strategic innovation for sustainability' – which focuses on the use of innovation to improve performance in environmental, economic and social dimensions of sustainable development - entails improvements that are not only technological/technical but also in business models, thinking, operating procedures and practices, processes, and systems. They consider that types of innovation for sustainability ranges from incremental innovations in products and services or eco-design (e.g. innovations that focus on improvement in eco-efficiency through reduction in resource inputs such as energy, materials, wastes and emissions), to radical innovations in value chains and processes, to 'game-changing systemic innovation'. Radical transformation of supply chains aims to take more account of the impacts of a company's products and operations, including environmental (e.g. raw materials sourcing, end-of-life), economic (e.g. competitiveness) and social (e.g. labour conditions) issues. Examples of 'game-changing innovation' include collaborative consumption (e.g. car sharing and sharing economy in general), that has been enabled by information technology, as well as social entrepreneurship (e.g. micro-credit to the poor). This reminds of the concept of ‘open innovation’ (e.g. CHESBROUGH, 2003; CHRISTENSEN *et al.*, 2005) that calls for a more open approach towards knowledge management and dissemination to assure a wider access to, and consequently use of, knowledge and innovation. Open innovation concept stresses innovation and knowledge as public goods (e.g. STIGLITZ, 2007) to which a wide range of stakeholders should have access. Open innovation is the opposite of closed innovation. Processes of closed innovation focus on in-house development of innovation before their dissemination to external stakeholders. On the contrary, open innovation focuses on “[...] *the use of purposive inflows and outflows of knowledge to accelerate Innovation*” (CHESBROUGH, 2003). However, finding a balance between reward (for innovation and creativity) and accessibility remains one of the fundamental challenges in science, technology and innovation ecosystems.

There is a growing emphasis on the concepts of ‘responsible’, ‘sustainable’, ‘social’ and ‘ecological’ innovation (Table 1).

Table 1. Some definitions of concepts of sustainable innovation, eco-innovation and social innovation.

Concept	Definition	Source
Sustainable innovation	Sustainable innovation is a process where sustainability considerations (environmental, social, financial) are integrated into company systems from idea generation through to research and development (R&D) and commercialisation. This applies to products, services and technologies, as well as new business and organisation models.	CHARTER and CLARK, 2007
	Creating new or improved products, services, technologies, processes and management techniques that produce environmental or social benefits along with economic value.	CHONKOVA, 2015
Sustainability-driven innovation	The creation of new market space, products and services or processes driven by social, environmental or sustainability issues.	KEEBLE *et al.*, 2005
Eco-innovation	The process of developing new products, processes or services which provide customer and business value but significantly decrease environmental impact.	FUSSLER and JAMES, 1996
	Eco-innovation is the production, assimilation or exploitation of a product, production process, service or management or business method that is novel to the organisation (developing or adopting it) and which results, throughout its life cycle, in a reduction of environmental risk, pollution and other negative impacts of resources use (including energy use) compared to relevant alternatives.	KEMP and PEARSON, 2008
	The production, assimilation or exploitation of a novelty in products, production processes, services or in management and business methods, which aims, throughout its lifecycle, to prevent or substantially reduce environmental risk, pollution and other negative impacts of resource use (including energy).	EC 2008 in CARRILLO-HERMOSILLA *et al.*, 2010
Social innovation	Social innovations ‘are new solutions (products, services, models, markets, processes etc.) that simultaneously meet a social need (more effectively than existing solutions) and lead to new or improved capabilities and relationships and better use of assets and resources.	CAULIER-GRICE *et al.*, 2012
	Social innovations are new ideas that meet social needs, create social relationships and form new collaborations. These innovations can be products, services or models addressing unmet needs more effectively.	EC, 2017
	Innovative activities and services that are motivated by the goal of meeting a social need and are predominantly developed and diffused through organizations whose primary purposes are social.	MULGAN *et al.*, 2007

Responsible innovations (RI) address so-called 'grand challenges' of our time, such as climate change (EC, 2013), but they are also associated with a range of socio-ethical issues. The NETWORK FOR BUSINESS SUSTAINABILITY (2012) identified multiple definitions relating to 'Sustainability-Oriented Innovation' (SOI): eco-innovation, ecological innovation, environmental innovation, frugal innovation, green innovation, green product innovation, inclusive innovation and social innovation. Sustainable innovation means paying attention to ecological integrity along social values diversity, promoting fairer und wider distribution of innovation benefits, encouraging plural innovation pathways, fostering inclusive and participatory governance of innovation processes (STEPS CENTRE, 2010). SOI is differentiated from conventional innovation in its purpose and direction as it adds environmental and social consideration to economic profit (BOS-BROUWERS, 2010). Key drivers of sustainable innovation include environmental and resource risks, sustainable consumption and production (SCP) policies, product environmental regulation and other product policy initiatives as well as market and financial drivers (CHARTER and CLARK, 2007). Also for CHONKOVA (2015), drivers of sustainable innovation include compliance with existing regulation or anticipating future regulations, cost savings by improving resource efficiency as well as social and supply chain pressures. Meanwhile, according to NIDUMOLU *et al*. (2009) to become sustainable, companies should go through five distinct stages of change: viewing compliance as opportunity, making value chains sustainable, designing sustainable products and services, developing new business models and creating next-practice platforms.

Sustainable innovation is widely recognised as a critical dimension of sustainable development as well as sustainable consumption and production (SCP), sustainable food systems included. In fact, the crucial importance of sustainable innovation in these contexts has been recognised since the 1980s and was reinforced since the 1990s not only by United Nations (UN) but also in the European Union (EU). However, sustainable innovation has remained mainly peripheral. Nevertheless, the subject is now rapidly moving to centre stage to meet sustainable development challenges of a growing population. In fact, the urgency of adopting sustainable innovation is nowadays recognised as a fundamental step towards a sustainable future (CHARTER and CLARK, 2007). For instance, the European Commission (EC) issued in 2016 two strategic notes dealing with innovation and sustainability namely '*Opportunity Now: Europe's mission to innovate*' (EPSC, 2016) and '*Sustainability Now! A European Vision for Sustainability*' (EPSC, 2016a).

GERHARDT and HUBBERT (2009) distinguish between conventional innovation (characterised by low sustainability, both environmental and social), green innovation (based on natural resources use and with positive or neutral environmental impact), social innovation (that contributes to social well-being and is accessible by consumers in emerging and developing countries) and sustainable innovation, that's to say addresses the triple bottom line (ELKINGTON, 1997) i.e. is environmentally, socially and economically sustainable. Sustainable innovation covers the spectrum of levels of innovation from incremental to radical. STEVELS (1997) defined four levels of innovation in the context of environmental improvement; from incremental, re-design or green limits, functional or product alternatives, to systems design. According to the NETWORK FOR BUSINESS SUSTAINABILITY (2012, 2012a), firms can adopt different pathways to become sustainable. These range from 'Operational Optimization' (small incremental changes to improve eco-efficiency) to 'Systems Building' (radical and disruptive changes that have a positive societal impact) through 'Organizational Transformation' (new products, services or business models).

A concept similar to green innovation, and to a certain extent also sustainable innovation, is that of eco-innovation (e.g. KEMP and FOXON, 2007; CHARTER and CLARK, 2007;

REID and MIEDZINSKI, 2008; CARRILLO-HERMOSILLA *et al*., 2010). However, CHARTER and CLARK (2007) pointed out that although the two terms, sustainable innovation and eco-innovation, are often used interchangeably, sustainable innovation embraces all dimensions of sustainability (environmental, economic, social/ethical) while eco-innovation addresses mainly environmental and economic dimensions. ANDERSEN (2005) distinguishes the following five categories of eco-innovations: add-on innovations, integrated innovations, eco-efficient technological system innovations, eco-efficient organizational system innovations, and general purpose eco-efficient innovations. Eco-innovation can be analysed in terms of its target (product, process, marketing method, organisation, institution), mechanism (modification, re-design, alternatives, creation) and impact (effect on the environment). Systemic changes, such as creation and alternatives, generally generate higher environmental benefits (OECD, 2009). The drivers for eco-innovation include cost reduction, increasing market share, pressure from regulation or communities, improving technical efficiency, green ethos, profits from commercialisation, and improving the company image (KEMP and FOXON, 2007).

Many companies as well as a number of governments use eco-innovation to describe their contributions to sustainable development. For instance, eco-innovation is considered to support the Lisbon Strategy for competitiveness and economic growth in the European Union (EU) while preventing or substantially reducing negative impacts on the environment, pollution and improving resource efficiency (OECD, 2009; EC, 2012). The Lisbon Strategy called for focusing on innovation and sustainable development and was, thus, a clear example of focus on sustainable innovation. This tendency to focus on sustainable innovation in the EU was reaffirmed in the declaration on the '*Strategy for smart, sustainable and inclusive growth*' that should drive the EU until 2020. Other key international organisms (e.g. OECD), UN agencies (e.g. ILO, WTO) as well as the World Economic Forum advocated similar approaches (VILANOVA and DETTONI, 2011). In fact, according to OECD (2008), eco-innovation is considered to have promise for improving environmental conditions without compromising economic growth. While eco-innovation has focused mainly on environmental technologies, there is a tendency to broaden the concept scope in order to accommodate more societal concerns (RENNINGS, 2000; METI, 2007). Therefore, the overarching concept of eco-innovation is seen as providing vision for pursuing sustainable development.

A further concept related to sustainable innovation is that of social innovation (e.g. NICHOLLS and MURDOCK, 2012; CAULIER-GRICE *et al*., 2012; OSBURG and SCHMIDPETER, 2013), that deals with the integration of environmental and social issues. As put by OSBURG (2013), social innovation is about adding the social element to innovation or the applied theory of innovation with addition of a relevant and significant social component. According to CHONKOVA (2015), social innovation can be both business oriented (product, process, organisational, marketing) and/or socially oriented (i.e. social innovation). Social innovations are considered good not only for the economy but also for society (CAULIER-GRICE *et al*., 2012) as they engage with social problems in a way that is more efficient, just, effective or sustainable than existing solutions (PHILLS *et al*., 2008). Nevertheless, social innovations are not value neutral but rather socially and politically constructed, and context dependent (CAULIER-GRICE *et al*., 2012). According to OSBURG (2013), open innovation is a must for social innovation, that cannot work with closed innovation processes, as solving current problems in today's societies requires a constant collaboration across sectors and between different categories of stakeholders. CAULIER-GRICE *et al*. (2012) identified eight common features of social innovation: cross-sectoral, open and collaborative, grassroots and bottom-up, *pro-sumption* (see, production-consumption) and co-production, mutualism, creating new roles and relationships, better use of assets and resources, and developing assets and capabilities.

In a Manifesto on innovation, sustainability, development, STEPS CENTRE (2010) called for a radical shift in how we think about and perform innovation towards a greater respect for and integration of cultural variety, democratic accountability and regional diversity. Moving towards innovation for sustainability and sustainable development means nothing less than a radical change in the whole innovation process including agenda setting, capacity building, governance, monitoring, evaluation accountability as well as funding. For that, three arrays of questions related to direction, distribution and diversity, should be addressed (Table 2). These three issues are interrelated. For instance, direction matters also because it shapes innovation benefits, risks and costs distribution. Meanwhile, the appraisal of innovation directions needs to take into account also benefits distribution and to address social equity and justice issues. Furthermore, taking seriously direction and distribution questions, means pursuing deliberately a diversity of innovation pathways to accommodate different needs and aspirations including those of marginalised and poor groups such as small-scale farmers. This, in turn, implies paying attention not only to technical dimension of innovation but also social and organisational ones (STEPS CENTRE, 2010). These questions are not only still actual but also particularly relevant in agro-food systems.

Table 2. Arrays of questions regarding innovation for sustainable development.

Issue	Questions to be addressed
Technical, social and political directions for change	What is innovation for? Which kinds of innovation, along which pathways? And towards what goals?
Distribution	Who is innovation for? Whose innovation counts? Who gains and who loses?
Diversity	What - and how many - kinds of innovation do we need to address any particular challenge?

Source: STEPS CENTRE (2010:9).

3. COMPLEX AND MULTIFACETED RELATION BETWEEN INNOVATION AND AGRO-FOOD SUSTAINABILITY

Innovation has become a key issue in the discussion about the relation between agriculture and sustainability (e.g. FAO, 2012; EIP-AGRI, 2013; FAO, 2013; IPES-FOOD, 2015; GLOBAL HARVEST INITIATIVE, 2016). In general, there is a broad consensus on the critical role of innovation to make agriculture not only more competitive but also sustainable. In fact, agricultural innovation is considered vital for meeting the challenges of agriculture development, adapting to climate change and achieving food security (IAASTD, 2009; IICA, 2014; EC, 2016; UNCTAD, 2017). Innovations and modern techniques can strengthen food system resilience, improve resource efficiency in agriculture, and secure social equity thus contributing to the achievement of sustainable food security (HLPE, 2017). The European Union has placed emphasis upon innovation as a key element in achieving transformation towards sustainable agriculture (HERMANS *et al*., 2010; DWYER, 2013).

Agricultural research and development (R&D) has been shown to very profitable (ALSTON *et al*., 2000; RAO *et al*., 2012) and to improve agricultural development, economic growth, and poverty reduction (IAASTD, 2009). However, assessing innovation in agriculture only through investment in agricultural R&D shows clearly that the linear model of innovation is still dominant in official arenas and, especially, when it comes to innovation statistics. Nevertheless, IAASTD (2009) highlights that "*There is ample evidence available from the literature that AKST investments have contributed significantly to organizational and institutional innovations in the form of methods, tools development, capacity strengthening, and understanding how institutes interact with each other in achieving developmental goals*" (p. 516).

The High Level Panel of Experts on Food Security and Nutrition (HLPE, 2017) identified in a recent note knowledge and technology as critical emerging issues for food security and nutrition. As there are diverging views on the suitability of different innovations and technologies to improve food security in a sustainable way in different contexts and for different kinds of users – for instance, in the context of small-scale agriculture and agri-food supply chains (e.g. PEANO *et al*., 2015; WETTASINHA, 2016), HLPE (2017) recommended to assess all innovations and technologies against their long-term environmental, economic and social impacts. Such an assessment should take into consideration not only technical sustainability and economic profitability but also environmental friendliness and social justice in each use context (e.g. DUNMADE, 2002; KRIESEMER and VIRCHOW, 2012).

The relation between innovation and sustainability (including sustainability transitions) in agro-food system is more complicated than in other systems and sectors. Although more recent sustainability transitions research has stressed that important sustainability innovations can be social rather than technological (SEYFANG and SMITH, 2007; KIRWAN *et al*., 2013), research on food systems change has long favoured a different vocabulary of civic initiatives, community development projects and social movements to reference what sustainability transitions researchers present as manifestations of 'grassroots innovations' for sustainable development (HINRICHS, 2014). It is widely admitted nowadays that to meet sustainability challenges, more attention should be paid to social innovations, grassroots innovation actors and processes (LEACH *et al*., 2012; MOULAERT, 2013; SMITH and SEYFANG, 2013; LOCONTO *et al*., 2017). Similarly, IAASTD (2009), suggests that future agricultural innovation needs to address not only simple technological and technical issues but also social ones and to innovate in scales of thinking and action in order to contribute more effectively in addressing pressing challenges such as climate change and food security. Likewise, innovation in rural development is widely understood, especially in the European Union, in terms of social innovation (i.e. encouraging collective learning cultures, networks, interactions) and cultural innovation (i.e. improving rural context) rather than in the narrow sense of technological innovation (DARGAN and SHUCKSMITH, 2008; DWYER *et al*., 2012; HERMANS *et al*., 2010). This broader understanding of innovation in agriculture is nowadays widespread predominantly in developed countries such as those of the European Union, as clearly stated by its Standing Committee on Agricultural Research (SCAR): "*Innovation is not restricted to a technical or technological dimension. It increasingly concerns strategy, marketing, organization, management and design*" (SCAR-EU, 2012).

Innovations imply different directions of development, not all of which are sustainable, and which should be subject to democratic debate (STRN, 2010). Critically inclined research on agricultural and food systems change has generally viewed capital-intensive technologies as contributing to the vast restructuring of food and agriculture and 'sustaining the unsustainable' (BUTTEL, 2006), especially referring to genetically modified (GM) crops. Because some technologies have abetted industrialization, consolidation, and global neo-liberalization of food and agriculture, technology may be categorically dismissed by some scholars and food system actors as a potentially productive analytical entry point for work on sustainability transitions in food and agriculture (HINRICHS, 2014). In fact, innovation and technology in agriculture may also negatively affect the environment and rural livelihoods and that may explain increasing mistrust in certain institutionalized forms and fields of science (MILLSTONE and van ZWANENBERG, 2000) such as genetics. Management of collective rights and intellectual property rights - IPR (cf. fields of big data and genetics) is particularly problematic and challenging in the agricultural sector (HLPE, 2017). In this regard, biotechnologies raise many ethical concerns as highlighted by the European Group on Ethics in Science and New Technologies (EGE, 2008:59): "*The current IPR system (for plant varieties and GM crops) could pave the way for market predominance where a few companies control much of agricultural production, with an impact on innovation and the growth of local economies in developing countries*".

The three perspectives on sustainable food security and food system sustainability analysed by GARNETT (2014) – namely efficiency, demand restraint, food system transformation – also reflect different values and ideologies on the role of technology and innovation in the agro-food arena. For the efficiency perspective, the boundaries of environmental limits can be expanded or overcome by using technology to accommodate humanity. The vision underlying the efficiency perspective is that technology can be used to deliver development goals (e.g. food security, well-being) with less environmental impact. Therefore, it can be assumed that advocates of this perspective have a positive attitude towards new technologies and innovation. Meanwhile, for the demand restraint perspective, technology is sometimes problematic and can be used by humans to further damage the environment and nature.

Nevertheless, innovation has always occurred in agriculture as farmers have adapted agricultural practices to changing climate and environment conditions. However, many scholars dealing with agro-food system do not feel comfortable with the current narrow definition of innovation meaning technological and commercialised innovation (LEVIDOW, 2015). This narrow 'technological-deterministic' understanding of innovation dates back to the early 20th century, when innovation was considered as synonymous of adoption of commercial technological inventions. This innovation model, that emphasises capital-intensive technology, has become profoundly entrenched in research and policy frameworks. The model ignores existing farmers' knowledge and marginalises their cooperative exchange and learning networks and undervalue their capacity to innovate while favouring a linear knowledge transfer (MOSCHITZ *et al*., 2015) based on the Transfer of Technology (ToT) model (LIONBERGER, 1960; HAVELOCK, 1969; CHAMBERS and JIGGINS, 1987). ToT model stimulates farmers to capture economies of scale and encourages externalization of significant environmental and social costs (e.g. LAL *et al*., 2005; MUKHERJEE and KATHURIA, 2006; IAASTD, 2009).

According to IAASTD (2009) report, in general, no recognition is given to farmers' local and traditional knowledge and their innovation in official systems of agricultural knowledge and science. In fact, the "*role of traditional, indigenous knowledge is already being undermined as a result of the growing disconnection with ongoing research activity*" (SCAR-FEG, 2007:11). The linear innovation model has privileged laboratory-based and scientific knowledge in research agendas at the expense of farmers' agro-ecological knowledge (VANLOQUEREN and BARET, 2009). This process was seen as causing profound social or cultural changes (GODIN, 2008, 2015), that are not always positive on farming and rural communities. Moreover, inequitable power relationships in agricultural knowledge and information system create barriers to farmers' innovation (SILICI, 2014). Therefore, in order to contribute more effectively to achieving sustainable food and nutrition security, agricultural research in particular and food-related research in general should adopt a 'food system approach' and address at the same time profitability, productivity and

sustainability in agricultural and food systems (GLOBAL PANEL ON AGRICULTURE AND FOOD SYSTEMS FOR NUTRITION, 2016).
Multinationals and consulting firms in the agro-food sector seem nowadays more aware about concerns regarding technology and innovation in agriculture. However, they stress the importance of classical technologies (fertilizers, crop protection products) in meeting food security challenge through increase in productivity. For them, the slowing down or plateauing of crop yields, especially cereals, is a big threat to future global food security that requires new breakthrough innovations for the 'sustainable' intensification of production. The latter include digital innovation (e.g. soil sensors, drones), biotech innovations (e.g. genetically engineered plants and animals) and process innovation (e.g. vertical farming, hydroponics, aquaponics). However, acceptance of these innovations and technologies is still a problem in agriculture sector: "*While these technological innovations have the potential to make a positive impact on agribusiness, the challenge is to find common ground between the significant social, political, and environmental concerns and the business interests surrounding these disruptive changes*" (ATKEARNEY, 2016:3). In order to gain such an acceptance, the advantages of such technologies for smallholder farmers in developing countries as well as relevance of these breakthrough innovations in reducing the carbon footprint of agriculture and its contribution to climate change are often highlighted. Further moves include global transfer of knowledge, also to developing countries, transparency and the democratization of data and better collaboration between agribusiness, government and the civil society (ATKEARNEY, 2016).
Many agro-food companies, including multinationals, are paying more attention to sustainability issues. A clear example of that is publication of periodical sustainability reports (e.g. MONSANTO, 2017) or dedicating a section of annual reports to sustainability (e.g. BAYER, 2017; BASF, 2017). In its Sustainability Report, Monsanto makes even a clear reference to SDGs and states that its key principles are: *act ethically and responsibly*, *advocate for biodiversity*, *advance product stewardship*, *create a great work environment*, *drive modern agricultural innovation*, *engage communities and society*, *foster collaboration and transparency*, *improve global food and nutrition security*, *reduce our environmental impact* (MONSANTO, 2017:5). Therefore, there is a clear intention to connect agricultural innovation and technology with societal challenges such as food security, environmental protection, biodiversity as well as ethical concerns. This move of agri-multinationals is often considered as an example of incremental change or even, worse, of 'greenwashing' (e.g. SCANLAN, 2013). However, for instance, SZEKELY and STREBEL (2012) consider the Unilever's transformation of its tea supply chain for its Lipton brand to certified sustainable tea as an example of 'radical innovation'. In fact, Unilever entered into a partnership with the Rainforest Alliance (RfA) to certify tea supplies focusing on the areas of environmental protection, employee welfare and farm management. This shows clearly the divergences in opinions regarding sustainability transitions in the agro-food arena. In fact, there are several contending paradigms and narratives about sustainable agriculture and way to achieve it (VAN DER PLOEG, 2009; LEVIDOW, 2011; ELZEN *et al*., 2017). A clear example of these contending agendas about agricultural innovation are the '*life sciences & global value chains*' (see, knowledge-based bio-economy) and '*agroecology & agro-food-energy relocalisation'* in the EU (LEVIDOW, 2011).
Sustainable intensification (e.g. GARNETT *et al*., 2013) is a good example to show diversity of visions, agendas and perspectives regarding sustainable agriculture. It clearly shows trade-offs not only between productivity and sustainability aspirations but also between innovation (that ideally should help improving, simultaneously, both productivity and sustainability) and sustainability. Sustainable intensification agendas promote a 'toolkit' of various options and techniques for reconciling higher productivity with environmental sustainability. The orthodox consensus on 'technological intensification' has been

challenged by a variety of concerns such as environmental protection, animal welfare, and food quality and safety (LOEBER and VERMEULEN, 2012). Nevertheless, some agri-companies have seized the 'sustainable intensification' momentum to rebrand their products as sustainable intensification tools (CONSTANCE *et al*., 2016). In fact, the *neoproductivist* agenda (e.g. ALMÅS and CAMPBELL, 2012) has been widely articulated under a sustainable intensification approach that encompasses various agroecological but also even biotechnological methods to increase yield, while also lowering the burdens on the environment (GARNETT and GODFRAY, 2012). In some contexts, the sustainable intensification toolkit is reduced to only biotechnological solutions such as GM crops (YOUNG, 2013). Meanwhile, counter-hegemonic global food movements embrace agroecology and community-based food systems. They promote a concept of 'eco-functional intensification' (NIGGLI *et al*., 2008). However, there are also some attempts to reconcile these two opposed agendas. For instance, a report from the Rural Investment Support for Europe (RISE) mentioned six systems to achieve sustainable intensification (BUCKWELL *et al*., 2014): agroecology, biodynamic, organic, integrated, precision farming and conservation agriculture. Similarly, in the European Union, where mainly capital-intensive technological innovation is emphasized, agroecology has been promoted as a different kind of practice that combines know-how, organizational, social and technological innovation (IFOAM EU GROUP *et al*., 2012). Disagreement about model of agriculture that allows reconciling productivity (cf. innovation) and sustainability (especially environmental one) was evident during the setting up of the European Innovation Partnership (EIP) for Agricultural Productivity and Sustainability (EIP-AGRI). In the end, the Strategic Implementation Plan of EIP-AGRI encompassed different approaches such as sustainable intensification, organic farming, low-external input systems (EIP-AGRI, 2013).

The attempt to show at least an apparent compatibility and harmony between productivity (see, technological innovation) and sustainability in agriculture is somehow exported to other world regions such as Sub-Saharan Africa. For instance, four different pathways to sustainable intensification of agri-food systems in Africa were identified in the PROIntensAfrica project (a Horizon 2020 coordination and support action): conventional agriculture pathway, eco-technical pathway, agroecology pathway, and organic agriculture pathway (PROIntensAfrica, 2017).

Generally speaking, some alternative agro-food movements (e.g. food sovereignty, Slow Food, agroecology) have a critical attitude towards innovation (especially technical/technological one) while others (e.g. organic agriculture) have evolved towards a more accommodating position. In the manifesto '*Food Sovereignty: A Future Without Hunger*', presented by Via Campesina at the 1996 World Food Summit (VÍA CAMPESINA, 1996), there is no reference to innovation. This clearly shows a critical attitude towards innovation of this peasant movement. However, access to technology by peasant families, especially women, is stressed. In the Declaration of Nyéléni (NYÉLÉNI, 2007), food sovereignty movement did again no reference to innovation, be it technical or social. However, it made it clear that it is fighting against "*Technologies and practices that undercut our future food producing capacities, damage the environment and put our health at risk. Those include transgenic crops and animals, terminator technology, industrial aquaculture and destructive fishing practices, the so-called white revolution of industrial dairy practices, the so-called 'old' and 'new' Green Revolutions, and the "Green Deserts" of industrial bio-fuel monocultures and other plantations*" (p. 3). Slow Food movement pays a particular attention to the ecological, economic, social and cultural sustainability of the local agro-food systems (e.g. SLOW FOOD, 2013; PEANO *et al*., 2014). It can be argued that the movement has a critical stance with respect to innovation and technology. In fact, references to innovation can be hardly found in the declarations of the movement (e.g. SLOW FOOD, 2008; Slow Food, 2009). The term innovation has also no place in Slow Food terminology (SLOW FOOD, 2015). The organic agriculture movement seems to have nowadays, as it was not always the case, a more accommodating attitude towards innovation. In fact, innovation is even part of the official definition of organic: "*Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved*" (IFOAM, 2010).

One of agro-food alternatives is agroecology that has been coined by scientists with the intention to open up scientific preoccupations and to contest technocratic governance of agricultural innovation, oriented towards commercial benefits, agricultural intensification and expansion of global trade (ELZEN *et al*., 2017). Agroecology is not against innovation in general but certain types of innovation. In fact, as the Institute for Agriculture and Trade Policy (IATP, 2013) points out agroecology *"is by definition an innovative, creative process of interactions among small-scale producers and their natural environments"*. However, agroecology faces the task of challenging the dominant models of innovation in agriculture. Beside technological-scientific innovation, it embraces also know-how, social and organisational innovation forms (IFOAM EU GROUP *et al*., 2012). Agroecology promotes social and organisational innovation as an alternative strategy across the whole agro-food chain with the aim of strengthening connection between agro-ecological farmers and consumers that support their agro-ecological innovations. These agro-ecological initiatives are variously known as short food supply chains (SFSCs) or alternative agro-food networks and they are clear examples of social innovation (GALLI and BRUNORI, 2013). Such new agro-ecologically-inspired local networks and citizen-community alliances can counterweight the dominant agri-food system (FERNANDEZ *et al*., 2013) provided that they professionalise their skills, maintain consumer loyalty, constantly learn and continuously innovate (KARNER, 2012) thus developing genuine and sustainable local food systems.

Agro-ecological innovation is key to the transition towards sustainability in the current agro-food system (LEVIDOW, 2015). Thanks to many social and grassroots movements (e.g. La Via Campesina), the Latin American agroecology agenda (e.g. National Plan of Agroecological and Organic Production, PLANAPO, in Brazil), inspired transformational strategies elsewhere such as in Europe. Indeed, according to the EU's Standing Committee on Agricultural Research (SCAR-FEG, 2009), agro-ecological principles should be given priority in agriculture research agendas in the European Union. In this context, the European organic sector promotes agro-ecological research with the concept of 'eco-functional intensification' linking farmers' knowledge and innovation with scientific research (NIGGLI *et al*., 2008). This new understanding of 'agro-ecological innovation' is promoted by a European alliance involving civil society organisations and farmers (ARC2020 and FRIENDS OF THE EARTH EUROPE, 2015).

Nevertheless, agro-ecological methods, but not necessarily agro-ecological principles, were adopted also by some conventional agriculture actors, such as agrochemical companies and some governments, that incorporated agro-ecological methods into 'sustainable intensification' agendas. Such a move and process was criticized by many farmers' organisations, NGOs and social movements (LEVIDOW *et al*., 2014; ARC2020 and FRIENDS OF THE EARTH EUROPE, 2015; LEVIDOW, 2015a).

LEVIDOW (2011) explained very nicely the complicated relation between innovation and sustainable agriculture: "*Nowadays most innovations are promoted under the banner of 'sustainable development', but there are different accounts of what is to be sustained. Likewise sustainable agriculture has different accounts, so it has become an ambiguous concept – even a* *contentious one"*. Accounts of what is to be sustained include agriculture growth, natural resources, current production patterns, livelihoods, ecosystem services, communities, etc. This adds to tensions between and multiple interpretations of environmental, social and economic sustainability in agriculture.

4. CONCLUSIONS

This paper argues that a better understanding of the complex and multifaceted relation between innovation and sustainability in the agro-food arena is crucial to make more effective transition towards sustainable food systems as perception towards innovation in agro-food can be an obstacle to or a lever for the deep and radical change that is needed.

It is clear nowadays that innovation is needed to foster sustainability transitions in food system from production to processing, distribution and consumption. While technical innovations are widely used and advocated for a sustainable intensification of food production, social and organisational innovations seem more relevant in the other stages of the food system as they allow improving food chain functioning and governance. However, although innovation and technological progress have had significant benefits in terms of achieving food security, relationship between innovation and sustainability is far from straightforward. In fact, the food system is for sure a contested arena where different worldviews and narratives are confronted and this applies also to innovation. Moreover, it should be highlighted that food has also a strong cultural connotation and, for that, all changes in agro-food arena are carefully scrutinised. Simply put, while many food system actors emphasize the positive role of innovation and technology in driving progress toward sustainable food systems; innovation (especially technical one) provides fertile ground for alternative agro-food movements (e.g. organic agriculture, Slow Food, agro-ecology, food sovereignty) to criticize the over-industrialization of food system. These movements seem more benign towards social innovations, especially grassroots ones, that are seen as a means to bring about the transition towards more sustainable, inclusive and equitable food systems.

Ultimately, it seems that the issue is not about questioning innovation *tout court*, but about what type of innovation should be promoted. In other words, it is not about being pro- or anti-innovation, but about addressing real, essential questions of innovation politics related to direction of change pathways in agro-food systems, distribution and equity, and diversity of options. While moving towards 'sustainable' or 'sustainability-oriented' innovation seems to be a good compromise and a step in the right direction, thus making transition towards sustainable food systems smoother and more effective, there is no doubt that this does not represent a panacea *per se* as even the concept of 'sustainability' is contested. In fact, one can always ask, a sustainable innovation for whom (as there are winners and losers in any transition or change), where (as sustainability is place- and context-specific), etc. Therefore, it can be assumed that innovation approaches need an increased dose of ethics, rules and values when they are applied to food systems and that is also true for all discourses and paradigms regarding sustainability transitions.

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OPTIMIZATION OF RICE-FIELD BEAN GLUTEN-FREE PASTA IMPROVED BY THE ADDITION OF HYDROTHERMALLY TREATED RICE FLOUR

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ABSTRACT

Rice and field bean semolina was used to obtain protein- and fiber-enriched gluten-free pasta. The tests covered the effect of pre-gelatinized rice flour used as a gluten-free pasta improver. A central composite design was applied involving the water hydration level and pre-treated rice flour level. Instrumental analyses of pasta (cooking loss, water absorption capacity, texture, hydration index, pasting and thermal properties, and microstructure) were carried out to assess the impact of experimental factors. The results showed that the application of hydrothermally treated rice flour improved the cooking and textural characteristics of pasta. The optimum recipe contained 5.845 g of pre-gelatinized rice flour and 59.266 mL of water, both selected based on the desirability function approach with the value of 0.775 corresponding to the optimum pasta properties.

Keywords: central composite design, gluten-free, pasta making, quality, starch, texture

1. INTRODUCTION

Semolina from durum wheat is a preferred substrate for the manufacture of pasta products. The quality characteristics of pasta such as texture, cooking properties, color, and sensory properties are under the influence of several conditions (SISSONS, 2008; PETITOT *et al.,* 2010). Both raw material quality and technological process have a major impact on the final product quality (DE NONI and PAGANI, 2010). Gluten protein plays an important role in determining the quality of pasta. Wheat semolina pasta products are required to exhibit the best quality (MARTI and PAGANI, 2013).

However, wheat pasta is not recommended for people with celiac disease because their immune system has a disorder in which the sentinel lesion is on enteropathy triggered by the ingestion of gluten proteins (LEFFLER *et al.,* 2017). It is known that the only possible therapy is a lifetime gluten-free diet, during which the damage to the intestinal mucosa regresses and the patient's well-being improves considerably. To ensure the manufacture of proper quality gluten-free (GF) foodstuffs, the proposed formula must be of such quality characteristics that resemble conventional pasta.

The degree of difficulty in producing GF products is closely associated with the technological role of gluten in the food-system (MARTI and PAGANI, 2013; LARROSA *et al.,* 2016). Although the demand for better-tasting, better-textured, and healthier GF products offers great market opportunities for food manufacturers, the replacement of gluten functionality still presents a major technological challenge (CHILLO *et al.,* 2007; SOZER, 2009; LOUBES *et al.,* 2016). Having that in mind, pre-treated flour could be used as the improving agents and key components to offer the effect of gluten in GF pasta (MARTI and PAGANI, 2013).

Moreover, improvement of the functional properties of starches is an important step in the manufacturing of GF pasta. To achieve this objective, natural treatment of flour through the passage of heating and cooling cycles can be applied. This passage facilitates the forming of a rigid network based on retrograded starch, which gives the dough a suitable texture (CHILLO *et al.,* 2009; MARTI *et al.,* 2010; CABRERA-CHÁVEZ *et al.,* 2012). The change in the organization of starch molecules by the application of physical and thermal treatment has been widely approved as a natural material with high food safety. The aim of this treatment is to promote the functional properties of starch without destroying its granular structure and, thus, lift any legislative limits as to its quantity in food (BEMILLER, 1997; ZAVAREZE and DIAS, 2011). The dominating properties of pre-gelatinized flour resulting from starch modifications under different treatments are associated with the re-organization of the macromolecular structure (LAI and CHENG, 2004). This re-organization of starch molecules in GF dough means better quality characteristics of the final product compared with those in durum semolina pasta.

In addition, pre-gelatinized rice flour was used as a major component and bulking or thickening agent in several food preparations (MOORE *et al.,* 1984; LAI and CHENG, 2004). Besides, as demonstrated by YOENYONGBUDDHAGAL and NOOMHORN (2002), the thermal treatment of rice flour improves the quality characteristics of rice noodles. HORMDOK and NOOMHORM (2007) applied the HMT (heat-moisture treatment) and the ANN (annealing) of rice starch for noodle preparation and obtained rice noodles with the desired features.

In many countries, celiac patients suffer from the lack of GF products, which makes it difficult for them to follow the recommended diet. One of the methods to improve the situation of celiac patients is to design new gluten-free products (GIUBERTI *et al.,* 2016; BOUREKOUA *et al.,* 2016). Also, as GF products are less available and less diverse, as well as being more expensive than gluten-rich food products, there is a strong urge to develop GF products that are technologically enhanced as well as economically sustainable. In

addition, there are fewer studies and information about the manufacture of traditional laminated pasta and its acceptability by consumers. Moreover, the supplementation of rice-based laminated pasta with leguminous is envisaged in order to improve the nutritional value of the proposed formulation (GIUBERTI *et al.,* 2016) and enhance the machinability of dough as well as the final quality of pasta products. Field bean is part of the leguminous group which is widely produced and consumed in the Mediterranean area and has an excellent potential to be used in ever new recipes because of its high nutritional quality. This plant is a rich source of fiber and protein and supplements the protein intake from cereals and tubers for a more beneficial amino acid balance (MICARD *et al.,* 2010; BENATALLAH *et al.,* 2012; CARACCIOLO *et al.,* 2016). Also, proteins of field bean have such functional properties as solubility, water retention capacity, foaming capacity as well as producing an emulsifying effect that plays an important role in food formulation and processing (DAKIA *et al.,* 2007; ROY *et al.,* 2010; BOYE *et al.,* 2010).
In addition, the need to achieve the appropriate quality and the possibility of production of more natural products for consumers with celiac disease are strong arguments behind the idea of a new formula of GF rice-field bean laminated pasta. For these reasons, the application of natural hydrothermal treatment of rice flour has been proposed.
The main objective of this study was to investigate the effect of the addition of hydrothermally treated rice flour as an improver in the manufacture of GF laminated pasta from rice supplemented with field bean semolina (RFBS). The optimization of water and pre-gelatinized rice flour (PGRF) levels needed to ensure the improvement of selected properties of GF pasta products was done by means of the response surface methodology (RSM) and following the desirability function approach (DFA).

2. MATERIALS AND METHODS

2.1. Raw materials

Cereal and leguminous semolina used for making GF laminated pasta was of rice and field bean. Rice (*Oryza sativa*) semolina was provided by Lubella Sp. z o. o. S.K. (Lublin, Poland). Field bean dehulled seeds (*Vicia faba minor*) were applied as supplement for improvement of the protein content and amino acids balance in GF pasta. Field bean seeds were purchased from Alamir Company (Albehera, Egypt) and ground. All semolina used in GF laminated pasta had particle sizes between 200 and 500 μm. Rice flour (Lubella Sp. z o. o. S.K., Lublin, Poland) with particles smaller than 200 μm was used for pre-gelatinization.

2.1.1 Chemical composition analysis

The raw materials and pasta samples were analyzed for fat, ash, and protein content using the standard procedures of the AACC methods (1995). Fat content was determined by Soxhlet extraction with n-hexane (AACC 30-10), ash by incineration in a muffle furnace at 550°C (AACC 08-01), the Kjeldahl procedure (AACC 46-10) was used for determining the protein content in three replications. Fiber was determined with the Scharrer-Kürschner method using the procedure of AOAC (2000). The method involved the acid digestion of the sample for the elimination of nutritive components; it was then flushed with ethyl alcohol and dried to dry matter. Based on that, the amount of fiber was calculated (AOAC 993.21). The measurements were performed in duplicate.

2.1.2 Hydrothermal treatment of rice flour

The heat treatment process of flour was performed according to the TangZhong method described by YVONNE (2007) and BOUREKOUA *et al.* (2016). Pre-gelatinized rice flour (PGRF) was made by mixing water and flour (1 to 5) in an aluminum pan. The mixture was then heated on a heating plate for 8-9 min and continuously stirred with a spatula until the temperature reached 65°C. Afterwards, the obtained paste was cooled down at ambient temperature for 1 h and stored in a fridge (4°C) for 24 h. Some preliminary experiments were made in a laboratory and indicated that the cooling of the slurries for 24 h at 4°C produces better cooking and textural properties when added to the pasta formula than PGRF kept for 1 h or used immediately after heating. Pre-gelatinized rice flour was added to pasta made with rice-field bean semolina by mixing with other ingredients of the formula after keeping at ambient temperature for 1 h.

2.2. Experimental design

A central composite design (CCD) was used, involving water hydration level (X_1) and pre-gelatinized rice flour (stored in a fridge for 24 h) level (X_2). The effects of two variables on the quality characteristics of laminated pasta were analyzed. The hydration range applied in the experimental design was determined by preliminary experiments using from 46.42 to 72.12 mL of water for 100 g of the recipe (46.42 mL/100 g is the minimum level of water necessary to make dough and 72.12 mL/100 g is the maximum level of water for obtaining sticky pasta). The level of pre-gelatinized rice flour was fixed from 0 to 11.70 g for 100 g of recipe based on the calculation of the water level added in each run. These values were incorporated into JMP software to determine the CCD matrix.
The optimization of the studied formula was carried out using the RSM. The factorial section is a 2^2 test; the star section includes four tests. Five replicates (runs 1, 4, 5, 10, 11, Table 1) at the center of the design were used to estimate the pure error at the sum of square, for a total of $2^2+2^2+5=13$ runs.

Table 1. Factors, levels and code values used in the Central Composite Design (CCD) for gluten-free rice-field bean semolina (RFBS) pasta.

Run	Hydration X_1 (mL)		PGRF X_2 (g)	
	Code	Value	Code	Value
1	0	59.26	0	5.85
2	-1	50.18	1	9.98
3	0	59.26	1.414	11.7
4	0	59.26	0	5.85
5	0	59.26	0	5.85
6	-1	50.18	-1	1.71
7	1	68.35	1	9.98
8	0	59.26	-1.414	0
9	-1.414	46.42	0	5.85
10	0	59.26	0	5.85
11	0	59.26	0	5.85
12	1	68.35	-1	1.71
13	1.414	72.12	0	5.85

A statistical analysis was performed for the experimental data for each response variable in order to select an optimized recipe using the desirability function approach (DFA). The DFA is a method of multi-criteria optimization showing some relationships between several responses. The desirability factor (D) varied from 0 to 1, where 1 meant a maximum satisfaction and 0 was complete refusal (LARROSA *et al.*, 2016; BOUREKOUA *et al.*, 2016).

2.3. Pasta making

According to the previous research and recommendations (FAO, 1982; MICARD, 2010), the optimum combination of cereal and legume required to achieve an excellent nutritional balance is 65% of cereal and 35% of legume. The GF formulation (rice-field bean semolina: RFBS) studied in this work was based on a mixture in a ratio of 2:1 (w/w) of cereal and leguminous semolina in order to ensure a technological approach to processing and enable good machinability of laminated rice-based dough as well as providing a good amino acids balance (BENATALLAH *et al.*, 2012).
The hydration range applied in the experimental design was determined by preliminary experiments (46.42 to 72.12 mL for 100 g of recipe). The level of pre-gelatinized rice flour was fixed up to 11.70 g for 100 g of the recipe. Hydration and PGRF levels were expressed based on the RFBS blend. The basic recipe of RFBS consisted of 66.66 g of rice semolina, 33.33 g of field bean semolina, 2 g of salt and the amount of distilled water defined in the experimental design data. Pasta produced without the addition of PGRF (run 8) was considered as control pasta (Table 1).
The optimum recipe was determined according to the desirability method performed by JMP software version 7 (SAS, USA). The selected optimum pasta was also prepared according to the procedure adapted both for traditional and industrial production.
In the first step, the ingredients were mixed for 15 min at 25°C using a KitchenAid mixer, model kPM5 (St. Joseph, Michigan, USA). The obtained dough was rounded, divided into balls of 50 g and covered with a sealed plastic wrap and then rested at 25°C for 1 h to let the starch hydrate. The dough was molded and passed through the reduction rolls of a pasta machine Marcato Ampia type 150 (Campodarsego, Italy) for four times from each pass and in all directions to produce a uniform dough sheet. The roller gap was maintained at 5 for the last sheeting. The final thickness of each dough sheet was 1.5 mm as determined with a caliper. Finally, pasta samples were dried in an oven with air circulation at 40°C for 4 h until it reached the final moisture content below 12%. They were stored in sealed plastic bags at room temperature.

2.4. Determination of pasta quality

2.4.1 Cooking quality

The cooking quality was determined according to the Approved Method 66-50 (AACC, 2000) with the modifications proposed by CHILLO *et al.*, (2007). In brief, 10 g of dried pasta was boiled in 300 mL of distilled water for an optimal cooking time (OCT), dripped for 3 min and weighed. The cooking water was evaporated by drying at 105°C overnight. The residue was weighed and the cooking loss (CL) was reported as a percentage of dry sample weight before cooking. The water absorption capacity (WAC) was expressed as a percentage increase of pasta weight after cooking compared with the weight of uncooked pasta. The measurements were performed in triplicate.

2.4.2 Hydration properties

The hydration properties of the optimum pasta and control sample were determined at ambient temperature designating the water absorption index (WAI), water solubility index (WSI), and swelling power (SP). Ground pasta samples (0.7 g) were mixed in centrifugal tubes with 7 mL of distilled water. After 5 min of rest, the samples were hydrated for 10 min and mixed every minute for uniform reconstitution, followed by centrifugation (15000 rpm, 10 min, 21°C) in T24 Centrifuge (VEB MLW MEDIZINETECHNIK, Leipzig, Germany). The supernatant was dried to constant weight at 105°C. The tests were carried out in four replications. The WAI and WSI calculations were made according to the method described by WÓJTOWICZ and MOŚCICKI (2014). SP was calculated as proposed by LAI and CHENG (2004).

2.4.3 Textural characteristics

The texture characteristics of pasta were achieved using the universal testing machine Zwick/Roell BDO-FB0.5 TH (Zwick GmbH& Co., Ulm, Germany). The cutting force (N) of single cooked strand of the optimum and control pasta was evaluated in five independent replications with a Warner-Bratzler blade (60 mm long, 3 mm thick, double face truncated at 45°), as described by WÓJTOWICZ and MOŚCICKI (2014). The measurements were carried out with a test speed of 8.33 mm/s using a 0.5 kN working head.
OTMS Ottawa cell was used for the evaluation of firmness, stickiness and cohesiveness of cooked pasta samples from each run of experimental design and for the optimum pasta properties (MARTINEZ *et al.,* 2007). For firmness (F) (N), stickiness (S) (mJ) and cohesiveness (mJ) of cooked pasta products, a double-compression test was applied. 50 g of cooked and drained pasta was put in the OTMS chamber and compressed with a test speed of 3.3 mm/s. The *testXpert*® 10.11 software was used to record data in three independent replications.

2.4.4 Pasting properties

The pasting performance of hydrothermally treated slurries were determined after 1 h of cooling at room temperature and after 24 h storage at 4ºC. The properties were adjusted according to BOUASLA *et al.* (2017) using a Brabender Micro-Visco AmyloGraph (Brabender OHG, Duisburg, Germany) under the constant measurement conditions: speed 250 rpm, sensitivity 235 cmg, and heating rate 7.5°C/min. The following characteristics were considered: pasting temperature (°C), temperature at the beginning of viscosity increase; initial viscosity (mPas), cold viscosity at 30°C; peak viscosity (PV) (mPas), the highest viscosity during the heating cycle; breakdown (BD) (mPas), corresponding to the difference between the PV and the viscosity at the end of the holding period at 93°C and an index of viscosity decrease during holding at 93°C; setback (mPas), corresponding to the final viscosity minus the viscosity at the end of the holding period at 93°C and an index of viscosity increase during the cooling cycle; final viscosity (mPas), i.e. viscosity reached at the end of the cooling period.

2.4.5 Thermal characteristics

For calorimetric measurements, the differential scanning calorimeter DSC Star System (Mettler Toledo AG, Greifensee, Switzerland) was used. The calibration of the instrument was done by means of the indium standard before the evaluation of the sample. All the measurements were performed under nitrogen gas atmosphere. The instrument was controlled with Star^e system. Ground samples of dried optimum and control pasta (3-5 mg) with a granulation below 250 μm were precisely weighed in aluminum crucibles with a pin (volume 40 μL). The sample-encapsulating press was used for the sealing of powder-filled pans. The samples were thermally inserted into the instrument, equilibrated at 25°C for 10 min and then heated at the rate of 10°C/min up to 180°C. An empty aluminum pan was used as the reference in all recorded thermograms. During the measurement, the temperature was controlled with an accuracy of ±0.1ºC by means of the high precision thermoregulation system TC 100 MT (Peter Huber Kältemaschinenbau AG, Germany) (BOUREKOUA *et al.*, 2017).

Heat flow during the whole process of sample heating was recorded. The following features of thermogram were analyzed: onset temperature (T_o), peak temperature corresponding to the maximum heat flow (T_p) and endset temperature (T_c). In order to compare the length of the transition, the difference between endset and onset temperatures ($T_r = T_c - T_o$) was calculated. The gelatinization enthalpy (ΔH) expressed per weight of dry sample (J/g) was assessed by integrating the area between the thermogram and the baseline under the peak. All thermal parameters were calculated using the evaluation mode of the Star^e system. Thermal scans were performed in three independent replicates.

2.5. Sensory evaluation

The sensory assessment was carried out on the optimal selected pasta and control sample. Pasta products were cooked in the optimum time and drained and placed in warm conditions until testing. The samples were served in a random order on a white ceramic plate to a panel of 55 untrained consumers (25-45 years old, 26 females and 29 males) who were the habitual consumers of pasta and were familiar with the definitions and references. They evaluated the products for appearance, taste, flavor, consistency, and stickiness on a 5-point scale (1 = poor, 5 = good) (WÓJTOWICZ and MOŚCICKI, 2014). For the preliminary tests of taste, five independent sessions were organized; each session involved a panel of 11 untrained consumers who were familiar with the terminology related to pasta (ISO 11036). For the final analysis, a single session was performed with the same pasta cooking conditions involving the presentation of the sample and specific environmental conditions. The results and observations of final sensory analysis were taken into account.

The overall acceptability was evaluated by the same panel, and each pasta sample was assessed using a verbal nine-point hedonic scale. The ratings were converted into numerical scores, where 1 was dislike extremely, 5 neither like, nor dislike, and 9 as like extremely. GF pasta was considered acceptable if the mean score for the overall quality was above 5 (WÓJTOWICZ and MOŚCICKI, 2014; BOUASLA *et al.*, 2017).

2.6. Pasta microstructure

The pictures of dry optimum and control pasta were taken using a scanning electronic microscope (SEM). A piece of dry pasta, attached to a carbon disc with a silver tape was sprayed with gold in the K-550X vacuum sublimator (Emitech, Ashford, England). The electron microscope VEGA LMU (Tescan, Warrendale, USA) was used to observe the surface and cross-section of the samples at different magnifications (×200, ×600 for surface, and ×200, ×600, ×1500 for cross-sections). The accelerating voltage of 30 kV was applied.

2.7. Statistical analysis

A second order complete polynomial equation was applied to fit the behavior of each measured variable as a function of dough composition (MYERS *et al.*, 2009; LARROSA *et al.*, 2016) by using the JMP software version 7 (SAS, USA). The models were used to determine response surfaces in Statistica, version 10 (StatSoft. Inc., USA). A one-way ANOVA analysis of variance was employed for the assessment of the effect of water (X_1) and pre-gelatinized rice flour (X_2) on dependent variables (Y) with a 0.05 significance level. The model proposed for each response was:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{12}X_1X_2$$

where: b_0 is the value of the fitted response at the center point of design, that is (0,0); b_1 and b_2 are the linear regression terms; b_{11}, b_{22} are the quadratic regression terms; and b_{12} is the cross-product regression term (interaction coefficients). Optimization was performed with JMP, version 7 to find the best formula of GF rice-field bean pasta with the DFA method. To compare the measured parameters (cooking and textural parameters, hydration index, pasting and thermal properties) between the optimized recipe and the control pasta, the results were analyzed with the ANOVA test at the level of significance of $p \leq 0.05$ followed by Fisher's LSD using Statistica 10.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

Table 2 shows the chemical composition of the raw materials and pasta samples. As expected, protein, fat, ash, and fiber increased significantly with the addition of field bean to rice-based pasta ($p<0.05$) because of the leguminous supplementation of rice flour (GIMENEZ *et al.*, 2012; GIUBERTI *et al.*, 2015; GIUBERTI *et al.*, 2016).

Table 2. Proximate composition of raw materials and gluten-free pasta samples (g/100 g).

	Protein	Fat	Ash	Fiber
Rice	6.72±0.02[c]	0.14±0.02[c]	0.42±0.026[c]	1.24±0.03[c]
Field bean	31.40±0.52[a]	1.38±0.07[a]	2.53±0.12[a]	10.88±0.18[a]
Rice pasta	6.90±0.02[c]	0.13±0.04[c]	0.37±0.05[c]	1.31±0.03[c]
Rice-field bean pasta	14.92±0.03[b]	0.61±0.03[b]	1.15±0.05[b]	4.31±0.10[b]

[a-c]Values followed by the same letter in the same column are not significantly different (p=0.05).

The enrichment of our formula based on rice with field bean semolina causes an increase of the protein content by 116% and the fiber level in the recipe by 229%. Celiac consumers have a low intake of dietary fiber (BOUASLA *et al.*, 2017), so the proposed formula could be beneficial if having an increased fiber content. Legumes supply an important dose of dietary fiber and protein with a good amino acids balance, and the supplementation of cereals with legumes could enhance the nutritional value of the final product.

3.1.1 Pasting performance of PGRF

The pasting properties of PGRF were investigated by the measurements through a viscoamylograph test. The viscograms of raw flour and PGRF were analyzed for comparison as shown in Fig. 1.

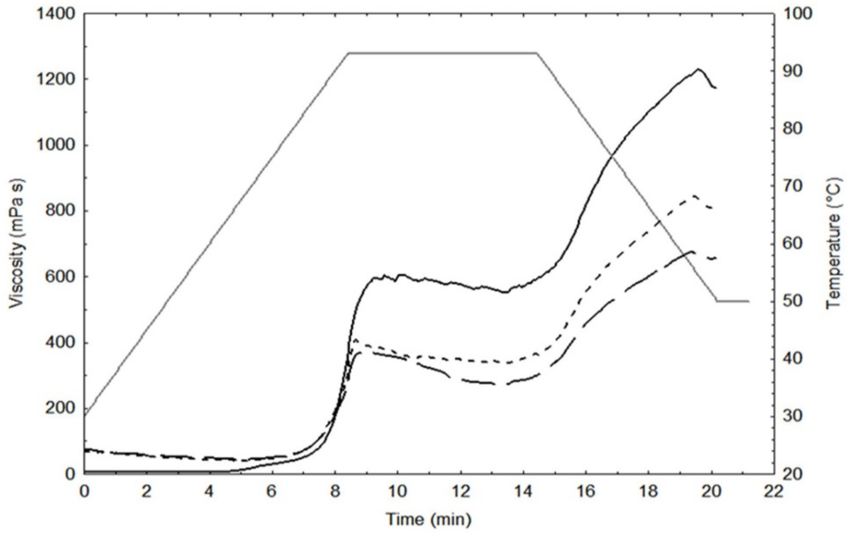

Figure 1. Pasting profile of native rice flour, and PGRF after 1h and 24 h of preparation and storage. Legend: (—) temperature profile; (—) rice flour; (·······) treated rice 24 h; (- - - - -) treated rice 1 h.

The results showed that changes in viscosity depended on the treatment conditions. The Brabender viscograms showed an increase in the IV of pre-gelatinized rice flour after 1 h and 24 h of preparation (54 mPas, 52 mPas, respectively) as a confirmation of partial gelatinization of starch under a hydrothermal treatment compared to untreated rice flour (17 mPas). The high IV for PGRF was attributed to a modification in the molecular organization of the starch during treatment, which resulted in the loss of granule integrity and breaking of the order of crystallinity of starch (LAI and CHENG, 2004; MARTI *et al.*, 2013). The hydrothermal treatment of rice flour exhibited a significant lowering of PV for both treatments (370 mPas and 407 mPas, respectively, for treated rice for 1 h and 24 h of storage) compared to the raw material (606 mPas). The decrease in PV might be related to the limited swelling capacity and its effect was the increase in PT (78.2°C and 78.1°C, respectively, for treated rice flour for 1 h and 24 h) compared to untreated rice flour (70°C). This corresponds with the increased temperature needed to gelatinize undamaged starch granules, as found by HORMDOK and NOOMHORM (2007). Moreover, the hydrothermal process induced the viscosity reduction during the cooling stage; the same behavior was observed in several studies (ADEBOWALE *et al.*, 2005; HOOVER and VASANTHAN, 1994; JACOBS *et al.*, 1995; STUTE, 1992; BOUREKOUA *et al.*, 2016). After treatment, the progression and continuation of retrogradation of the starch molecules during storage will take place, which leads to a significant ($p<0.05$) increase in the viscosity of treated rice flour 24 h after the beginning of storage (827 mPas) compared to treated rice flour after 1 h (655 mPas). This phase is commonly described as the setback region and is related to the retrogradation and reordering of starch molecules. All these observations indicated that a hydrothermal treatment may affect the granule rigidity and starch molecular re-association. Therefore, various treatment conditions applied would give the possibility to obtain a different form of pre-gelatinized flour with different pasting properties that should be used for various applications.

3.2. The effect of pre-gelatinized rice flour on the laminated GF pasta quality

3.2.1 Verification of the fitted models

For each model, linear, quadratic and interaction effects were calculated. Regression coefficients are shown in Table 3. Adequacy of the model was verified by estimating the coefficient of determination (R^2) and the lack-of-fit test.

Table 3. Regression coefficients for the predictive models for cooking loss, water absorption capacity, firmness and stickiness.

Model term	CL (%)	WAC (%)	F (N)	S (mJ)
X_1	-0.3115	+0.0829	-0.0073*	-0.0086*
X_2	-0.1631	-0.0755	-0.2998	-0.0028*
X_1X_1	+0.4966	+0.4503	-0.0280*	+0.1112
X_2X_2	+0.0056*	-0.0323*	-0.0192*	+0.0009*
X_1X_2	+0.6541	+0.7510	+0.6851	-0.0149*
Lack of fit	NS	NS	NS	NS
F	3.87^{NS}	3.39^{NS}	6.05^{NS}	14.96^{NS}
R^2	0.73	0.71	0.81	0.91

CL: cooking loss, WAC: water absorption capacity, F: firmness, S: stickiness, X_1: water, X_2: PGRF, X_1X_1, X_2X_2: quadratic coefficients, X_1X_2: interaction between coefficients,
F: variance Ficher-Snedecor, R^2: coefficient of determination,
^{NS}: not significant (p>0.05),
* significant at p≤0.05.

The statistical analysis proved that the fitting models were adequate because they yielded satisfactory values of R^2, and the lack-of-fit test was not significant for all responses. If the values of a predicted model are not significantly different from real values, and the lack-of-fit is not significant, the model is adequate (GOUPY, 2013). The results of the discussed experiment confirmed that the model was adequate to envisage the responses of CL, WAC, F and S.

3.2.2 Cooking quality

The CL and WAC represent the crucial parameters of the cooking quality of pasta. The effect of a range of water and PGRF on these responses was shown on Fig. 2A and 2B, respectively. The CL of pasta samples ranged from 9.35 to 14.26%. The CL decreased with an increased addition of PGRF up to the incorporation level of 7 g/100 g, which raised the maximum incorporation as its quadratic effect was positive (p<0.05, Table 3). Moreover, the chart (Fig. 2A) and the data in Table 3 demonstrate the absence of the linear and quadratic effect of water on the value of CL.

As reported in a paper presented by BOUASLA *et al.* (2017), a cooking loss below 10% indicates good quality pasta. Moreover, MARTI *et al.* (2013) reported a cooking loss for pasta ranging between 3.5 and 11.3%; CHAM and SUWANNAPORN (2010) found the CL of rice noodles varying from 6.5 to 10.25%; and BOUASLA *et al.* (2017) presented the range of 3.5 - 5.93% of the CL for extruded GF pasta. According to these values, it could be concluded that pasta with an addition of PGRF has sufficient cooking quality as a GF product.

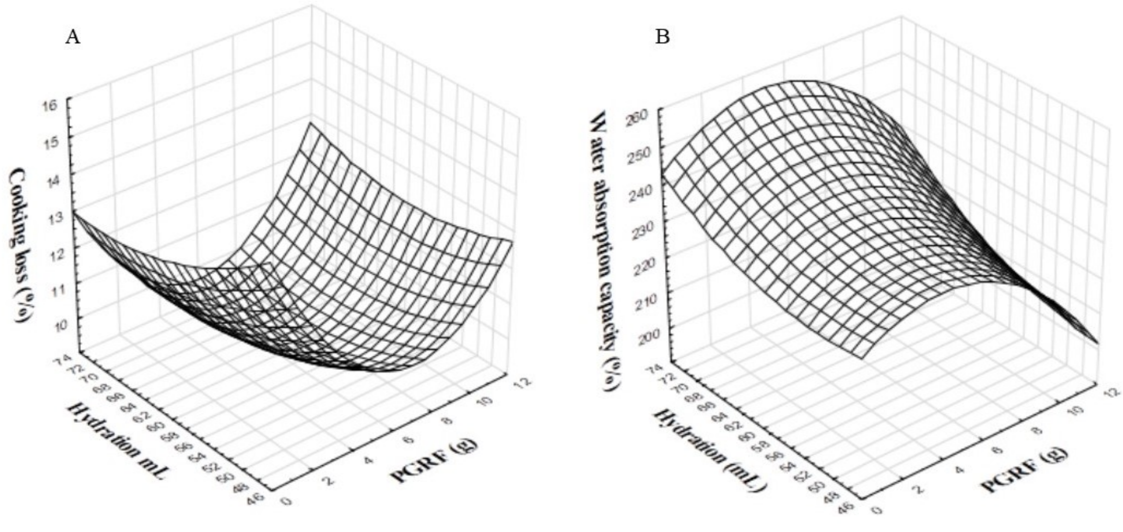

Figure 2. Response surface of CL (A) and WAC (B) of gluten-free RFBS pasta depend on hydration level and PGRF amount.

As starch is the decisive component of GF pasta, its applicability can be noticeably enhanced by a convenient re-organization (BEMILLER, 1997). The cooking quality of pasta could be improved by the addition of treated flour containing an appropriate amount of modified starch (HORMDOK and NOOMHORM, 2007). The hydrothermal treatment of rice flour discussed in this study probably leads to the formation of a permanent and solid network of retrograded starch within gelatinized starch, which may produce the effect of lesser solubilization of amyloceous components and, therefore, results in a less cooking loss, as found also by RESMINI and PAGANI (1983). Recently, MARTI and PAGANI (2013) announced that the incorporation of 50% of treated rice flour improved the characteristics of pasta in terms of cooking properties. As hypothesized by the above authors, pre-gelatinized flour could perform the role of a binding agent leading to the establishment of a new network around starch granules, thus increasing their resistance to cooking stress, as suggested by PAGANI (1986).

The WAC of RFBS laminated pasta ranged from 215.83 to 255.00% (Fig. 2B). The WAC grew with an increasing addition of PGRF up to the incorporation level of 6 g/100 g, which decreased with the maximum incorporation as its negative quadratic effect ($p<0.05$, Table 3). In addition, the chart (Fig. 2B) showed that the WAC of the samples increased with an increasing amount of water.

The WAC is a primordial measured parameter, which depends on the fragments of damaged starch and the fragility of its granules (BOUASLA *et al.,* 2017). The observed water uptake dynamics suggests that hydrothermal treatment conditions of rice flour in our study might possibly promote a less hydrophilic structure of starch resulting in low water absorption, as suggested by MARTI *et al.* (2013). In addition, HORMDOK and NOOMHORM (2007) reported that rice starches thermally treated by both HMT and ANN slightly reduced the WAC of composite noodles. Moreover, the low WAC indicates a poor quality of cooked pasta due to chewy texture (WANG *et al.*, 2012).

3.2.3 Textural characteristics

The textural properties of pasta are related to the ability to maintain consistency after cooking (LARROSA *et al.*, 2016). The response surface obtained for the texture measurement showed that firmness ranged from 216.50 to 344.72 N (Fig. 3A). The chart showed an increased firmness of samples along with the increasing amount of PGRF up to the incorporation level of 7 g/100 g, which decreased with the maximum incorporation as its negative quadratic effect ($p<0.05$, Table 3). However, the chart (Fig. 3A) showed a decreased firmness of samples with the increased amount of water as its negative linear and quadratic effect ($p<0.05$, Table 3).
As demonstrated by the results of BOUASLA *et al.* (2017), firmness of extruded gluten-free pasta supplemented with legumes ranged between 199.5 and 326.5 N. MARTI *et al.* (2013) reported that firmness of rice pasta ranged from 188 to 902 N., ZHAO *et al.* (2005) regarded firmness as work required to shear 4 cooked strands of spaghetti that ranged between 5.72 to 8.64 g/cm. The firmness characteristic of pasta can be evaluated with various methods, so the results are difficult to compare.

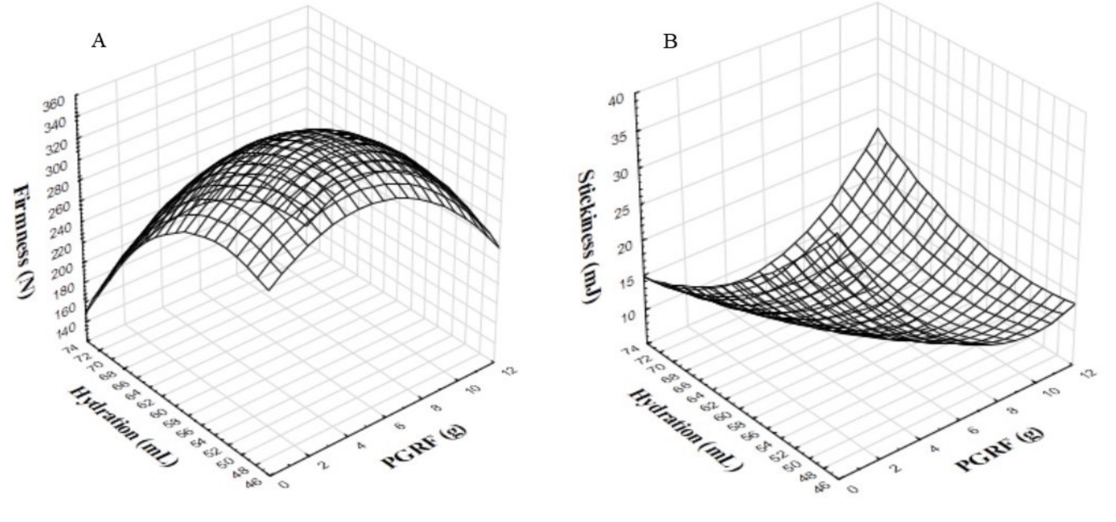

Figure 3. Response surface of firmness (A) and stickiness (B) of gluten-free RFBS pasta depend on hydration level and PGRF amount.

The response surface in Fig. 3B for RFBS pasta showed the stickiness values varying from 9.10 to 25.65 mJ. The chart demonstrated decreased stickiness of samples as the amount of PGRF and water increased. Moreover, the addition of treated rice represents a negative linear effect, a positive quadratic effect and a positive interaction effect between the two variables (water and PGRF). Also, water induced a negative linear effect on this parameter ($p<0.05$, Table 3).
Heat treatment of rice flour seems to generate a higher regulated crystalline area in starch granules; this induced a lesser leaching of amylose fractions and, consequently, low

stickiness. Moreover, the modification and re-organization of starch macromolecules as a result of the applied treatment gives rise to the formation of a rigid network. Consequently, the strengthened starchy matrix could protect the amyloceous components from increased leaching and, thus, giving an improved texture as exhibited by the low CL values and high firmness.
The hydrothermal treatment discussed above partially eliminates the swelling of granules by slowing down gelatinization and enhancing the stability of starch paste (HOOVER and VASANTHAN, 1994; HORMDOK and NOOMHORM, 2007) and, thus, enhancing the aptitude of cooking and consistency of rice pasta (YOENYONGBUDDHAGAL and NOOMHORN, 2002; MARTI *et al.*, 2013). In addition, RAINA *et al.* (2005) showed that the application of pre-gelatinized rice flour activated the starch arrangements, which guaranteed a good textural quality of both uncooked and cooked rice pasta. FIORDA *et al.* (2013) also reported a significant enhancement and elevated firmness of pasta after the incorporation of pre-gelatinized flour made from blends of cassava starch and bagasse, and the level of pre-gelatinized flour was the most significant component when it comes to pasta stickiness.

3.3. Optimization and characteristics of optimum GF pasta

3.3.1. Cooking and textural quality

The optimum recipe was determined based the desirability method performed by JMP, version 7 (SAS, USA). Desirability is a useful approach for the optimization of multiple responses in order to select the best recipe. The main objective of optimization was to determine the levels of variables (X_1, X_2) that produce the best characteristics of GF pasta. Good quality of pasta is a combination of high water absorption, low cooking loss, and good texture parameters (high firmness and low stickiness) (BRUNEEL *et al.*, 2010). The criteria used to obtain individual desirability functions, predicted responses and real results are presented in Table 4.

Table 4. The criteria used to obtain desirability functions, predicted responses and individual desirability functions (di).

Parameter	Objective	Predicted response	Individual desirability values (di)	Experimental response
CL (%)	Minimum	9.53 [a]	0.956	9.49 [a]
WAC (%)	Maximum	237.49 [a]	0.603	234.14 [a]
F (N)	Maximum	338.09 [a]	0.891	339.85 [a]
S (mJ)	Minimum	9.14 [a]	1	9.05 [a]

CL: cooking loss, WAC: water absorption capacity, F: firmness, S: stickiness,
[a]Experimental responses with the same superscript than predictive response indicate there are not significant differences ($p>0.05$).

For optimization, the global desirability was selected, and the DFA method for selection of the optimum recipe involved the CL and S as minimized, the WAC and F as maximized. Based on such a desirability approach, the optimum amounts were found to be 5.845 g of PGRF and 59.266 mL of water for 100 g of RFBS with a value of 0.775. A hydration level is another key factor to be considered in order to obtain good quality pasta. This may be directly related to the fact that hydrating goes with formulation having an impact on the textural properties of pasta. Moreover, in the opinion of LARROSA *et al.* (2016), the amount of water used in pasta production should be optimized to achieve the acceptable quality of pasta. It was demonstrated that the insufficient water content produces streaky and/or flaky dough sheet surfaces, resulting in softer texture of cooked pasta. Using the above optimum levels of water in the formulation has also adverse quality effects, as dough will be stickier to handle and difficult to sheet tending to produce poor quality finished pasta. The cooking behavior, textural parameters (cutting force, firmness, stickiness and cohesiveness) of the optimized and control GF pasta are shown in Table 5.

Table 5. Characteristics of optimum and control gluten-free RFBS.

Parameters	Optimum pasta	Control pasta
Cooking quality		
Cooking loss (%)	9.49±0.03[a]	14.26±0.01[b]
Water absorption capacity (%)	234.14±7.21[a]	217.11±0.10[b]
Textural parameters		
Cutting force (N)	1.21±0.03[a]	0.62±0.03[b]
Firmness (N)	339.85±0.03[a]	295.50±1.12[b]
Stickiness (mJ)	9.05±0.07[a]	25.00±0.5[b]
Cohesiveness (mJ)	28.14±0.15[a]	14.12±0.15[b]
Pasting properties		
Pasting temperature (°C)	77.50±0.04[a]	73.80±0.13[b]
Initial viscosity (mPa s)	18.00±0.10[a]	14.00±0.01[b]
Peak viscosity (mPa s)	195.00±0.02[a]	256.00±0.01[b]
Final viscosity (mPa s)	351.00±0.03[a]	456.00±0.26[b]
Setback (mPa s)	160.00±0.07[a]	205.00±0.02[b]
Breakdown (mPa s)	2.05±0.05[a]	5.00±0.07[b]
Hydration properties		
WAI (g/g)	2.93±0.07[a]	1.15±0.21[b]
WSI (%)	7.38±0.13[a]	10.85±0.13[b]
SP (-)	3.22±0.95[a]	5.60±0.01[b]
Thermal characteristics		
T_o (°C)	50.80±0.04[a]	46.93±0.11[b]
T_p (°C)	102.84±0.00[a]	95.58±0.00[b]
T_c (°C)	171.57±0.02[a]	169.76±0.24[b]
T_r (°C)	120.77±0.01[a]	123.76±0.03[b]
ΔH (J/g)	123.64±1.09[a]	180.70±1.23[b]

[a-b]Means with the same superscript within line are not significantly different (p > 0.05).

As described by BRUNEEL *et al.* (2010), good quality pasta should be characterized by the low CL and stickiness, the high WAC and firmness. For commercial durum wheat spaghetti, they reported that the cooking loss ranged between 3.9 and 6.1%, stickiness between 0.2 and 119.9 mN/mm, water absorption capacity between 1.83 and 2.13 g/g dm, and firmness between 5.8 and 7.8 N/mm.

The addition of hydrothermally treated rice flour reduced the CL significantly (9.49%) and improved the WAC (234.14%) of optimum pasta, and these were found comparable or superior to the control pasta (14.26% of CL, 217.11% of WAC, respectively) (p<0.05). For texture measurements, the optimized pasta exhibited higher and better values of cutting force (1.21 N), F (339.85 N) and cohesiveness (28.14 mJ) with lower S (9.05 mJ) than the control pasta (0.62 N, 295.5 N, 14.12 mJ, 25.00 mJ, respectively). The presented results of GF pasta are difficult to compare with the durum pasta properties. A good and firm structure of pasta means less quantity of substances going into cooking water (PADALINO *et al.,* 2013). A hydrothermal treatment of rice flour might stimulate the variation in the physical and chemical properties of starch. These modifications of starch organization are responsible for the new macromolecular structure, resulting in reduced stickiness and cooking loss and an improved texture.

3.3.2 Pasting properties

Integrity of starch granules is commonly investigated by measurements of the pasting behavior of cereals before and after modification under specific treatment conditions. The results of pasting parameters presented in Table 5 show some significant differences in the pasting characteristics of optimized formula (PT, IV, PV, FV, BD and Set) in comparison with the control pasta without PGRF ($p<0.05$). Higher PT, IV and lower PV, FV, BD and Set were noted for the optimum pasta. The observed differences can be explained by the modification and alteration of rigidity of treated starch granules (MARTI *et al.,* 2010). The reported increase in pasting temperature, a decrease in PV, FV and BD may be attributed to the alterations of the pasting properties in treated flour possibly due to the production of bonds between the chains in the amorphous regions of starch granules as well as modification of crystallinity during the hydrothermal treatment, as reported previously by other authors (ADEBOWALE and LAWAL, 2002; CHUNG *et al.,* 2009; HORMDOK and NOOMHORM, 2007; OLAYINKA *et al.,* 2008; ZAVAREZE and DIAS, 2011). Additionally, the low PV of the optimum pasta may ensue from the limited starch swelling capacity (HAGENIMANA *et al.,* 2006). The strengthening of intra-granular bonds means that more heat is required for the structural disintegration of starch and formation of paste (OLAYINKA *et al.,* 2008; ZAVAREZE and DIAS, 2011). A reduction in BD after thermal treatment indicates a higher stability of starch exposed to continuous heating, as reported by ADEBOWALE *et al.* (2005). Moreover, the lowest recorded value of BD may indicate that PGRF may have a good potential as a food improver for food exposed to heat and mechanical treatment. Setback (Set) indicating the retrogradation tendency was apparently lower in the optimized recipe than in the control ($p<0.05$). Retrogradation is influenced by the amount of extracted amylose, the size of granules, and the presence of stiff, non-disintegrated swollen granules (LAN *et al.,* 2008).

Additional amylose–amylose and/or amylopectin–amylopectin chain interactions may occur upon the applied hydrothermal treatment, which can result in the cut-down of amylose extraction and decrease of retrogradation. A similar phenomenon of the modifications of pasting parameters of heat-moisture treated potato starch was reported by STUTE (1992). The reported alterations in pasting characteristics indicated some degree of re-association of starch molecules during the hydrothermal treatment. Moreover, the high IV of sample incorporated with PGRF indicates a good WSI, consistent with the value of hydration parameters shown in Table 5. This is combined with the effect of heat treatment on the properties of pre-gelatinized rice flour and dextrinization of starch molecules to a high extent, as reported by LAI and CHENG (2004). Upon heating, the bonding force within starch granules can affect the swelling behavior, which explains the rapid increase in viscosity. Moreover, the starch hydration properties were greatly affected by the thermal treatment as a consequence of macromolecular disorganization and degradation (NAKORN *et al.,* 2009; MARTI *et al.,* 2013).

3.3.3 Hydration properties

Starch solubility is the result of amylose leaching from starch during swelling as dissociating from and diffusing out of granules. This process represents a transition from the ordered to disordered structure of starch granules that occurs during heating in the presence of water (ZAVAREZE and DIAS, 2011). Macromolecular disorganization and degradation is the result of alteration of starch hydration properties by the thermal treatment (NAKORN *et al.*, 2009). The hydration properties differed significantly between the optimized and control recipe ($p<0.05$) – the results are shown in Table 5. A significant increase of the WAI in the optimum sample as compared to the control results probably from a greater possibility of exposed hydrophilic groups to create bonds with water molecules during the formation of gel. In addition, this is the result of partial starch gelatinization occurring during the treatment, as suggested by LAI and CHENG (2004). Moreover, the reduction in the SP and WSI were observed compared to the control sample without PGRF. WADUGE *et al.* (2006), JACOBS *et al.* (1995) and HOOVER and VASANTHAN (1994) explained that a decrease in the SP may result from the increased crystallinity and interactions between amylose and amylopectin molecules. The authors also showed that this phenomenon could be attributed to enhanced intra-molecular bonds and modifications of the crystalline structure of starch.
In addition, GOMES *et al.* (2005) described the effect of increased molecular organization on the reduction of hydration properties of starch. Also, the same group suggested that the reduction in the solubility of annealed starch was due to the enhancement of the formation of bonds between amylose and amylopectin or between amylopectin molecules, inhibiting the extraction of starch granules. The hydrothermal treatment applied in our work probably leads to the re-organization of the structure attributed to the changes in starch granule rigidity under treatment, which entails other specific interactions between starch molecules (HOOVER and MANUEL, 1996; LAI, 2001) such as the formation of more ordered double helical amylopectin side-chain clusters and amylose–lipid complexes located within granules. A similar phenomenon of decreased swelling power and solubility was reported in other studies (HOOVER and MANUEL, 1996; OLAYINKA *et al.*, 2008).

3.3.4 Thermal properties

The differential scanning calorimetry (DSC) is useful in the characterization of starch gelatinization as it detects the temperature of the different stages of this process. The onset temperature (T_0) indicates the beginning of the gelatinization process, peak temperature corresponds to the maximum heat flow (T_p), thus indicating the temperature of main phase transition, and the endset temperature indicates the end of the process of gelatinization (T_c). The difference between endset and onset temperatures is the information about the length of the gelatinization process. The thermal parameters characterizing the gelatinization process of the optimum and control pasta measured by the DSC are presented in Table 5.
The obtained parameters significantly varied between the control and optimized recipe ($p<0.05$). As shown in Table 5, the optimum pasta was characterized by a higher onset, endset and peak temperatures (T_o=50.80, T_c=171.57 and T_p=102.84°C) as compared to the control (46.93, 169.76 and 95.58°C, respectively). This indicates that a higher temperature is needed to start the gelatinization process, and that the main transition occurs at a higher temperature. On the other hand, a significant decrease in the length of transition (gelatinization process) was observed – as indicated by the shortening of T_r distance in the optimum sample.

Crystalline and amorphous transitions influence the shape of experimental curves representing the gelatinization process (CHAM and SUWANNAPORN, 2010). The analysis of thermal results showed some distinctive changes in the heat-treated internal granular structure of starch, which revealed a higher stability. Similarly, the hydrothermal treatment of rice flour admixed to pasta produced a more homogenous structure of starch crystallite during melting, swelling and hydration and resulted in the formation of new kinds of starch crystallites displaying dissimilar heat stability, as indicated by HORDMOK and NOOMHORM (2007). Besides, the neighboring amorphous region can govern the melting temperatures of starch crystallite indirectly. An addition of treated rice flour to pasta would increase the stability of amorphous regions during crystallite melting. As a consequence, a higher temperature is required to melt crystallites of PGRF. Increased T_o, T_p, and T_c can be explained by the structural changes of starch granules and the associated interactions between amylose or amylose–lipid (HOOVER and VASANTHAN, 1994). ADEBOWALE *et al.* (2009) found that for starch granules, the hydration and swelling of the amorphous regions is driven by gelatinization, which involves the melting of crystalline regions and double helices. During the swelling of the amorphous regions, stress is induced on the crystalline regions as well as on the polymer chains and the former is removed from the starch crystallite surface. Subsequently, treated starches require a higher temperature for the swelling and breaking of crystalline areas leading to the increased T_o, T_p, and T_c which was also confirmed in our study.

As an accompanying effect, a decrease in enthalpy of the temperature-induced gelatinization process was observed in the optimum sample (123.64 J/g) as compared to the control (180.70 J/g). It is obvious that the reduction in ΔH following the hydrothermal treatment indicated partial gelatinization of some fractions of molecules having smaller heat stability, as suggested by STUTE (1992) and HORDMOK and NOOMHORM (2007). Also, the decrease in ΔH can be interpreted as a presence of disorganized double helices of starch granules revealing a crystalline and non-crystalline character under the treatment conditions. Thus, some reductions in the fraction of unbend and melted double helices during gelatinization would be noticed after treatment.

3.4. Sensory attributes

The sensory characteristics of cooked GF pasta are reported in Table 6. The sensory evaluation showed no significant difference (p>0.05) in taste, color and flavor between the GF control and the optimum pasta. The obtained results showed that the optimum recipe pasta was significantly better with the highest scores for appearance and stickiness.

Table 6. Sensory evaluation and overall acceptability of gluten-free pasta.

	Appearance[1]	Color[1]	Flavor[1]	Taste[1]	Stickiness[1]	Overall acceptability[2]
Control pasta	3.75±0.34[b]	4.18±0.21[a]	3.45±0.34[a]	4.40±0.43[a]	3.17±0.40[b]	4.42±0.33[b]
Optimum pasta	4.45±0.46[a]	4.22±0.31[a]	3.35±0.33[a]	4.42±0.42[a]	4.52 ±0.48[a]	6.42±0.24[a]

[1]5-point scale, [2]9-point hedonic scale (n = 55).

[a-b]Means with the same superscript within a column are not significantly different (p > 0.05).

The best scores were recorded for appearance and stickiness of the optimum pasta, as confirmed by the texture measurements; it can be attributed to the enhancing effect of the hydrothermal treatment of rice flour added to the pasta recipe in our study. Moreover, the selected optimum pasta gathered superior scores (values above 5) in the overall acceptability in comparison with the control sample without the addition of PGRF.

3.5. Microstructure of dry pasta

The microscopic pictures can be helpful in analyzing the appearance, texture or integrity of food products (GORINSTEIN *et al.*, 2004). The surface of traditional laminated GF pasta is presented in Fig. 4.

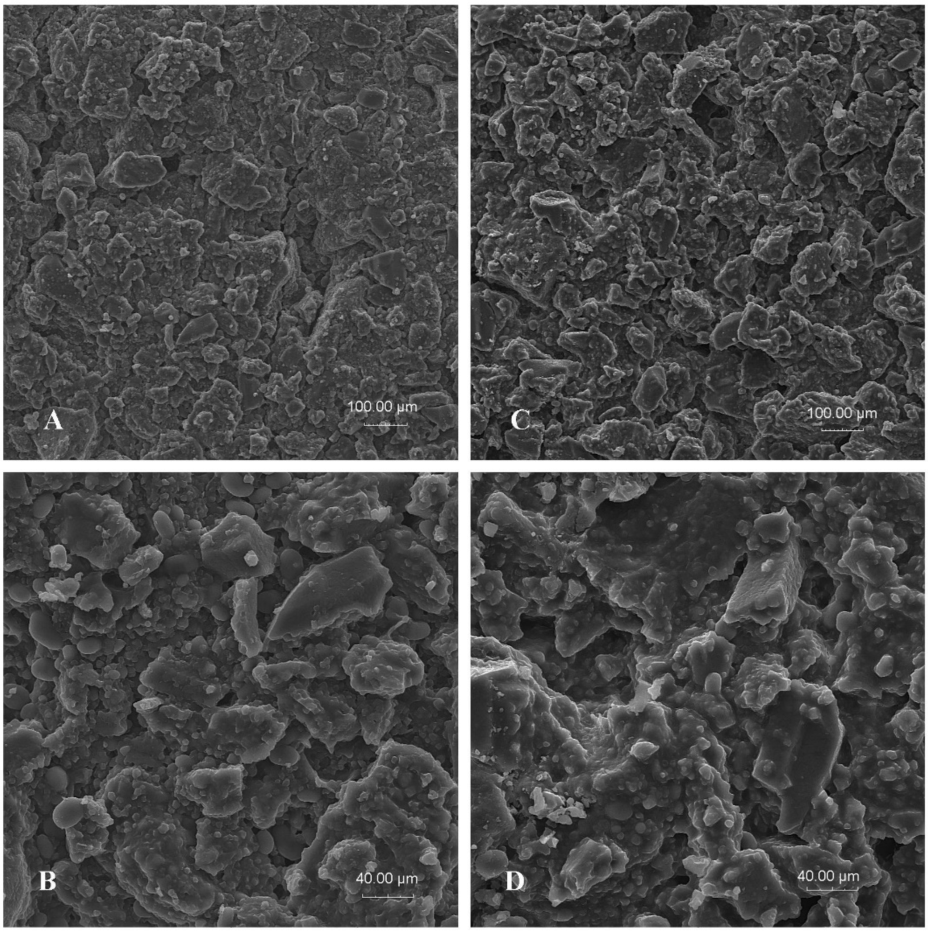

Figure 4. SEM surface of laminated pasta from control and optimized recipe at different magnifications; control pasta: A) x200, B) x600, optimum pasta: C) x200, D) x600.

The analysis of the micrographs of pasta manufactured from the RFBS recipe without PGRF (Fig. 4A) exhibits the presence of small cracks with a small amount of empty spaces, which suggests some lack of continuity due to the absence of gluten. Also, the graphs showed the structure of poorly agglomerated starch granules and separate granules visible on the pasta surface (Fig. 4B). The microstructure images, presented in Fig. 4C, highlighted the differences in starch organization and collocation observed on the surface of traditional pasta laminated from the optimized recipe with the addition of PGRF. It was characterized by a compact and homogeneous matrix. The agglomerates of starch and proteins are surrounded by pre-gelatinized starch, which forms a continuous phase responsible for the better results of the CL and a low quantity of starchy components passing into the cooking water. Pre-gelatinized rice flour forms aggregates combine all pasta components, which is clearly visible at high magnification (Fig. 4D). Similar morphological features could be observed and confirmed by analyzing the cross-sectional microstructure of pasta (Fig. 5).

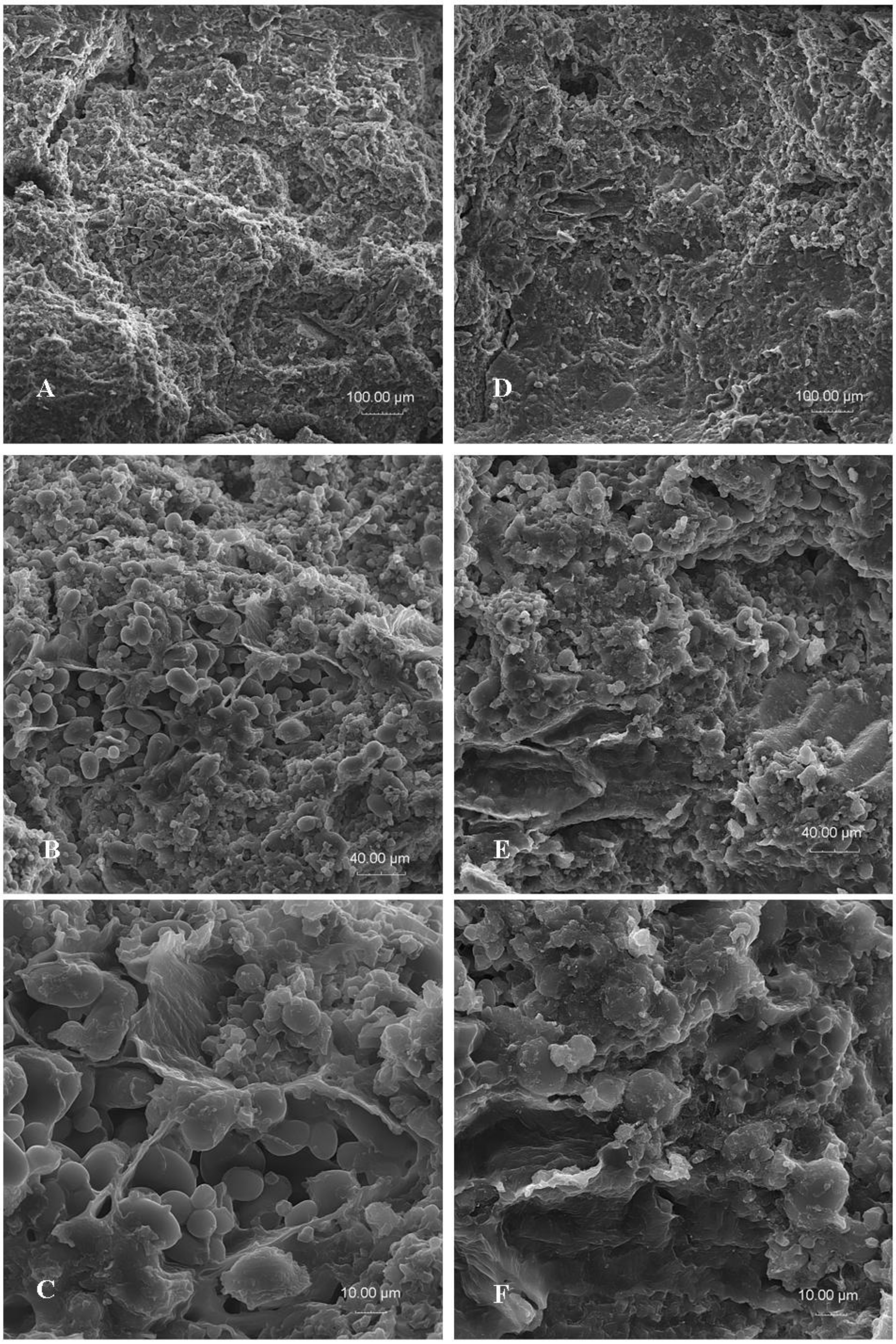

Figure 5. Cross-section of control RFBS pasta: A) x200, B) x600, C) x1500, and optimum pasta with the addition of PGRF: D) x200, E) x600, F) x1500 observed with SEM.

Fig. 5A and 5B show the irregular and discontinuous internal structure of RFBS pasta with a small amount of swollen starch granules and visible higher dimensions, and only few places are linked by a protein fibrous fraction that comes from field bean flour (Fig. 5C). A more compact and uniform structure can be observed if pre-gelatinized rice flour was used as a pasta improver (Fig. 5D). The incorporation of PGRF caused the ultra-structural reorganization of matrix and influenced the excellent microstructure. Comparing the size and amount of visible starch granules, pasta with the addition of treated rice flour showed the lower amount of free starch and a more uniform distribution of components inside the tread (Fig. 5E). Starch granules are surrounded by a continuous phase of gelatinized starch responsible for the limited leaching of components during cooking and the enhanced consistency of PGRF-improved pasta (Fig. 5F).

4. CONCLUSIONS

Laminated pasta based on rice and enriched with field bean semolina provides an important quantity of dietary fiber and protein with a positive amino acids balance. The supplementation of cereals with legumes is likely enhance the nutritional value of gluten-free pasta products. Optimization made by an experimental design showed that the amount of water and improver are the important factors having an influence on the quality of gluten-free pasta. The optimum formulation of rice and field bean containing 5.845 g of pre-gelatinized rice flour and 59.266 mL of water was selected based on the desirability function approach with the value of 0.775, which showed the optimum pasta properties. The hydrothermal treatment of rice flour resulted in the changes to the starch structure and the physical properties of PGRF pasta, such as the cooking and texture parameters, pasting and hydrations properties, thermal features, microstructure, and sensory profile. The obtained results also showed that the addition of pre-gelatinized flour induced significant differences ($p<0.05$) in all parameters in comparison with the control pasta. The pasta processed with the optimized formula was characterized by the improved cooking quality and texture. As regards the sensory evaluation, the optimum recipe showed acceptable scores for all the sensory attributes and the overall quality of gluten-free pasta. Optimized GF rice and field bean pasta obtained using hydrothermally treated rice flour as the improver allow the manufacture of gluten-free laminated pasta with improved nutritional characteristics and appropriate quality suitable for celiac people. Hydrothermal treatment, as an environmentally friendly technique of modifying starch functionality at low cost, fits the public demand for natural products and is highly promising in the long perspective.

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PAPER

DESCRIPTIVE SENSORY PROPERTIES OF *CECINA DE LEÓN*

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ABSTRACT

Cecina de León is the Protected Geographical Indication of a dry-cured beef produced in Northwest Spain. A Quantitative Descriptive Analysis (QDA)® of three types of *Cecina de León* pieces (thick flank or *babilla*, silverside or *contra*, and topside or *tapa*) was performed by a trained 10-member sensory panel using an intensity non-structured 10-cm length scale. Average sensory scores varied between 3.14±1.54 (beef flavour) and 6.95±1.26 (brightness of lean). Contra pieces showed lower percentage of unacceptable scores (9.57%) than *babilla* (10.24%) or *tapa* (13.09%). Frequency of unacceptable values was lower for appearance (4.09%) than for flavour (15.32%) or texture (8.79%) attributes.

Keywords: *cecina*, dry-cured meat, quantitative descriptive analysis

1. INTRODUCTION

Cecina de León is a high value intermediate moisture meat (approximately 50% humidity) produced exclusively in the province of León (Northwest Spain) from hind leg pieces (*babilla* or thick flank, *cadera* or rump, *contra* or silverside, and *tapa* or topside) of beef cattle, with a minimum age of 5 years old and weight of 400 kg. This food product has the quality label, Protected Geographical Indication (PGI; OJEC, 1996).
Cecina de León is manufactured following a processing scheme based on the preparation of pieces and profiling (excision from the carcass and rubbing in order to eliminate any remaining blood, and shaping of the pieces for adjusting); salting (with common salt at 3-5ºC for 0.3-0.6 days per kg weight); washing (with lukewarm water in order to eliminate any remaining salt); settling or post-salting (for 30-45 days in a cold room to allow for a homogeneous distribution of salt within the meat mass); smoking (optional, with oak or holm-oak wood, between 12 and 16 days), and drying (in natural drying kilns with adjustable windows to control the temperature and humidity using the traditional system of "opening and closing windows", or in industrial drying installations). The whole process takes a minimum of seven months after salting.
The production of *Cecina de León* has increased over the last few years from 1,500 manufactured pieces in 1994 to more than 100,000 pieces in 2012. *Cecina* from *babilla*, *contra* and *tapa* make up more than 95% of the production (Supervisory Council of Protected Geographical Indication *Cecina de León*, private communication). The Supervisory Council of PGI *Cecina de León* has to control the sensory quality of the *cecina* pieces in order to detect the presence of defects in the product as well as to certify its typicality in such a way that it can be differentiated in comparison with non-labeled products.
Most reports on *Cecina de León* refer to physicochemical and microbiological characteristic (GARCÍA *et al*. 1998; MENÉNDEZ *et al*., 2015; MOLINERO *et al*., 2008). The hedonic and descriptive sensory properties have been scarcely studied (RUBIO *et al*., 2007; MOLINERO *et al*., 2008). To the best to our knowledge, the influence of the type of piece used for manufacture on the descriptive sensory attributes of *Cecina de León* has yet to be reported. This study was designed to describe the sensory properties of *Cecina de León*; to investigate whether the type of meat used for manufacturing has a significant influence on sensory attributes of this foodstuff, and to determine the frequency of intensity scores outside specifications for each attribute and type of meat piece tested.

2. MATERIALS AND METHODS

2.1. Samples

Eleven *Cecina de León* pieces (three *babilla*, five *contra* and three *tapa* pieces) were randomly obtained from normal production in different processing plants in the Province of León (Northwest Spain). *Babilla* or thick flank is made up of *vastus lateralis, vastus intermedius, vastus medialis* and *rectus femoris* muscles, *contra* or silverside is composed by *semitendinosus* and *gluteobiceps* muscles, and *tapa* or topside contains the *quadratus femoris, semimembranosus, adductor, gracilis, pectineus* and *sartorius* muscles and a fragment of *obturatorius externus* muscle.

2.2. Sensory evaluation

A trained 10-member sensory panel (eight males and two females, ranging in age from 23 to 47 years, with experience in sensory evaluations) was used to evaluate attributes of each sample. The trained assessors were selected and trained for two years according to International Organization for Standardization regulations (ISO 6658:2005, ISO 8586:2012, ISO 11132:2012).

A Quantitative Descriptive Analysis (QDA; ISO 13299:2016) was used to describe *cecina* pieces, which were evaluated in a tasting room equipped with white fluorescent lighting (ISO 8589:2007). Scores were given for appearance (cherry colour, brightness of lean, marbling and fat colour), flavour (odour characteristic, flavour characteristic, persistence of flavour, taste characteristic, saltiness, beef flavour and smokiness) and texture (tenderness, juiciness and fibrousness) attributes on a non-structured 10-cm length scale with anchor points one cm from each end, where 0 means absence (white for fat colour attribute) and 10 means great intensity (yellow for fat colour attribute). Scores were the distances (cm) from the left extreme. The panelists were also asked to indicate the heterogeneity of the colour (Table 1). *Cecina* pieces showing different intensities were used to define the scale for the descriptors (reference standards).

Table 1. Description of the sensory attributes considered in this work.

Attribute	Definition
Cherry colour	Visual assessment relating to the hue and the lightness (intensity) of the typical red colour of *cecina*
Brightness of lean	Brightness intensity (attribute of a glossy surface showing bright reflection) of the lean surface
Marbling	Level of visible intramuscular fat
Fat colour	Colour intensity of subcutaneous fat
Odour characteristic	Assessment relating to the odour before eating the sample, associated with the ripening and smoking process
Flavour characteristic	Assessment relating to the olfactory/gustatory sensation caused by salt, ripening and smoking process
Persistence of flavour	The time during the olfactory/gustatory sensation is perceptible after the bolus has been swallowed or ejected
Taste characteristic	Assessment relating to the taste associated with the salt, ripening and smoking process
Saltiness	Basic taste sensation elicited by NaCl
Beef flavour	Flavour after cooking/heating of beef
Smokiness	Assessment relating to the olfactory/gustatory perception caused by the smoking of these products with smoke obtained from wood burning
Tenderness	Softness and ease of chewing before swallowing
Juiciness	Perception of the amount of water released by the product during the first chews
Fibrousness	Perception of the amount of muscle fibers detected during chewing
Heterogeneity of the colour	Assessment of the uniform distribution of colour on the slice

A portion of each piece of *cecina* was presented to the panelists at room temperature (21±1ºC) for evaluation of visual attributes and odour characteristic, and slices of approximately 2 mm thick were presented for evaluation of the remaining sensorial attributes. Samples were randomly labeled with three digit codes and panelists were asked to evaluate each sample in randomized order. Mineral water at room temperature was used to cleanse the palate between successive samples.

The testing of the eleven *cecina* pieces was carried out in four sessions (four sets of two or three samples, randomly chosen) at daily intervals. Each sample was evaluated by all panelists in the same session. A replication for each *cecina* piece was carried out in a different session; each session lasting approximately 2 hours. The performance of panel

and panelists was confirmed by their reliability, reproducibility and discrimination in sensory descriptive tests (ROSSI, 2001; RODRÍGUEZ-LÁZARO *et al*., 2002b, c).
Sensory specifications (represented by the range of intensities tolerated for each attribute) were established by correlating descriptive data with scores from consumer hedonic evaluation (nine-point hedonic scale). Intensities of attributes in the QDA were considered acceptable when they were associated with scores ≥ 5 in the hedonic evaluation. Attributes of positive evaluation (the better the intensity, the better the quality): cherry colour, brightness of lean, marbling, odour characteristic, flavour characteristic, persistence of flavour, taste characteristic, tenderness and juiciness, were deemed as unacceptable if a score lower than 5 was given by the panelist, according to the 10-cm scale. The beef flavour (attribute of negative evaluation) was considered unacceptable when scores were higher than 5. For the remaining attributes (fat colour, saltiness, smokiness and fibrousness), scores lower than 3 and higher than 8 were considered as unacceptable values (RODRÍGUEZ-LÁZARO *et al*., 2002d).
Statistical analysis. Mean and standard deviations for all *cecina* samples data were calculated. Sensory panel evaluation averages were analyzed by an analysis of variance (ANOVA). Mean separation was carried out using the Duncan's multiple range test. Pearson's correlation coefficients were calculated. The Statistica® 8.0 (Statsoft Ltd., Tulsa, OK, USA) software package was used.

3. RESULTS AND DISCUSSION

Analysis of variance of the four factors (replication, attribute, type of meat piece and panelist) showed statistical differences ($P < 0.001$) between scores from different attributes. Replication, panelist, type of meat piece or their interactions did not influence ($P > 0.05$) descriptive scores.
The score values obtained by the attributes tested (the mean data of replications were considered) are given in Table 2.
Values differed markedly between samples, as indicated by the standard deviations (STD) calculated on all groups of samples, which were much greater than the STD obtained from replicate analysis (data not shown). The absence of significant differences between *babilla*, *contra* and *tapa* pieces are probably due to the relatively high standard deviations found, which are mainly due to the heterogeneity of the samples. According to REYES-CANO *et al*. (1994), there are differences between animals (age, breed, sex) that may have an influence on the sensory properties of *cecina* pieces.
Even though significant differences were not found between types of piece, *contra* pieces showed the best behaviour because they scored higher ($P > 0.05$) than *babilla* and *tapa* pieces in five (55.6%) of the nine attributes of positive evaluation (cherry colour, odour characteristic, flavour characteristic, persistence of flavour and taste characteristic), and lower ($P>0.05$) in beef flavour (attribute of negative evaluation). Moreover, *contra* pieces showed the lowest mean percentage of unacceptable scores: 9.57%, as opposed to 10.24% and 13.09% for *babilla* and *tapa* pieces, respectively (Fig. 1). The only *cecina* without any unacceptable (outside specifications) score was a *contra* piece.
No substantial differences were found for average scores between *babilla* and *tapa*. However, *tapa* pieces showed a higher percentage of unacceptable scores, and heterogeneity in colour was detected in 10% of tests. This attribute is an important and desirable sensory property of dry-cured meats when they are sliced (ARNAU *et al*., 1998).

Table 2. Average values for the sensory properties of three different types of *Cecina de León* pieces.

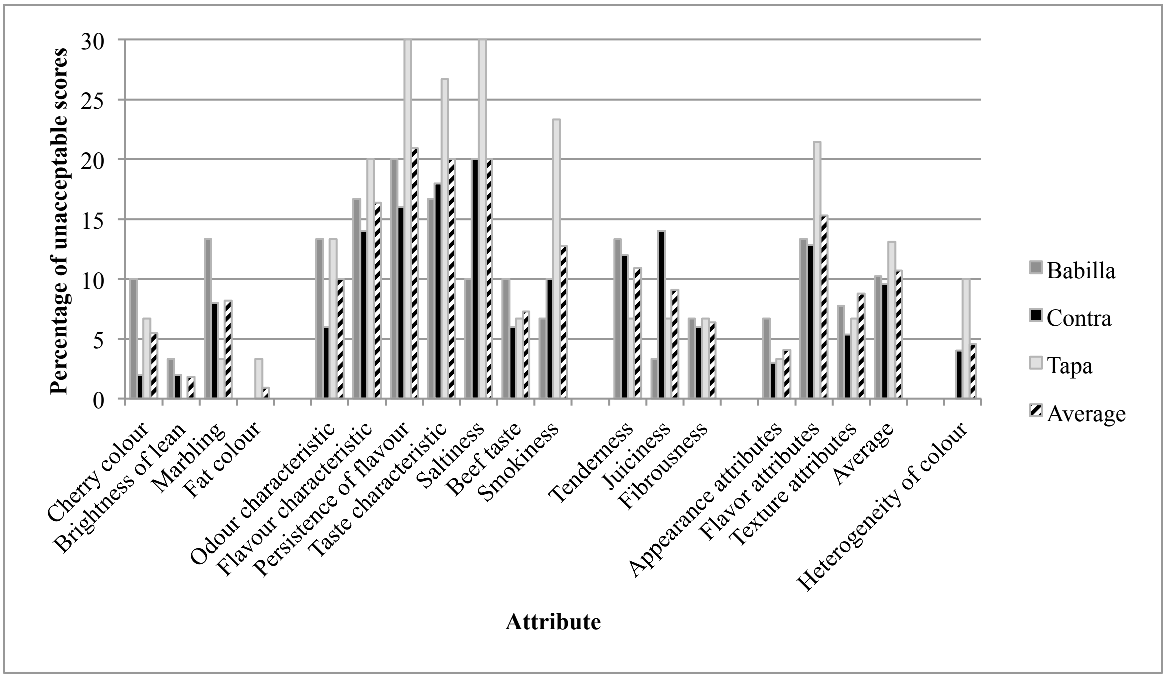
Attribute	Sensory modality for the attributes	Meat piece			Average
		Babilla	*Contra*	*Tapa*	
Cherry colour	Appearance attributes	6.70±1.56a	6.94±1.35a	6.87±1.33ab	6.85±1.39ab
Brightness of lean		7.00±1.39a	6.96±1.19a	6.90±1.27b	6.95±1.26ab
Marbling		6.63±1.88a	6.64±1.59ab	6.90±1.24ab	6.71±1.58abc
Fat colour		5.47±1.09bc	5.49±1.14cd	5.49±1.02cd	5.48±1.09d
Odour characteristic	Flavour attributes	6.57±1.59ad	6.66±1.36ab	6.57±1.72ab	6.61±1.51bc
Flavour characteristic		6.07±1.66bd	6.24±1.49ac	5.97±1.79ace	6.12±1.61ef
Persistence of flavour		6.00±1.55bd	6.34±1.57ab	5.87±1.81ace	6.12±1.63ef
Taste characteristic		6.07±1.64bce	6.10±1.70bc	5.67±1.88ace	5.97±1.73e
Saltiness		3.68±0.83f	3.70±0.95ef	3.48±1.15f	3.63±0.97g
Beef flavour		3.30±1.64f	3.04±1.54e	3.13±1.48f	3.14±1.54h
Smokiness		4.04±0.85f	4.04±1.01f	3.76±1.11f	3.96±1.00g
Tenderness	Texture attributes	6.26±1.48ad	6.30±1.50ab	6.50±1.41abc	6.34±1.46ce
Juiciness		6.63±1.27ade	6.36±1.53ab	6.37±1.45be	6.44±1.44cf
Fibrousness		5.04±1.25c	4.83±1.26d	5.01±1.24d	4.94±1.24i
Grouping attributes[1]					
Appearance attributes		6.45±1.60a	6.51±1.45a	6.54±1.35a	6.50±1.46a
Flavour attributes		5.10±1.90b	5.16±1.97b	4.92±2.04b	5.08±1.97b
Texture attributes		5.98±1.49c	5.83±1.59c	5.96±1.51c	5.91±1.54c

[1],mean score for the attributes in each modality.
A 10-cm non-structured scale was used by trained assessors.
Average values within a column (for single attributes or for grouping attributes) that are not followed by the same letter are significantly different ($P<0.05$). No significant differences were found between means in the same row.
Data are the means of 60, 100, 60 and 220 determinations for the first (3 *cecina* pieces x 10 panelists x 2 replications), second (5 *cecina* pieces x 10 panelists x 2 replications), third (3 *cecina* pieces x 10 panelists x 2 replications) and fourth columns, respectively.

The lower number of unacceptable scores was obtained by a contra piece (0%) and the higher by a *tapa* piece, which showed a total of 24 of 140 (14 attributes x 10 panelists) unacceptable values (17.14%). It must be noted that scores considered as unacceptable were close to the intensity range tolerated for each attribute.

Average scores of appearance attributes were significantly ($P<0.05$) higher than those of flavour and texture (Table 2). Moreover, unacceptable values were substantially lower for appearance (4.09%) than for flavour (15.32%) and texture (8.79%) attributes. MARTÍN *et al*. (1999) also observed the best scores for appearance attributes in *Cecina de Maestrazgo* pieces.

Significant ($P<0.001$) Pearson's correlations were found between marbling and odour characteristic (0.542), flavour characteristic (0.431), persistence of flavour (0.407) and taste characteristic (0.399). These results coincide with findings of DE ANDA-SERRANO *et al*. (1999) in ham samples, and may be explained by taking into account the fact that fat compounds are important components of flavour in meat products. According to KAUFFMAN (1993), marbling is required to adequately provide flavour attributes. The high correlation coefficients detected between smokiness and characteristic flavour and taste attributes ($P<0.001$; $r>0.7$) also agrees with previous findings in smoked meat products (SINK and HSU, 1979).



For cherry colour, brightness of lean, marbling, odour characteristic, flavour characteristic, persistence of flavour, taste characteristic, tenderness and juiciness, a value was considered as unacceptable when a score lower than 5 was given by the panelists, according to the 10-cm scale. The beef flavour was considered unacceptable for scores higher than 5. For fat colour, saltiness, smokiness and fibrousness, scores lower than 3 and higher than 8 were considered as unacceptable values.

Figure 1. Percentage of unacceptable scores for each attribute tested in three different types of *Cecina de León* pieces.

The negative correlation coefficient found between saltiness and brightness ($P<0.01$; r=-0.270) and tenderness ($P<0.05$; r=-0.197) may be explained by considering the influence of salt on proteolysis activity (GUERRERO *et al*., 1996). Lower salt levels are related to a higher proteolytic activity and consequently with a higher tenderness and brightness. It should be noted that the normal salt concentration in *Cecina de León* (5.6%) is generally lower than that of ham (RODRÍGUEZ-LÁZARO *et al*., 2002a).
Finally, a significant ($P<0.001$) correlation was found between fibrousness and flavour, persistence of flavour and taste attributes (r=0.428 to 0.467). These results are similar to previous findings by BUSCAILHON *et al*. (1994) in dry-cured ham. According to these authors, higher fibrousness induces longer chewing time, which allows for better extraction and stronger perception of some compounds responsible for taste and flavour.
To summarize, sensory properties of *Cecina de León* are not significantly influenced by the type of meat piece used for manufacturing, which is a positive aspect for producers. However, *contra* pieces showed the best behaviour, with the lowest percentage of outside specifications (unacceptable) scores. Flavour attributes showed the highest, and appearance attributes the lowest, percentage of unacceptable scores for all *cecina* pieces examined. On average, one tenth of the scores for each piece were outside (although close to) the range of intensities tolerated.

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PAPER

APPLE SLICES ENRICHED WITH *ALOE VERA* BY VACUUM IMPREGNATION

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ABSTRACT

Recently, the interest in *Aloe vera* has been increased accordingly with its content in polymannas, which show many healthy effects. The vacuum impregnation (VI) was used to enrich apple slices with *Aloe vera* gel. The effects of vacuum level, vacuum and relaxation times on the main chemical and physical attributes were described. Results showed as, applying the best operating conditions, VI allowed to introduce *Aloe vera* gel into the pores of apple tissue reaching a content of polymannan between 1 and 8 mg/100 g of fresh apples.

Keywords: fresh cut apples, polymannans, enriched fruit, aloe vera gel

1. INTRODUCTION

Aloe vera (*Aloe barbadensis* Mill.) is a perennial xerophyte belongs to the *Liliaceae* family, which consists in about 360 species. The internal fraction of the leaves is a large thin-walled parenchyma cells in which the water is held as a gel (NEWTON, 2004). The *Aloe vera*'s gel consists of water (99.5%) and solids (0.5%) such as polysaccharides, vitamins, minerals, enzymes, phenolic compounds and organic acids (ESHUN and HE, 2004; BOUDREAU and BELAND, 2006). However, a mass fraction of 60% of the total solids is constituted by polysaccharides (MCANALLEY, 1993). Recently, the interest on *Aloe vera* gel has increased on the basis of its healthy properties. Back in the past, peoples used the *Aloe vera* for its curative and therapeutic properties. Recently the intake of the gel has been proved to have positive effects in the treatment of gastrointestinal, kidney and cardiovascular deseases. Also, the gel has used to reduce the cholesterol and triglyceride levels in human blood (LIM *et al.*, 2003; GEREMIAS *et al.*, 2006). Furthermore, anti-inflammatory and antibiotic properties, as well as positive effects against several diseases, have been reported (REYNOLDS and DWECK, 1999; ESHUN and HE, 2004). Several scientific papers (HAMMAN, 2008; RODRIGUEZ *et al.*, 2010) proved the most of the health benefits may be attributed to polysaccharides (DAGNE *et al.*, 2000; NI *et al.*, 2004; HABEEB *et al.*, 2007) such as cellulose, hemicellulose, glucomannans, mannose derivatives and acetylated mannans (ROBERT and TRAVIS, 1995; FEMENIA *et al.*, 1999; LEE *et al.*, 2001). Given these considerations, food industries have been attracted from the use of *Aloe vera* as an ingredient to improve the functional properties of food products. Moreover, the FDA approved the use of *Aloe vera* gel extracted as a "*dietary supplement*" (RAMACHANDRA and SRINIVASA RAO, 2008). Thus, a wide number of enriched foods with *Aloe vera* such as yogurt, juice, pasta are available on the market. However, food processing could induce irreversible modifications of the polysaccharides, reducing or inactivating the health effects (FEMENIA *et al.*, 2003; ESHUN and HE, 2004; CHANG *et al.*, 2006; VEGA-GÁLVEZ *et al.*, 2011).

The vacuum impregnation (VI) is a very interesting technique that allows to introduce, dissolved or suspended substances in the void fraction (i.e. the pores) of food in a controlled manner (GRAS *et al.*, 2002). VI occurs at room temperature, avoiding the thermal degradation of nutritional and functional compounds. In the last years, several authors studied the application of VI to obtain foods enriched with structural compounds (MARTINEZ-MONZO *et al.*, 1998), probiotic microorganisms (PUENTE *et al.*, 2009), fruit juices (BETORET *et al.*, 2012; CASTAGNINI *et al.*, 2015), phenols (SCHULZE *et al.*, 2014), folic acid (MORENO *et al.*, 2016), sugars (NERI *et al.*, 2016), etc. SANZANA *et al.* (2011) evaluated the effects of VI with *Aloe vera* on the respiration rate of some vegetables (endive, cauliflower, broccoli and carrots). In some previous papers we applied the vacuum impregnation in order to accelerate the acidification of some vegetables (DEROSSI *et al.*, 2013a,b) as well as to improve the quality of thawed truffles by introducing anti-freezing proteins (DEROSSI *et al.*, 2015a).

Given these considerations, the main aim of this paper was to study the application of vacuum imprengation to improve the functional properties of apple slices by using an extract of *Aloe vera gel*. Specifically, the effects of the main process variables of VI on the level of enrichment as well as on the main physical properties of apples slices were studied using the response surface methodology.

2. MATERIALS AND METHODS

2.1. Fresh apple and Aloe vera plant

Fresh apples (cv. *Golden Delicious*) were purchased to the local market in September 2015 and stored for a maximum of 3 days at 4°C. Before treatments, the apples were equilibrated at room temperature. *Aloe vera* plant (*Aloe Barbadensis Mill.*) of three years old was supplied by Ricciotti Gardens (Foggia, Italy). The gel was prepared by cutting the fresh leaves and separating the outer green rind from the inner parenchyma.

2.2. Vacuum impregnation treatments

After washing and peeling, the apples were manually cut in slices with a thickness of 0.5 cm. The *Aloe vera* extract was prepared by dissolving 25 g of the *Aloe vera* gel in 125 mL of distilled water at room temperature under agitation at 300 g^{-1} for 90 min. A product/solution mass ratio of 1:5 (w/w) was used for each experiment.

2.3. Experimental design

A factorial design was used to study the effects of three variables (pressure, p, vacuum time, t_1, and relaxation time, t_2) on the main quality attributes of apples (BOX and BEHNKEN, 1960). The pressure was modified between 50 and 450 mbar while vacuum times and relaxation times were studied in the ranges of 1-5 min and 5-15 min, respectively. These values were chosen on the basis of preliminary experiments performed in order to define wide ranges able to significantly increase the weight of apple slices that is a rough index of the filling of pores. More specifically, a $3^{(k-p)}$ fractional factorial design was implemented with $k = 3$ and $p = 1$ obtaining a total of 9 experimental conditions. Each VI test was repeated in triplicate by using the experimental conditions reported in Table 1.

Table 1. Experimental conditions of vacuum impregnation experiments.

Experiments	Pressure (mbar)	Vacuum time (t_2, min)	Relaxation time (t_1, min)
1	450	5	15
2	450	1	10
3	450	3	5
4	250	1	15
5	250	5	5
6	250	3	10
7	50	1	5
8	50	3	15
9	50	5	10

2.4. Chemical and physical analysis

Moisture (x_w) and solid (x_s) content of samples were gravimetrically measured by drying 5 g of vegetable tissue at 65°C until a constant weight (CABEZAS-SERRANO *et al.*, 2009).

2.5. Changes in porosity

Porosity values of fresh and VI samples were obtained comparing apparent (ρ_a) and real solid-liquid (ρ_r) density values. All porosity values were expressed as kg/m^3. Pycnometer method was used to determine the apparent density (ρ_a) using an isotonic sucrose solution as a reference. Real (ρ_r) density and porosity fraction (ε) were estimated as reported by GRAS *et al.* (2002). The weight increase (DE) was expressed as relative differences on the basis of fresh weight.

2.6. Determination of total polymannans content

The total polymannans content was determinated by using the colorimetric assay proposed from EBERENDU *et al.* (2005) with some minor changes. A volume of 400 mL of aqueous extract, 500 mL of NaOH (0.1 M) and 1 mL of Congo Red (Sigma Aldrich) dye (diluted by a factor of 500) were mixed and agitated for 2 h, then the changes in colour were analysed at 540 nm with a spectrophotometer (Perkin Elmer Lambda 25 UV/VIS). The calibration curve was performed by using a solution of β-glucan (Sigma Aldrich Inc.) in the range between 3.2 and 100 mg/L as reported by PELLIZZONI *et al.* (2012).

2.7. Statistical analysis

The effect of each independent variable on the quality indexes of apple samples was evaluated by ANOVA test with a significant level of 0.05. Also, the results were described by Pareto's charts. Moreover, 3D plots describing the changes of each dependent variable as a function of experimental conditions were obtained by fitting the experimental data with a polynomial model as reported by DEROSSI *et al.*, (2015b). All statistical analyses were performed using Statistica ver. 10.0 (Statsoft Tulsa, USA).

3. RESULTS AND DISCUSSION

Table 2 shows the main physico-chemical attributes of fresh apples. Average values of moisture and solids content of *Golden delicious* were of 0.88±0.01 g H_2O /g f.w. and 0.12±0.01 g H_2O /g f.w., respectively. These values were in good agreement with other authors (MUJICA-PAZ *et al.*, 2003; PAES *et al.*, 2007; WU *et al.*, 2007).

Table 2. Physico-chemical properties of fresh apples.

Parameter	Value ± SE
Moisture content (X_w) (g H_2O/g f.w.)	0.88±0.01
Solids content (X_s) (g/g f.w.)	0.12±0.01
Apparent density (ρ_a) (kg/m^3)	822±0.04
Real density (ρ_r) (kg/m^3)	1047±0.04
Porosity fraction (ε) (%)	21.5±0.04

Moreover, a porosity fraction of 21.4±3.69 % indicates slight variability in porosity as a consequence of their biological variance, which includes the ripening degree, the environmental conditions, varieties, etc. In general, literature has reported a significant variability in porosity of apples with values between ~18 % and ~27 % (SALVATORI *et al.*, 1998; MARTINEZ-MONZÒ *et al.*, 2000; MUJICA-PAZ *et al.*, 2003; PAES *et al.*, 2007) which are in agreement with our data. As expected, polymannans were not revealed in fresh apples. With the aim to quantify the content in polymannans in aqueous extracts of *Aloe vera* the distribution function of about 40 measurements performed on extracts prepared from different part of *Aloe vera* plant, is reported in Fig. 1. A normal distribution was proved by the Shapiro-Wilk's test that exhibited a value of 0.98. The mean value was of 0.355 ± 0.078 mg/g enables to state that the most of the observation fell in the range of 0.3 and 0.4 mg/g. Also, minimum and maximum values of 0.188 mg/g and 0.546 mg/g were also observed.

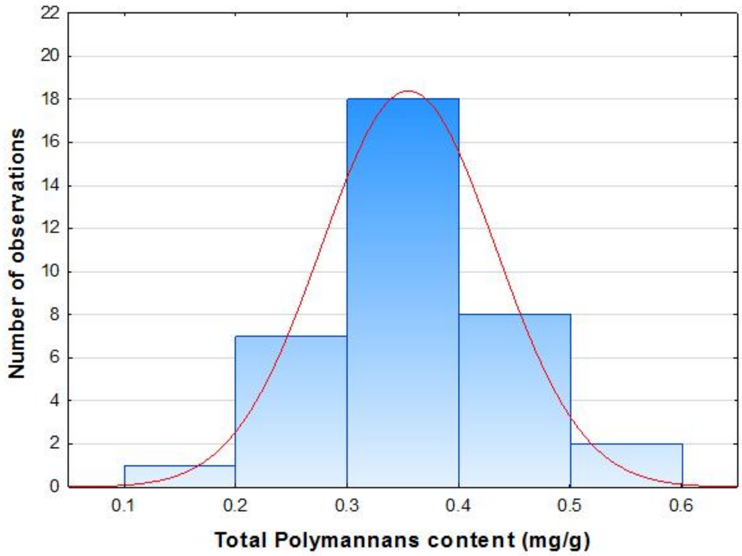
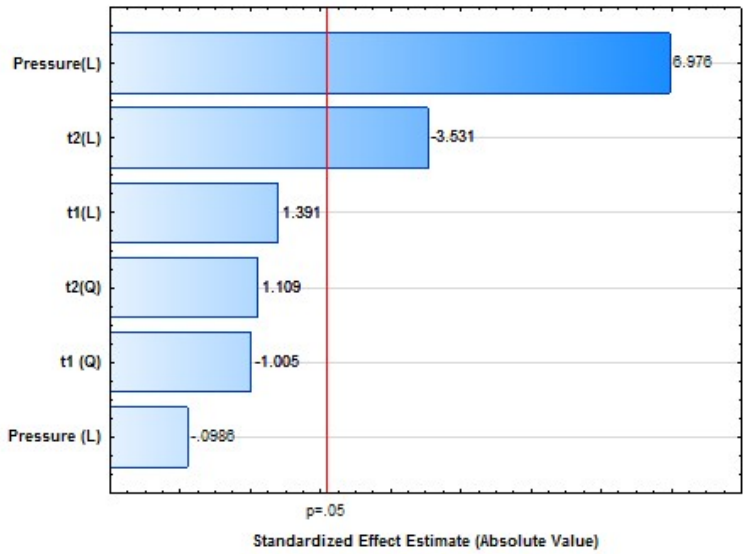


Figure 1. The normal probability function of polymannans content of the *Aloe vera* gel extracts.

The results of statistical analysis showed that the pressure value and the relaxation time linearly affected the changes in porosity of apples showing standardized effects of 6.97 and -3.53, respectively (Fig. 2a). This means that as the pressure decreased as the porosity decreased too, while the relaxation time showed an inverse relationship with the void fraction of apple tissue. Since that the driving force of VI is the difference between internal (i.e. inside the pores) and external pressures, the higher was the vacuum, the greater was the impregnation. Also, the longer was the relaxation times, the higher was the reduction in porosity, because there was more time for impregnation and relaxation phenomenon (GRAS *et al.*, 2002; MUJICA-PAZ *et al.*, 2003; NERI *et al.*, 2016). Figure 2b shows the 3D plot describing the effects of relaxation time and pressure on the porosity fraction of apples. At first, taking into account the treatment performed at the lower vacuum of 450 mbar and the minimum relaxation time of 5 min, a significant reduction in porosity from fresh apple (ε = 21.4±3.69%) to 14% was observed.

Moreover, according to Pareto chart of Fig. 2a the pressure had the highest effect in the reduction of porosity fraction of the samples. According to this results porosity fraction reduced from 0.14 to ~0.08% when the pressures of 450 and 50 mbar were applied, respectively, with a minimum t_2 of 5 min (Fig. 2b).



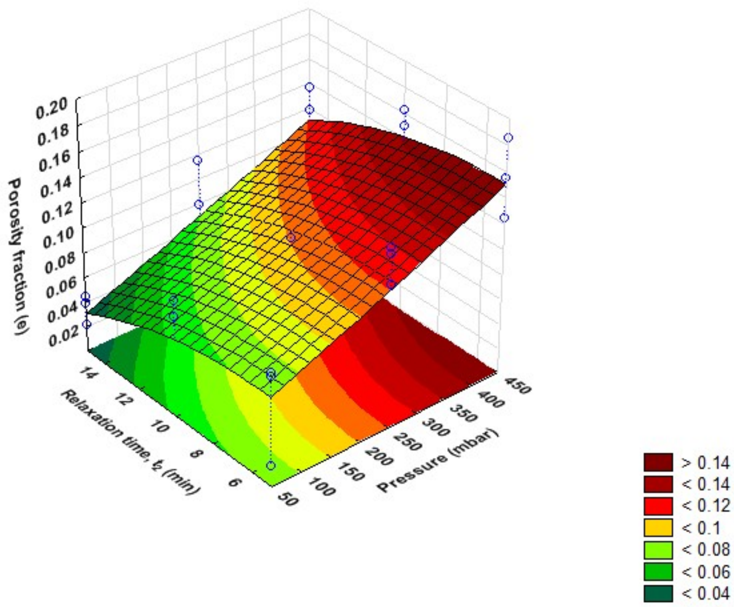


Figure 2. a) Estimated effects of the independent variables on the porosity fraction of apple slices submitted to VI treatments with *Aloe vera* gel extract. L and Q refer to the linear and no linear (quadratic) effect, respectively. b) 3D Plot describing the effects of relaxation time and pressure on the fraction porosity of apple slices submitted to VI treatments with *Aloe vera* gel extract.

On the other hand, a negligible reduction in porosity was observed by applying relaxation time from 5 to 15 min for some pressure applied. For instance, appling a pressure of 50 mbar, values of 0.08 and 0.03 were measured progressively increasing t_2. Moreover, the results stated the very high reduction in porosity fraction of apples tissue independently from the length of vacuum time; in fact, by reducing t_1 a change in porosity only of 0.4% was achieved (data not shown). However, experimental data show a high variability, which cannot be underestimated. This could be the result of the variance in microstructure properties such as the porosity of fresh apples, the presence of closed pores, the size and dimension of capillaries, their tortuosity, etc. However, the decrease of the porosity fraction after VI treatment cannot assure that the impregnation by external solution occurred. As well known, during relaxation time the impregnation and the compression of capillaries are involved, both allowing to reduce the porosity fraction of fruit. The equilibrium between the impregnation and compression level is controlled by several variables such as the viscosity of the solution, the rigidity of vegetable tissue, etc., most of which cannot be manually controlled. The Pareto chart (Fig. 3a) shows the effect of the independent variables on the weight increase of apples. The pressure linearly affected the weight increase of apple samples exhibiting a standardized effect of -3.58, while the other variables did not show any effect. The variation in weight as a function of vacuum time and pressure are shown in Fig. 3b.

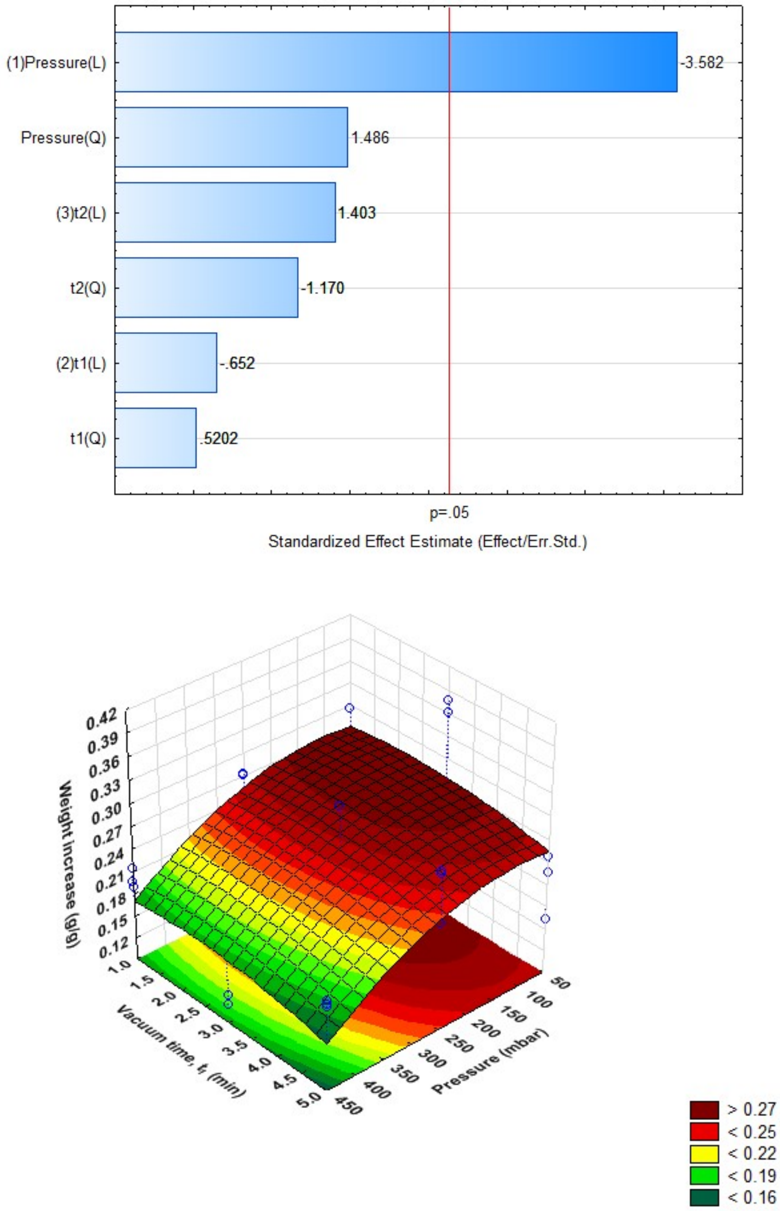
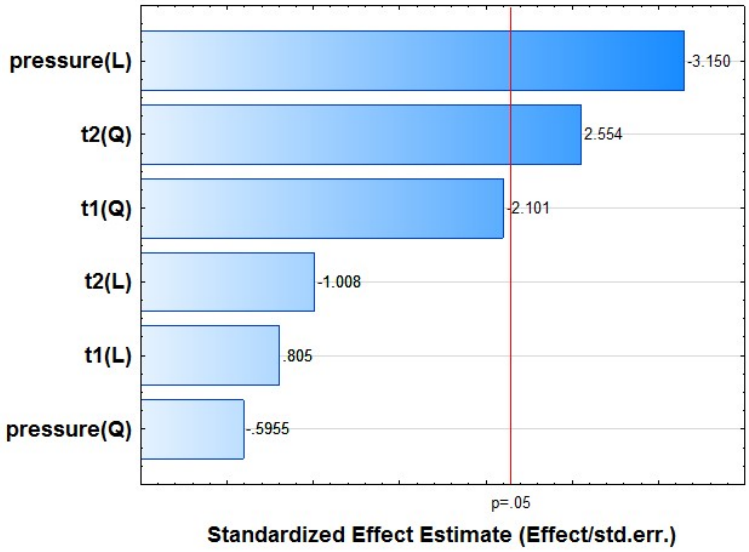


Figure 3. a) Estimated effects of independent variables on the weight increase of apples slice submitted to VI treatment with *Aloe vera* gel extracts. L and Q refer to the linear and no linear (quadratic) effect, respectively. b) 3D plot describing the effect of vacuum time and pressure on the weight increase of apple slices.

According to pareto chart, only the pressure exhibited a significant effect. Even considering the lower t_1 = 1 min the weight of apple slices increased from ~0.18 to ~0.26 g/g by reducing the pressure from 450 to 50 mbar. That means that a significant amount of *Aloe vera* extract was introduced in the void phase of apple. On the other hand, any differences were not observed increasing the vacuum time until 5 min. Also in this case, the experimental data showed a not negligible variability that could be the result of differences in microstructure (porosity, connectivity, pore size distribution, etc.). Fig. 4a reports the standardized effects of the independent variables on polymannans content of apple slices. The pressure and the relaxation time were the most important variables affecting the enrichment of apple slices. The standardized effects of -3.15 and 2.55 showed as the pressure exibhited the greater effect on enrichment of apple samples. Fig. 4b shows the effect of the vacuum level on the polymannans content of apples.



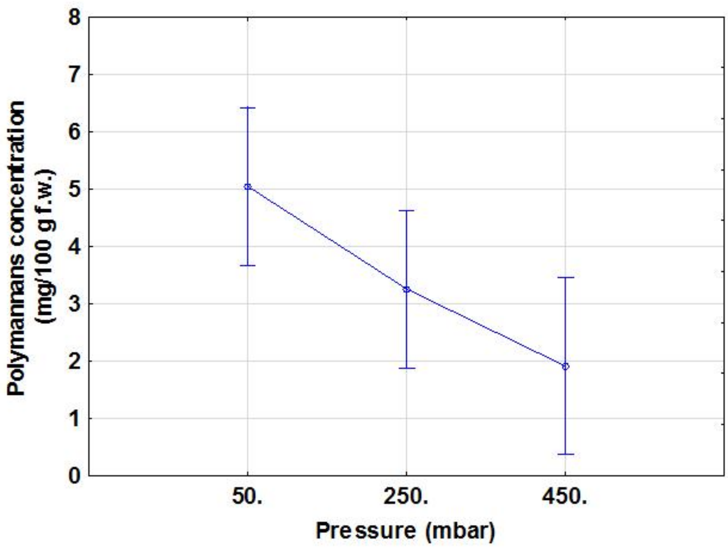


Figure 4. a) Standardized effect of the independent variables on the polymannan content of apple slice submitted to vacuum impregnation with *Aloe vera* gel extract. L and Q refer to the linear and no linear (quadratic) effect, respectively b) Changes in polymannas content of apple slices submitted to vacuum impregnation as a function of pressure.

More specifically, the figure was obtained by grouping all experimental data for the pressure values used during experiments while the other two variables, t_1 and t_2, were free to change. The positive effect of the vacuum impregnation is clearly observed. By reducing the pressure from 450 to 50 mbar, the average content in polymannans increased from 2 to 5 mg/100 g f.w. This means that VI treatments may be considered a useful method to enrich apples with the bioactive compounds of *Aloe vera*. Of course, the high variability of the data (i.e. error bars) was caused by the different relaxation times as well as by the effects of the microstructure variability of fresh apple.
Fig. 5 shows the 3D plot describing the effects of pressure and relaxation time on the polymannans content of apple slices. The enrichment in polymannans was obtained for any experimental condition with values ranged between 1 and 8 mg/100 g f.w.. For any relaxation time, by increasing the vacuum level, it was possible to enrich apple slices with polymannans. An average increase of 7 mg/100 g f.w. was obtained decreasing the pressure, progressively, with a fixed relation time of 5 or 15 min. For a t_2 of 10 min a peak of polymannans content was reached according to the non-linear effect reported in figure 4a. This means that a negative effect was observed for relaxation time longer than 10 min, probably because a prolonged compression damaged apple tissue favoring the release of *Aloe vera* extracts from the capillaries. On these bases, further experiments, focused on the changes in void and solid matrix phases as well as in terms of microstructure, would be necessary to improve the understanding of the behavior of apple tissues under VI treatment and to optimize the enrichment in polymannans.

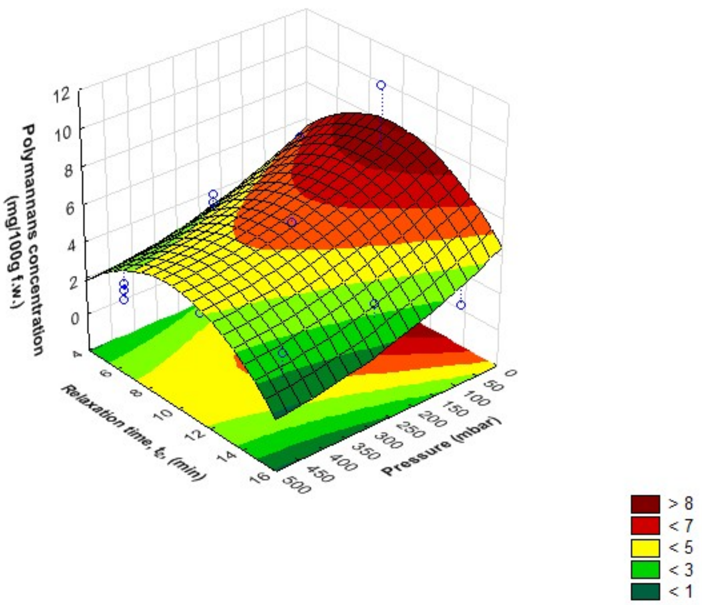


Figure 5. 3D Plot describing the effect of relaxation time and pressure on the changes in polymannas content of apples slice submitted to vacuum impregnation treatments.

4. CONCLUSIONS

By vacuum impregnation is possible to obtain fresh-cut apple slices with improved healthy properties by filling their pores with an *Aloe vera* gel extract. Pressure and relaxation time significantly affected the porosity fraction and the polymannans content of apples, while any effect was not observed modifying the vacuum time. From an initial porosity of 21.4 % of fresh apples, values of 16% were obtained for the weakest treatments, while a value of 2% was reached for the strongest experimental conditions. The weight increase ranged between 0.16 and 0.25 g/g proved the significant impregnation of the pores by the *Aloe vera* extract. The best operative conditions were a pressure of 50 mbar and a relaxation time of 10 min by which a polymannans content of 8 mg/100 g was measured in apple slices. On the other hand a further increase in t_2 produced a decrease in polymannans content probably caused by the damage on the vegetable tissues, which reduced the capacity of the capillaries to retain the external solution.

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PAPER

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF FERMENTED MEAT PATTY WITH *LACTOBACILLUS* STRAINS

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ABSTRACT

The effect of fermentation by *Lactobacillus fermentum* PTCC 1638, *Lactobacillus plantarum* subsp. *plantarum* PTCC 1745 and *Lactobacillus sakei* subsp. *sakei* PTCC 1712 on antimicrobial activity against Alternaria alternate PTCC 5224, *Aspergillus parasiticus* PTCC 5018, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* O157 H7 ATCC 35150 and *Salmonella Typhimurium* ATCC 14028 as well as antioxidant properties (carbonyl assay, peroxide and anisidine value) in a beef patty during 24 h of fermentation and further storage at 4°C for 8 days were investigated. Results indicated that *L. plantarum* subsp. *plantarum* had the highest radical scavenging activity (54.3±1.7%) before fermentation. During the fermentation process, DPPH and ABTS activities of the meat patty were improved in comparison to the control. The highest antioxidative value was observed for *L. plantarum* subsp. *plantarum*. All of three strains had a strong antimicrobial effect against pathogenic bacteria and fungi. Oxidation products were enhanced in fermented and non-fermented samples. However, the increasing trend of the oxidation process was mitigated in all fermented samples. In particular, the lowest protein and lipid oxidation values were observed in the samples treated by *L. plantarum* subsp. *plantarum*. Generally speaking, fermentation improves the antioxidative and antimicrobial effect of meat patty and lengthens its storage period.

Keywords: antimicrobial activity, antioxidant activity, fermentation, *Lactobacillus* strains, meat product

1. INTRODUCTION

Lactic acid bacteria (LAB) are the primary microorganisms involved in fermentation (SALMINEN *et al*., 2004) by consuming simple sugars such as glucose as the substrate (YADAV, 2017). LAB possess antimicrobial and anticancer activities and play a critical role in the balance of gut microbial flora, synthesis of vitamins, improvement of immunes system, reduction of cholesterol level, prevention of food allergy, improvement of lactose absorption and so forth (MANSOURIPOUR *et al*., 2013). Moreover, as antioxidant and anti-inflammatory agents are used for treatment of various diseases such as diabetes, Alzheimer's, Parkinson's, high blood pressure, liver disorders (WOO *et al*., 2014). Also, application of LAB cultures to promote antibacterial and antioxidant properties of foods has been recommended by many investigations (LI *et al*., 2012; PISANO *et al*., 2014).

Fermentation was a cheap and simple method to conserve meat since ancient time. Production of acid (pH reduction), H_2O_2 and bacteriocins alone or in combination with starter cultures prevents from the growth of meat deteriorating microorganisms. Improving in aroma is another advantage of fermentation. Considering limitation of chemical additives, application of fermentation techniques in meat has been increased (SAKHARE and RAO NARASIMHA, 2003). One of oldest meat products created to increase meat conservation is fermented sausage in which fermenting microorganisms especially LAB are used. The mentioned microorganisms (single species or a mix of different microorganisms) are added into meat paste as starter cultures (YILMAZ and VELIOGLU, 2009). LEROY *et al*. (2005) demonstrated that the application of functional meat starter cultures in fermented sausages promotes product's safety via production of bacteriocins and other antimicrobial compounds (JAFARI *et al*., 2017).

LAB species used in the production of dry fermented sausages possess antibacterial properties against *Listeria monocytogenes*, *Staphylococcus aureus* (PAPAMANOLI *et al*., 2002,2003). Various strains of *Lactobacillus plantarum* show strong antioxidant and antibacterial properties during the fermentation process (HASHEMI *et al*., 2017). Furthermore, use of *Lactobacillus* in fermented pork prevents the growth of different *Clostridium* species (DI GIOIA *et al*., 2016). However, based on the some investigations, other microorganism such as probiotic *Bacillus* could pose similar effects (JAFARI *et al*., 2017).

Considering above mentioned issues, the present study was conducted to investigate the antioxidant and antimicrobial effect of meat patty fermented by *Lactobacillus fermentum* PTCC 1638, *Lactobacillus plantarum* subsp. *plantarum* PTCC 1745, *Lactobacillus sakei* subsp. *sakei* PTCC 1712 during fermentation process and storage period.

2. MATERIALS AND METHODS

2.1. Microbial culture

Lactobacillus fermentum PTCC 1638, *Lactobacillus plantarum* subsp. *plantarum* PTCC 1745, *Lactobacillus sakei* subsp. *sakei* PTCC 1712, *Alternaria alternat*e PTCC 5224, and *Aspergillus parasiticus* PTCC 5018 were purchased from the culture collection at Iran Institute of Industrial and Scientific Research. Reactivation of the *Lactobacillus* strains was done in the MRS broth (Oxoid, UK) at 37°C for 48 h. The mold cultures were cultivated on yeast extract dextrose chloramphenicol agar (Lab M, UK) slants for 9 days at 25°C. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* O157 H7 ATCC 35150 and *Salmonella Typhimurium* ATCC 14028 were obtained from microbial culture stock of Veterinary

School, Shiraz University. The strains were reactivated in defined Mueller Hinton broth (Oxoid, UK) and left for incubation at ~37°C.

2.2. Fermented meat patty preparation and storage

Fresh ground beef was obtained from a local supermarket in Shiraz city (Fars, Iran). Ground beef and irradiated herb spice were pasteurized at 80°C for 15 min. Fermented meat patty was prepared by mixing ground beef (2 kg), herb spice (10 g), pasteurized brine (8 mL, 10% w/v) and each *Lactobacillus* strain culture (~10^5 CFU/g). This preparation was subsequently placed into glass vessels (3 L) and kept in an incubator (Shimazu, SHI1 55 AL, Iran) to ferment at 35°C for 24 h. A time course analysis was carried out prior to fermentation and 4, 8, 16, 20 and 24 h during the fermentation process. A control (non-fermented) sample was also prepared. After fermentation, fermented and non-fermented meat patty samples were kept at 4°C in refrigerator for 8 days. Approximately, 120 samples were prepared and all of the experiments were carried out in triplicate.

2.3. DPPH free radical scavenging activity of *Lactobacillus* strains

The DPPH content was determined using the described method of KAO and CHEN (2006). After centrifugation at 3500 × g (Hettich, EBA21, Germany) for 20 min, the absorbance of cell samples was determined using spectrophotometer (UV/Visible Philips Cambridge, UK) at 517 nm. The blank sample corresponded only to the cells emerged in methanol.

2.4. Meat patty analysis during fermentation

- The pH of fermented and non-fermented samples was determined during the 24 h period of fermentation using a pH-meter (model 520A, Orion Research Inc., MA, USA).
- The enumeration of *L. fermentum*, *L. plantarum* subsp. *plantarum*, and *L. sakei* subsp. *sakei* was done during 24 h period of meat patty fermentation. Enumeration of *Lactobacillus* strains was carried out using MRS agar (Oxoid, UK) after incubation under anaerobic conditions (35°C, 72 h).
- DPPH radical scavenging activity of fermented meat sauce samples was performed according to the method of KATO *et al*. (1988). About 1 mL of the filtrated sample was mixed with 1mL of DPPH reagent (250 mM) and 1mL of 0.1MTris-HCl buffer (pH 7.4) in test tubes. After incubation at room temperature, the absorbance of sample was assessed at 517 nm. Ethanol was used as a blank.
- $ABTS^+$ activity was measured according to the method of SHIRWAIKAR *et al*. (2006). About 100 mL of sample extract was blended with 4.9 mL of $ABTS^+$ working standard solution and absorbance was determined after 20 min at 734 nm. The $ABTS^+$ activity was evaluated by using equation:

$$ABTS^+ \text{ activity } (\%) = \left[\frac{Absorbance(0) - Absorbance\ (20)}{Absorbance\ (0)}\right] \times 100$$

- The antimicrobial activity of fermented samples against *Alternaria alternata*, *Aspergillus parasiticus*, *Staphylococcus aureus*, *Escherichia coli* O157 H7 and *Salmonella* Typhimurium was measured at the end of fermentation, by means of the well diffusion method. For bacterial cells, the inoculum (10^6 CFU/ mL) was spread on plates containing Mueller-Hinton Agar (Oxoid, UK). For molds, well was created on yeast extract dextrose chloramphenicol agar (Lab M, UK) plates, which had been previously inoculated by 0.1 mL of inoculums containing indicator molds in the range of 10^4-10^5 spores/mL. Subsequently, aliquot solutions (80 μL) from meat patty samples were forwarded into the wells. The agar plates were incubated at 37°C for 24 h and at 25°C for 48 h for pathogenic bacteria and molds, respectively. Then, the disk diameter of inhibition zones was measured.

2.5. Meat patty analysis during storage

Protein and lipid oxidation of samples were measured during storage.
- Protein carbonyls of fermented and non-fermented meat patty samples were measured according to the described method of LEVINE *et al*. (1994). Carbonyl groups were measured by precipitation of 100 μL of sample with 50 μL of trichloroacetic acid (100%, w/v). After preparation of samples, the absorbance was determined at 280 and 370 nm for measurement of carbonyl content of samples.
- Lipids were extracted according to the described method of BLIGH and DYER (1959) using chloroform/methanol (1:1, v/v). The ferric-thiocyanate technique described by SHANTA and DECKER (1994) was conducted for the measurement of peroxide value (PV). Anisidine value (AnV) of the samples was measured according to the AOCS method (1998).

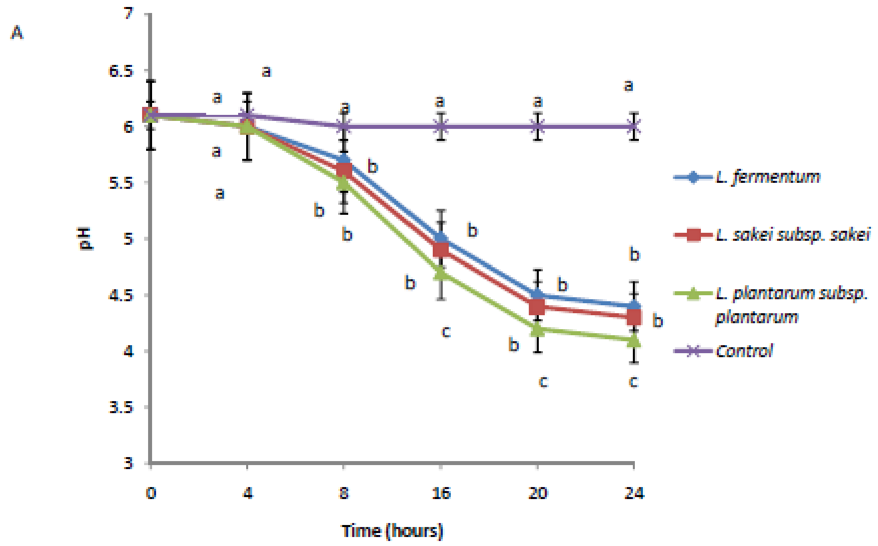
2.6. Statistical analysis

Statistical analysis was conducted with one-way ANOVA and Duncan's multiple range tests (SPSS package program; v. 20.0 for Windows, SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. *Lactobacillus* growth and pH changes during fermentation

The reduction of pH, production of lactic acid and antimicrobial compounds including bacteriocins, non-bacteriocins and non-lactic substances can be considered as the major mechanisms of LAB activities include (FAYOL-MESSAOUDI *et al*., 2005). In current study, pH variation of meat patty during 24h fermentation was investigated as depicted in Fig.1A. pH variation trend was similar in the three species *L. fermentum*, *L. plantarum* subsp. *plantarum* and *L. sakei* subsp. *sakei*. Initial pH was 6.1 in all of the three species and decreased to 4.1, 4.3 and 4.4 at the end of the fermentation. Significant variation ($p<0.05$) occurred between 4 and 20 hours of the fermentation process as shown in the Fig. 1A. pH reduction after fermentation is due to the growth of fermenting microorganisms and production of lactic acid from available carbohydrates by these strains (DROSINOS *et al*., 2007). YADAV (2017) reported that application of *L. plantarum* significantly ($p<0.05$) reduced pH during fermentation of chicken sausages. Similar results also have been reported the direct correlation between pH reduction and LAB growth during meat fermentation (COCOLIN *et al*., 2001a, 2001b). According to the United States Department of Agriculture, pH should be lower than 5 in highly stable fermented meat products (LEISTNER and RODEL, 1975).



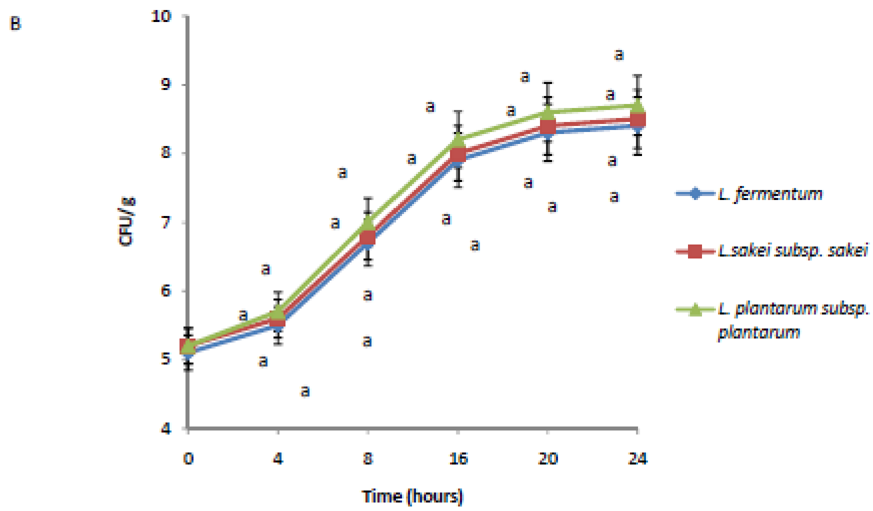


Figure 1. pH changes (A) and changes in cell viability of *Lactobacillus* strains (B)of meat patty samples during fermentation. Means in each hour with the same superscript lowercase letters are not significantly different at $P<0.05$.

The growth of LAB in current investigation was promoted during fermentation (Fig.1B). An initial number of the three strains in fermented meat patty was about 5.2 CFU/g that reached to 8.5 CFU/g at the end of fermentation (24 h). All strains showed similar logarithmic growth during the first 16 hours and a constant and slow growth afterward. Increased LAB growth coinciding with pH reduction suggests resistance and compatibility of them against pH variation (CEBECİ and GÜRAKAN, 2003). Indeed, there is a direct correlation between microbial cells population and pH (JOHNSON and STEELE, 2013). According to COMI *et al*. (2005), LAB population increased at early stages of production of dry fermented sausages and then remained at a constant value of 7-9log CFU/g. Moreover, in French fermented sausages, the population of fermenting strains increased and reached the same value at the end of the fermentation process (REBECCHI *et al*., 1998). Increased LAB growth is attributed to pH reduction during early stages of fermentation (COCOLIN *et al*., 2001a, 2001b).

3.2. Radical scavenging activity of *Lactobacillus* strains and fermented meat patty

Radical scavenging activity was measured, and the corresponding results are presented in Table 1. As seen, there is a significant difference ($p<0.05$) among the bacterial species, and the highest value was observed in *L. plantarum* subsp. *plantarum* (54.3±1.7%); followed by *L. sakei* subsp. *sakei* and *L. fermentum*.

Table 1. Radical scavenging activity of *Lactobacillus* strains against DPPH radicals.

***Lactobacillus* strains**	**Radical scavenging activity (%)**
L. fermentum	38.2 ± 1.1^{c}
L. sakei subsp. *sakei*	44.6 ± 1.5^{b}
L. plantarum subsp. *plantarum*	54.3 ± 1.7^{a}

[a]Means in the column with different superscript letters differ significantly ($P < 0.05$).

Moreover, radical scavenging activity (RSA) was measured in fermented meat patty during the 24h period (Fig. 2A). The results indicated that RSA was increased through the time. By progress and completion of fermentation, RSA value was elevated. The highest RSA value was observed in the sample treated by *L. plantarum* subsp. *plantarum* that increased from 12.1% to 48.2% at the end of fermentation, followed by *L. sakei* subsp. *sakei* (increase from 12.1% to 41.3%) and *L. fermentum* (from 12.1% to 33.5%). Thus, *L. plantarum* had better antiradical properties. For example, RSA value obtained in fermentation by *L. sakei* subsp. *sakei* can be achieved by fermentation with *L. plantarum* subsp. *plantarum* for 12 hours. LI *et al*. (2012) investigated antioxidant activity of traditional Chinese fermented food and concluded that *L. plantarum* had the highest hydroxyl radical and DPPH scavenging activities (44.31% and 53.05%; respectively). Moreover, it was found out that proteins and polysaccharides residing at the surface of *L. plantarum* provide the species with antioxidant power; hence, degradation of these compounds reduces DPPH free radical scavenging capacity of *L. plantarum*. Evaluation of antioxidant activity of *L. plantarum* isolated from Marcha of Sikkim indicated that this strain possesses high DPPH scavenging activities (DAS and GOYAL, 2015). Similar investigations support our findings regarding application of *L. plantarum* in the fermentation of various foods (KULLISAAR *et al*., 2002; WANG *et al*., 2009; HASHEMI *et al*., 2017).

Evaluation of $ABTS^{+}$ activity in meat sauce during fermentation revealed that $ABTS^{+}$ value was enhanced by an increase in time and the highest value of this parameter was achieved by *L. plantarum* subsp. *plantarum* that increased from 26.3% at the first hours to 64.24% at the 24^{th} hour (Fig. 2B). The last two assays suggest that *Lactobacillus* strains especially *L. plantarum* had high antioxidant power because the results show that the highest value of this parameter obtained by 24h fermentation with *L. sakei* subsp. *sakei* and *L. fermentum* can be achieved by 14 h and 10 h fermentation with *L. plantarum* subsp. *plantarum*. These findings accord with those reported by YADAV (2017) who found out that application of *L. plantarum* in sausage fermentation promotes $ABTS^{+}$ activity and DPPH activity.

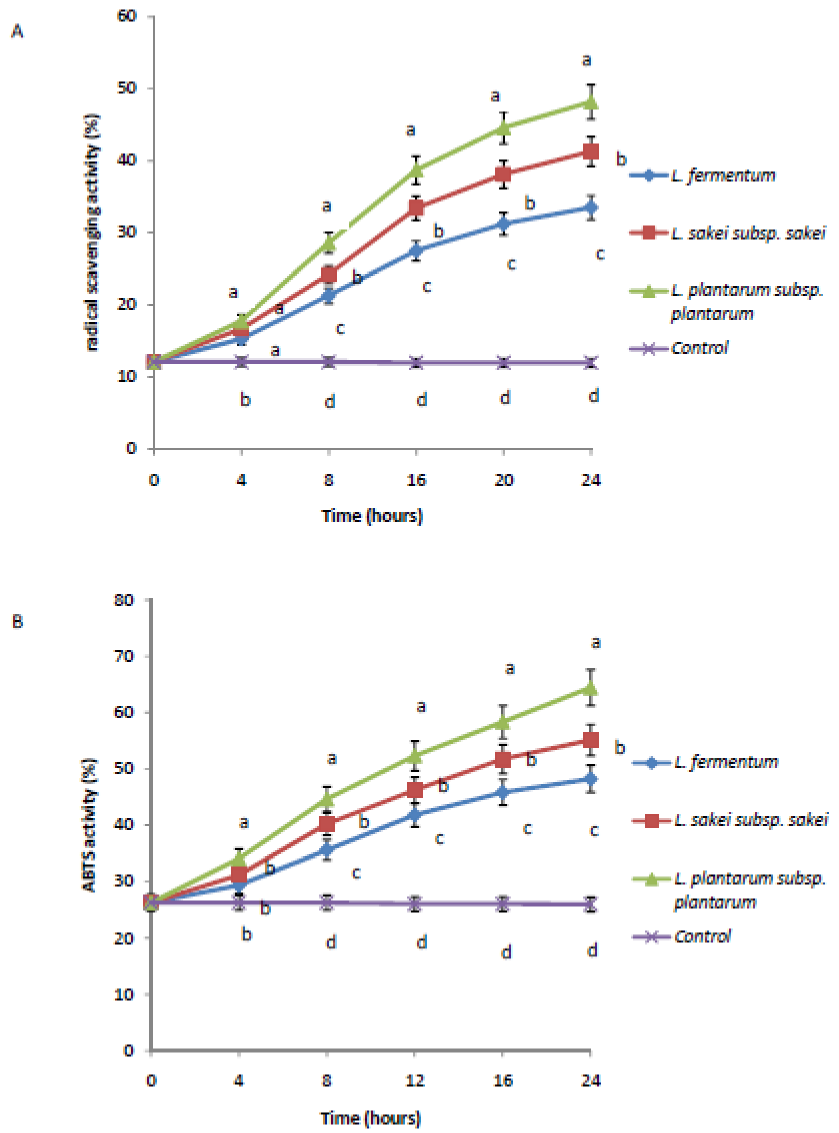


Figure 2. Changes in DPPH activity (A) and ABTS+ activity (B) of meat patty samples during fermentation with Lactobacillus strains. Means in each hour with the same superscript lowercase letters are not significantly different at P<0.05.

3.3. Antimicrobial activity of fermented meat patty

Antimicrobial activity of meat patty during fermentation was investigated against *A. alternat*e, *A. parasiticus*, *S. aureus*, *E. coli* O157 H7 and *S. Typhimurium*, and the result were represented in Table 2. The highest antimicrobial activity was corresponded to sample fermented with *L. plantarum* whose inhibition zone diameters (Table 2). Inhibition zone diameters of *L. plantarum* against *E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, A. *parasiticus* and *A. alternat*e were 22.6±0.3mm, 20.9±0.4mm, 25.9±0.4mm, 28.5±0.5mm, and 27.9±0.8mm; respectively. The highest inhibition zone diameter of *L. plantarum* was 28.5±0.5mm that obtained for *A. parasiticus*; followed by *L. sakei* subsp. *sakei* and *L. fermentum*. Inhibition zone of *L. plantarum* often had significant difference (p<0.05) with that of the two other strains. LABs prevent microbial growth and meat deterioration by acid production (pH reduction), H_2O_2 and bacteriocins production. YADAV (2017) reported that *L. plantarum* plays a critical role in the prevention of microbial growth during sausages chicken fermentation. YILMAZ and VELIOGLU (2009) found out that LAB was the leading cause of microbial growth inhibition in fermented products. The author observed that the number of *Bacillus* strains was significantly (p<0.05) lower in the samples treated by 24h fermentation, while the number was increased in control group. REA *et al*. (2013) investigated antimicrobial properties of species used in fermented sausages and concluded that the main reason behind the reduction of *Bacillus* in these products was pH reduction and bacteriocins production by the fermenting bacteria. Application of *L. plantarum* in fermentation has antimicrobial activity against *E. coli* O157: H7 and *S. aureus* (HASHEMI *et al*., 2017).

Table 2. Antimicrobial activity of meat patty samples with different *Lactobacillus* strains.

Meat samples	**Inhibition zone diameters (mm)**				
	***E. coli* O157:H7**	***S. Typhimurium***	***S. aureus***	***A. parasiticus***	***A. alternata***
L. fermentum	18.4±0.5^{cC}	15.7±1.1^{bD}	20.5±0.6^{cB}	23.7±0.6^{bA}	24.1±0.7^{cA}
L. sakei subsp. *sakei*	20.3±0.9^{bD}	16.3±0.8^{bE}	23.1±1^{bC}	27.4±1.2^{aA}	25.5±0.4^{bB}
L. plantarum subsp. *plantarum*	22.6±0.3^{aC}	20.9±0.4^{aD}	25.9±0.4^{aB}	28.5±0. 5^{aA}	27.9±0. 8^{aA}
Control (non-fermented)	0±0^{d}	0±0^{d}	0±0^{d}	0±0^{d}	0±0^{d}

[a]Values represent means ± standard deviations of inhibition zones. Means within a column with the same superscript lowercase letters are not significantly different at P<0.05 and means within a row with the same superscript uppercase letters are not significantly different at P<0.05.

3.4. Lipid and protein oxidation of meat sauce samples during storage

PV and AnV were measured to evaluate oxidative stability in fermented meat patty at 4°C for 8 days. Lipid oxidation includes continuous formation of hydroperoxide as primary oxidation products that can be degraded to various volatile substances as secondary oxidation products (ADEGOKE *et al*., 1998). PV indicates primary oxidation products that are odorless materials that are degraded during the reaction and converted to a wide range of substances including carbonyl, hydrocarbons, furans and other products creating an unsuitable taste of the foods (YANISHLIEVA and MARINOVA, 2001). PV was measured during 8-day period after fermentation. As depicted in Fig.3A, the samples fermented with three LAB strains had lower PV than the control group (p<0.05). Among fermented samples, the sample fermented with *L. plantarum* subsp. *plantarum* had the lowest PV (p<0.05), suggesting antioxidant properties of the LAB. In this research, the highest antioxidant activity was observed for *L. plantarum* subsp. *plantarum*. PV increased with time, but this increasing trend for *L. plantarum* was at the lowest rate compared to other fermented and control samples (p<0.05).

As an indicator of secondary oxidation, AnV was measured during the 8day period. A similar trend was observed for this parameter (Fig. 3B), meaning that the parameter was increased in all fermented and control samples, but *L. plantarum* was the best species considering resistance against formation of secondary oxidation products. AnV was increased by the time but the lowest value on the first and eighth days was observed in the sample treated by *L. plantarum* subsp. *plantarum* (p<0.05). These results accord with those reported by other authors indicating that food fermentation by LAB especially by *L.*

plantarum improves the oxidative stability of the products (TSENG and ZHAO, 2013; HASHEMI *et al*., 2017; YADAV, 2017).

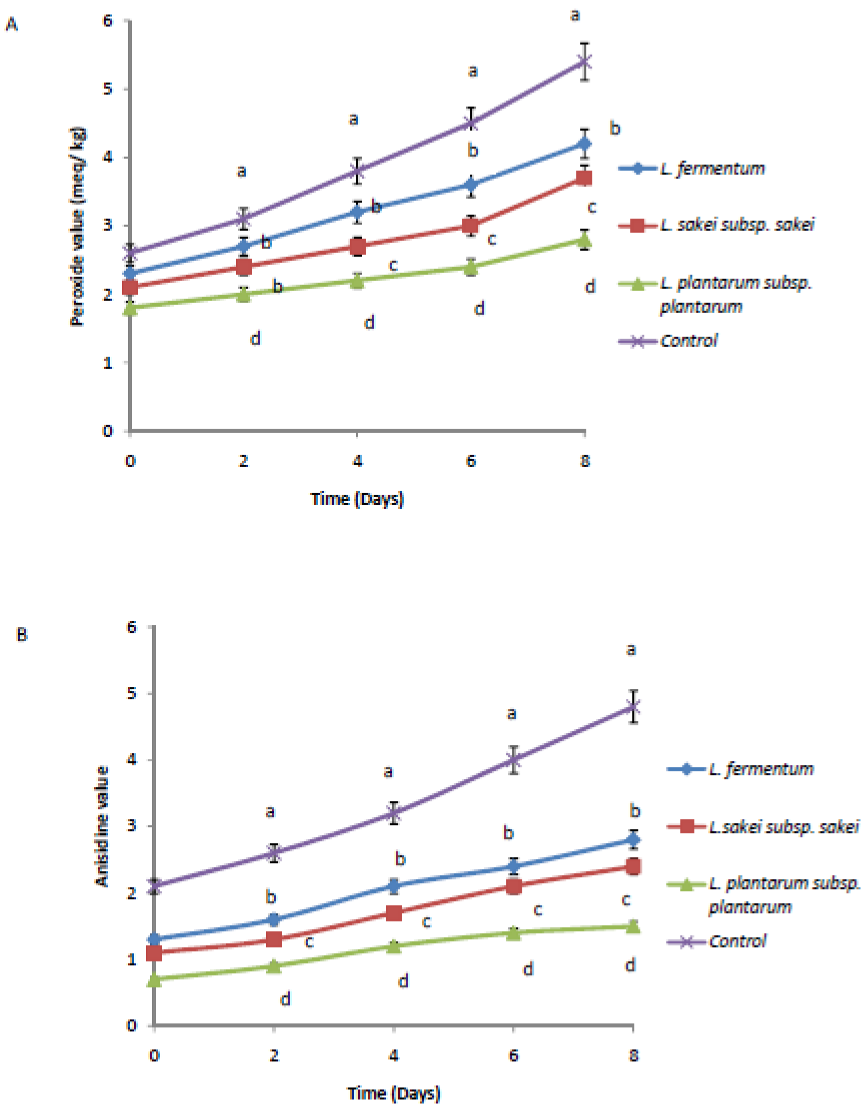


Figure 3. Peroxide value (A) and Anisidine value (B) of meat patty samples during storage at 4°C for 8 days storage. Means in each day with the same superscript lowercase letters are not significantly different at $P<0.05$.

Carbonyl value was measured during 8 days of storage to determine protein oxidation. As depicted in Fig. 4, carbonyl formation was increased by the progress of storage time. The highest value of this parameter was observed in non-fermented (control) sample, and the lowest value was obtained in meat patty fermented with *L. plantarum* subsp. *plantarum* as 0.9 and 2.1nmoL/mg on the first and eighth days, respectively. Indeed, protein oxidation rate was much more decreased in this sample. Protein oxidation may occur naturally during cold storage of foods. Indeed, protein oxidation occurs in side chains of amino acids including thiol and aromatic hydroxyl that results in the formation of carbonyl groups (STADTMAN, 1990). The concentration of carbonyl groups can be considered as an index of oxidative activities (HASHEMI *et al*., 2015). HASHEMI *et al*. (2017) concluded that application various strains of *L. plantarum* in fermentation reduced protein oxidation rate, which accords with the results obtained in the present study.

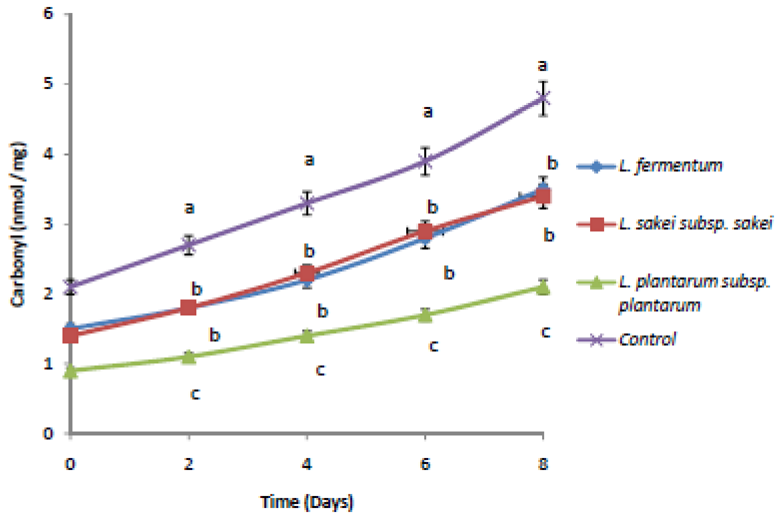


Figure 4. Carbonyl value of meat patty samples during storage at 4°C for 8 days storage. Means in each day with the same superscript lowercase letters are not significantly different at P<0.05.

4. CONCLUSIONS

The results obtained in this research revealed that meat patty fermentation using *Lactobacillus* strains improved its antimicrobial and antioxidative properties. Antiradical activity was enhanced during fermentation, and the fermented product had antimicrobial activity against pathogenic bacteria and fungi. Moreover, during storage of meat patty, lipid and protein oxidation was lower in fermented samples compared to non-fermented ones. Future studies can focus on the antioxidant mechanism of *Lactobacillus* strains.

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PAPER

THE INFLUENCE OF QUINOA (*CHENOPODIUM QUINOA* WILLD.) FLOUR ON THE PHYSICOCHEMICAL, TEXTURAL AND SENSORIAL PROPERTIES OF BEEF MEATBALL

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ABSTRACT

In this study, beef meatballs were produced by using different percentages of quinoa flour as functional ingredient. The effects of quinoa flour levels on physicochemical, textural, sensorial properties of meatballs were examined. Quinoa level had significant effect on fat and moisture contents ($p<0.0001$). The protein content was improved by adding quinoa flour. Cohesiveness, springiness, gumminess, redness(a*), yellowness(b*) values were significantly affected by addition of quinoa flour. According to sensorial analysis, meatball samples had high acceptability and favorable scores. Consequently, quinoa flour has high potential as gluten-free ingredient for use in meatball production in addition to nutritional value and health benefits.

Keywords: beef, meatball, quinoa flour

1. INTRODUCTION

Celiac disease is one of the most common lifelong disorders worldwide with as estimated mean prevalence of 1% of the general population. The only acceptable treatment to date for celiac disease is the strict elimination of gluten from the diet. Gluten-containing wheat proteins and/or starches are often added to many commercial products such as ready meals, convenience food products (meatball etc.), some medicines for technological reasons, to act as fillers, thickeners, binders and stabilizers (ALVAREZ- JUBETE *et al.*, 2010).

The increasing consumer demand for foods that combine extra benefits in addition to common nutrients imposes on the food industry. Therefore, it is needed to advance the new ingredients and formulations, particularly for the production of functional foods. Quinoa (*Chenopodium quinoa* Willd) is a gluten-free pseudo-cereal that contains a high amount of fibre, high biological-value proteins and essential fatty acids (ω-3 and ω-6). It is consumed in the raw or processed as flakes and flour (BRITO *et al.*, 2015).

Quinoa, is a good source of minerals, vitamins and natural antioxidants like vitamin E. The most important characteristic of this pseudocereal is the high amount and quality of its protein. Studies have been carried out to investigate the use of quinoa as a food ingredient to increase the protein level and for taste improvement (SCHUMACHER *et al.*, 2010).

Currently, natural extracts, vegetable and fish oils can be used in order to develop the functional properties of meat products (BILEK and TURHAN, 2009). Non-meat ingredients such as bean flour, corn flour, oat flour have been used to binding and extending in comminuted meat products in previous studies. However, quinoa flour have not been used in meatballs before.

In this study, the effects of quinoa flour on the properties of beef meatballs were presented. The results of chemical composition, pH, cooking yield, Texture Profile Analysis, sensory analysis and Hunter (L*, a*, b*) were obtained.

2. MATERIALS AND METHODS

2.1. Preparation of meatballs

Meatballs were prepared in duplicate according to the following traditional recipe. Quinoa flour was provided from a local market in Manisa. Medium-fat (max. 15% fat) ground beef meat were purchased from a local butcher shop in Manisa 20 kg of ground beef were used in each batch. Ingredients were as follows; 2% salt, 3% ground onion, 2% red pepper, 0.3% black pepper, 3% cumin, 0.8% garlic powder. In the first batch (control) 5% bread crumbs was added; in the second batch 2.5% quinoa flour was added; in the third batch 5% quinoa flour was added, in the fourth batch 7.5% quinoa flour was added to the formulation. Therefore, four different quinoa flour levels (0%, 2.5%, 5% and 7.5%) were used in meatball preparation. All ingredients were mixed at Research Laboratories of Manisa Celal Bayar University-Food Engineering Department. Each batch was kneaded for 15 min by hand to obtain homogenous dough. The doughs were stored in a refrigerator (+4°C) for 12 hours and then shaped in to ball with a diameter of 3 cm and a weight of 20 g. The meatballs were cooked for 20 min in a preheated hot air oven at 180°C. For every treatment two replicates were maintained.

2.2. Cooking yield

Cooking yield was determined by measuring the difference in the sample weight before and after cooking and was calculated according to following equation (ULU, 2006).

$$Cooking\ yield\ (\%) = \frac{Cooked\ meatball\ weight\ (\%)}{Uncooked\ meatball\ weight\ (\%)}\ x\ 100$$

2.3. Proximate analysis

Ash, moisture, protein and fat contents were determined according to AOAC methods (AOAC, 2000). Protein content was measured by the Kjeldahl method (Nx6.25). Fat was determined extracting samples in a Soxhlet apparatus using diethyl ether as a solvent. Moisture content was measured by the weight difference before and after oven drying at 105°C. Ash was determined after incineration in a furnace at 500°C. Carbohydrate content was calculated by computing the difference.

2.4. pH

10 g meatball and 100 mL distilled water were blended for two minutes to obtain pH value by using pH meter (HANNA INSTRUMENTS MODEL HI 221, USA) (AOAC, 1984).

2.5. Color

The color of the surface of meatball samples was measured with a colorimeter (Minolta CR-300) on three different points using D-65 illuminant. The L*, a*, b* values were recorded.

2.6. TPA (Texture profile analysis)

Texture profile analyses (TPA) of cooked beef meatballs were determined by using TA.XT Plus Texture Analyzer (Godalming, England). Six cores (diameter 25 mm) were taken from random cooked beef meatballs per treatment. 50 kg of load cell was applied. P/25 cylindrical probe was used. The pretest speed was considered as 1mm/s with 2 mm/s of test and posttest speed. Double compression was applied with 50% of compression rate. The results of hardness, cohesiveness, springiness, chewiness and gumminess were achieved (BRUNA *et al.* 2000).

2.7. Sensory analysis

The sensory attributes (color, taste, odor, texture, appearance and overall acceptability) of cooked meatball samples were evaluated by 12 well trained panelists from the staff members of Manisa Celal Bayar University using a 9-point Hedonic scale. The analysis was performed in the Food Engineering Research Laboratory under white fluorescent lights. The samples were scored on scale of 1-9. The results between 1-3 are considered as unacceptable; 4-5 are acceptable; 6-7 are good; 8-9 are excellent.

2.8. Trial plan and statistical analysis

The data obtained from two replications were processed by analysis of variance (ANOVA) using Statistical Analysis System (SAS) (SAS Institute, 2001). PROC GLM procedure was done. The level of statistical significance is $p \leq 0.05$.

3. **RESULTS AND DISCUSSION**

Table 1 presents the results of moisture, ash, fat, protein and carbohydrate contents of quinoa flour and bread crumbs. The moisture content for quinoa flour and bread crumbs were found as 13.77 and 7.73, respectively. The results show that quinoa flour is good source of protein and carbohydrate. The similar results of quinoa flour composition were obtained in previous studies by OSHODI *et al*. (1999), ALVAREZ-JUBETE *et al*. (2009), OGUNBELLE (2003).

Table 1. Chemical composition of quinoa flour and bread crumbs (%).

Component	Quinoa flour	Bread crumbs
Moisture (%)	13.77±0.80	7.73±0.15
Ash (%)	2.46±0.06	1.28±0.04
Fat (%)	4.93±0.15	2.69±1.09
Protein (%)	13.60±0.22	10.75±0.71
Carbohydrate (by difference)	65.26±0.67	77.56±0.57

All the values indicate mean ± SD.

Mean percent of moisture, fat, protein, ash, cooking yield and pH values of raw meatballs with quinoa flour are given in Table 2. Meatballs had moisture contents ranging from 44.06% to 52.50%. The maximum content moisture content was determined in meatballs with the addition of 7.5% quinoa. The quinoa level used in meatball production had very significant effect on moisture content of meatball samples ($p<0.05$). The moisture contents increased by the percentage of quinoa flour was increased. In fact, the increase of the moisture content with the increase of quinoa flour percentage is related to the water holding capacity of quinoa flour. OSHODI *et al*. (1999) reported that the water absorption capacity for quinoa is 147%. According to OGUNGBENLE (2003), the water absorption capacity for quinoa seed is 14%. It is higher than soy flour, pumpkin seed and pigeon pea flour. The present findings of moisture contents are agreeing with the findings of BILEK and TURHAN (2009) and TURHAN *et al.* (2005).
Fat contents of meatballs having 0% (control), 2.5%, 5% and 7.5% quinoa flour were found as 12.66%, 12.09%, 9.86% and 9.80%, respectively. The amount of quinoa flour affected the fat content of meatball samples significantly ($p<0.05$). When the quinoa flour was increased, the fat content was decreased. The decrease of fat content with the increase of quinoa flour percentage is related to the composition of the flour. And also, OGUNGBENLE (2003) was determined that the oil absorption capacity of the quinoa flour (46.0%) was lower than wheat flour (84.2%). This is consistent with the findings of YıLMAZ (2005) who reported wheat bran addition at the level of 20% resulted in a significant ($p<0.05$) reduction in the fat content of meatballs. On the contrary, MODI *et al.*

(2009) reported that the fat content of uncooked kofta did not affected by different levels of carrageenan and out flour (p>0.05).

Protein contents of meatballs with 0% (control), 2.5%, 5% and 7.5% quinoa flour were found as 33.38%, 33.81%, 34.36% and 38.49%, respectively. Analysis of variance showed that the differences of protein amounts of the meatballs were non significant (p>0.05). Similarly, SERDAROĞLU and DEĞIRMENCIOĞLU (2004) determined that the protein content of uncooked meatballs did not affected by corn flour addition (p>0.05). Also, AUKKANIT *et al.* (2015) found that protein contents in corn silk added low fat meatballs have not significant difference(p>0.05).

Table 2. Moisture, fat, protein, ash contents and pH, cooking yield (%) and Lab values of beef meatballs formulated with different percentages of quinoa flour.

Parameters	Control (0)	Quinoa flour level (%)		
		2.5	5	7.5
Moisture (%)	44.06 [d]	46.45 [c]	48.93 [b]	52.50 [a]
Fat (%)	12.66 [a]	12.09 [b]	9.86 [c]	9.80 [c]
Protein (%)	33.38 [b]	33.81 [ab]	34.36 [ab]	38.49 [a]
Ash (%)	2.60 [d]	3.23 [c]	3.89 [b]	4.39 [a]
pH	5.57 [a]	5.59 [a]	5.59 [a]	5.59 [a]
Cooking yield (%)	70.49 [a]	68.44 [b]	66.39 [c]	66.32 [c]
L*	44.32 [a]	43.06 [a]	43.87 [a]	45.38 [a]
a*	9.91 [c]	14.00 [a]	10.94 [b]	11.50 [b]
b*	11.03 [a]	10.20 [b]	9.94 [b]	10.44 [ab]

[a-d]Mean in the same row with different letters are significantly different (p<0.05).

The ash contents of meatball samples formulated with quinoa flour were presented in Table 2. The highest ash content was observed in 7.5% quinoa flour added meatballs as 4.39%. The percentage of quinoa affected the ash content of meatball samples significantly (p<0.05). Similar results of ash contents were obtained by several researchers (BILEK and TURHAN, 2009; AUKKANIT *et al.*, 2015; YILMAZ, 2005). Utilization of quinoa flour affected the pH values of the samples non-significantly (p>0.05). The pH values ranging from 5.57 to 5.59. This is similar with the data of BAUGREET *et al.* (2016) who reported that there was no treatment effect or interaction between treatments among pH values. Also, BILEK and TURHAN (2009) found that the pH values of raw and cooked beef patties enhanced with flaxseed flour at different levels (3%, 6%, 9%, 12% and 15%) were not significantly different between treatments.

From the perspective of meatball production process, cooking yield is the most important factor to guess the characteristic of final products during cooking considering non-meat ingredients (AUKKANIT *et al.*, 2015). It was determined that cooking yield values did not affected by the quinoa flour percentage significantly (p<0.05). Cooking yield value decreased with the increase of quinoa flour percentage. The maximum value was obtained in control samples and the minimum value was found in 7.5% quinoa flour added samples. Similarly, AUKKANIT *et al.* (2015) found that cooking yields of low fat meatballs decreased with the addition of corn silk powder (1-4%). Also, SERDAROĞLU *et al.* (2005) observed that cooking yields ranged between 85.2% and 93.2% for meatballs having

blackeye bean flour and lentil flour resulted in the maximum cooking yield values ($p<0.05$).
The color (L*, a*, b*) values were given in Table 2. The redness and yellowness values were significantly ($p<0.05$) affected by quinoa flour percentage. The lightness of meatballs was measured by Hunter-L. Amount of quinoa had no significant effect on lightness (L*) values ($p>0.05$). The maximum L* values were displayed for 7.5% addition of quinoa flour, which means that the addition of quinoa flour resulted in a lighter-colored product. YıLMAZ and DAĞLıOĞLU (2003) found similar results with oat bran added meatballs. a* (redness) values were also different ($p<0.05$) for different amount of quinoa flour. Therefore, a* values were higher in the samples with quinoa flour than in the control. The highest a* value was for the samples with 2.5% quinoa. Also, the lowest redness value was determined in control group samples. Similarly, BILEK and TURHAN (2009) reported that redness values were the lowest in the uncooked control beef patties (20% fat) when compared with different amounts of flaxseed flour added samples ($p<0.05$).
All values for yellowness were higher in control samples than in the samples formulated with quinoa flour. Quinoa addition appears to decrease product yellowness. TURHAN *et al.* (2005) obtained similar results of yellowness values in low-fat beef burgers produced with hazelnut pellicle.

Table 3. Texture Profile Analysis (TPA) of cooked beef meatballs formulated with different levels of quinoa flour.

Parameters	Control (0)	Quinoa flour level (%)		
		2.5	5	7.5
Hardness (N)	46.382 ^{a}	46.460 ^{a}	51.356 ^{a}	56.359 ^{a}
Cohesiveness	0.466 ^{c}	0.494 ^{bc}	0.499 ^{b}	0.638 ^{a}
Springiness	75.143 ^{b}	78.512 ^{a}	72.795 ^{c}	68.016 ^{d}
Chewiness (N)	15.853 ^{a}	20.130 ^{a}	23.632 ^{a}	20.768 ^{a}
Gumminess (N)	21.614 ^{b}	22.951 ^{b}	25.627 ^{b}	35.957 ^{a}

$^{a-d}$Mean in the same row with different letters are significantly different ($p<0.05$).

TPA parameters of meatball samples were given in Table 3. The maximum hardness value was detected in samples of 7.5% quinoa flour and the minimum value was obtained in control samples. The differences of hardness values of the meatballs were nonsignificant ($p>0.05$). Table 3 indicates that increasing quinoa flour level increased hardness. Similar results of hardness values were found by SARıÇOBAN *et al.* (2009) and ULU (2006). Also, AUKKANIT *et al.* (2015) observed that corn silk powder did not affect the hardness values significantly.
Cohesiveness is defined as the degree to which the sample can be deformed before it breaks. Results of statistical analysis demonstrated that the amount of quinoa flour affected the cohesiveness values significantly ($p<0.05$). When the quinoa flour was increased, the cohesiveness values were increased.
Springiness can be defined as the rate at which the deformed beef meatball springs back after the compression (BAUGREET *et al.*, 2016) Quinoa level had a very significant ($p<0.05$) effect on springiness of meatballs. The minimum springiness value was determined in the samples with 7.5% quinoa flour. On the other hand, the maximum springiness value was found in the samples with 2.5% quinoa flour. Similarly, ULU (2006)

determined that guar gum significantly affected the springiness of cooked meatballs produced with 15% and 10% fat levels.
In this study, chewiness values of meatball samples varied between 15.853 N to 23.632 N. Quinoa flour level did not affect the chewiness values significantly (p>0.05). Meatballs with 5% quinoa flour had the maximum chewiness value and the control meatballs had the lowest chewiness value. AL-JUAHIMI *et al.* (2016) reported that the chewiness of uncooked meatballs increased with increment of moringa seed flour.
The amount of quinoa flour displayed a significant (p<0.05) effect on the gumminess values of meatball samples. When the amount of quinoa flour in meatball formulation increased, the gumminess values were increased. All gumminess values were higher in the samples with quinoa flour than in the control groups. The highest gumminess value was found in the samples with 7.5% quinoa flour. Consistent results of gumminess values were determined by AUKKANIT *et al.* (2015). They observed that gumminess increased as the increment of corn silk powder amount.
Duncan's multiple range test results for sensory scores of meatball samples were given in Table 4. All values for color (sensorial) were lower (p<0.05) in the samples with quinoa flour than the control samples.

Table 4. Sensorial Characteristics of cooked beef meatballs formulated with different levels of quinoa flour.

Sensory scores	Control (0)	Quinoa flour level (%)		
		2.5	5	7.5
Color	7.85^{a}	7.13^{b}	7.07^{b}	6.83^{c}
Taste	7.78^{b}	8.28^{a}	8.40^{a}	7.07^{c}
Odor	8.00^{a}	8.34^{a}	8.00^{a}	5.36^{b}
Appearance	8.58^{a}	7.46^{c}	7.83^{b}	6.69^{d}
Texture	7.61^{a}	7.57^{a}	7.93^{a}	6.75^{b}
Overall acceptability	8.00^{a}	8.00^{a}	7.93^{a}	6.75^{b}

$^{a-d}$Mean in the same row with different letters are significantly different (p<0.05).

Quinoa flour level had a significant effect on scores of taste (p<0.05). The highest score of taste was determined in meatball samples with 5% quinoa flour. A significant correlation *(r =+ 0.88, p<0.05)* was found between taste and odor scores.
The quinoa flour amount affected the odor significantly (p<0.05). The lowest odor score was found in meatball samples with 7.5% quinoa flour. On the contrary, SERDAROĞLU *et al.* (2005) reported that no differences in flavor scores were observed among treatments containing legume flours (p>0.05). A significant correlation *(r =+ 0.74, p<0.05)* was found between odor and appearance scores.
The mean values of appearance scores were shown in Table 4. The amount of quinoa flour affected the appearance scores significantly (p<0.05). The highest score was determined in the control samples and the lowest appearance score was found in samples with 7.5% quinoa flour. The present findings are agreeing with the findings of TURHAN *et al.* (2005) who reported that increasing the pellicle level resulted in beef burgers with decreased appearance scores. Also, SERDAROĞLU and DEĞIRMENCIOĞLU (2004) determined that adding corn flour (4%), affected the appearance scores (p<0.05) significantly; meatballs with 4% corn flour had lower appearance scores. In this study, a significant correlation *(r =+ 0.91, p<0.05)* was found between appearance and color scores.

Quinoa flour level had significant effect on the texture scores ($p<0.05$). The meatball samples with 5% quinoa flour had the highest texture score and the lowest score was determined in the samples with 7.5% quinoa flour. Similarly, AUKKANIT *et al.* (2015) concluded that meatballs with 4% corn silk powder had the lowest texture scores compared with control and the meatballs with 1%, 2%, 3% of corn silk powder. Also, a significant correlation *($r =+ 0.90$, $p<0.05$)* was determined between texture and taste scores. On the other hand, a significant correlation *($r =+ 0.81$, $p<0.05$)* was found between texture and odor scores.

The overall acceptability score was shown in Table 4. The quinoa level affected the overall acceptance scores significantly ($p<0.05$). The maximum score was observed in the control and samples with 2.5% quinoa flour (8.00). On the other hand, the lowest score was found in the samples with 7.5% quinoa flour (6.75). The scores of overall ACCEPTABILITY decreased as the quinoa flour was increased. Similar results were found by TURHAN *et al.* (2005). A significant correlation *($r =+ 0.92$, $p<0.05$)* was found between acceptability and odor scores. Also, a significant correlation *($r =+ 0.86$, $p<0.05$)* was obtained between acceptability and taste scores.

In this study, 2.5% and 5% of quinoa flour is considered optimum for use an enhancer to the functional properties in beef meatballs. Similarly, SARıÇOBAN *et al.* (2009) concluded that patties enriched with <7.55% wheat bran were determined as more suitable with respect to sensorial overall quality.

4. CONCLUSIONS

In conclusion, the addition of quinoa flour had significant and variable effects on moisture, fat, ash, cooking yield, cohesiveness, springiness, gumminess values and all sensorial characteristics (color, taste, odor, appearance, texture and overall acceptability) of beef meatballs. The addition of quinoa flour improve the protein content compared to control. Considering sensorial analysis, all meatball samples had high acceptability and favorable scores (5.36 and above). In this study, it is found that when the functional properties of beef meatballs are considered, the samples with 2.5% and 5% of quinoa flour give the best results. It is concluded that, in addition to its nutritional value and health benefits of quinoa flour, it has high potential as gluten-free ingredient to use in meatball production instead of bread crumbs.

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PAPER

BEVERAGES BASED ON RICOTTA CHEESE WHEY AND FRUIT JUICES

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ABSTRACT

For studying antioxidants, sugars and organic acids compositions and their impact on Sourness Index (SI) and Total Sweetness Index (TSI), beverages were produced by blending Ricotta-cheese whey (RCW), by-product of Ricotta cheese production, with fruit juices. Pear-RCW had higher malic acid and sorbitol, blueberry-RCW higher citric acid, total phenolics and anthocyanin pigments, apple-RCW higher sucrose and fructose, and strawberry-RCW higher glucose. Blueberry-RCW had the highest SI and the lowest TSI, while apple-RCW had the lowest SI and the highest TSI. Higher quality beverages may be obtained by using apple juice ('Yellow' type) and the apple:blueberry (50:50) blend ('Red' type).

Keywords: monomeric anthocyanin pigment, percent polymeric color, sourness index, total phenolic compounds, total sweetness index

1. INTRODUCTION

The whey-based fruit juice drinks could be considered novel functional beverages in which the nutraceutical components coming from fruit are combined with those of whey, so strengthening the functional value of the resulting product (HÖZER and KIRMACI, 2010). By changing the type of fruit juice used in the formulation, the functional properties of the beverage can be modulated. The presence of anthocyanins, flavonols, catechins and phenolic acids in blueberries and strawberries has been related to the prevention of oxidative stress by scavenging reactive oxygen species and free radicals (BRAMBILLA *et al*., 2008; ZAFRA-STONE *et al*., 2007; GRANATO *et al*., 2016), and risks reduction of several diseases, such as cardiovascular diseases and cancer (NAVINDRA, 2010). Pear juice, being rich in hydroxycinnamic acids, glycosides of the flavonols quercetin and isorhamnetin, catechins and procyanidin polymeric units with chain lengths of up to 25 units, has a potent antioxidant power, and its singlet oxygen quenching abilities have been linked to its protective activities against both human erythrocyte lyses and protein oxidation (FERREIRA WESSEL *et al*., 2016). Apple juice, containing polyphenols, including flavonoids (quercetin glycosides, procyanidins, epicatechins, chlorogenic acids, and phloretin glycosides) and phenolic acids, and apple fruit may reduce the risk of chronic disease by various mechanisms, including antioxidant, antiproliferative, and cell signaling effects (HYSON, 2011).

Several researches have focused on the development of whey-based fruit blends with various formulations exploiting acid whey and sweet rennet-based whey in order to select the optimal mixture based on sensory perception (DJURIĆ *et al*., 2004; GOUDARZI *et al*., 2015; SAKHALE *et al*., 2012), as the poor sensory profile of whey protein beverages still remains a challenge to consumer acceptance.

RIZZOLO and CORTELLINO (2017) replaced whey with Ricotta-cheese whey (RCW, *scotta*), the main by-product of Ricotta cheese production obtained after flocculation of whey proteins induced by thermal treatment of cheese whey at 85-90°C for about 20 min, and produced 'yellow' and 'red' fruit-RCW-based beverages (juice/RCW ratio: 80/20, 14% soluble solids content), using apple, pear, blueberry and strawberry pectin-free juices. Furthermore, CORTELLINO *et al*. (2015) showed that the type of fruit juice used in the RCW-based beverage impacted on sensory perception, and suggested blending the blueberry juice with the apple ones in order to buffer the high sourness of the blueberry-based drink, which on the other hand was preferred for color, and at the same time to enrich the apple-based drink, which was preferred for taste, with anthocyanin compounds from blueberry.

In this study, we investigated how antioxidants, sugars and organic acids compositions of the RCW-fruit juice beverages could be modulated by changing the type of fruit juice used in the formulation, considering pear-RCW and apple-RCW beverages for the 'yellow' type and blueberry-RCW, strawberry-RCW, apple:blueberry 50:50-RCW; apple:blueberry 75:25-RCW beverages for the 'red' ones.

2. MATERIALS AND METHODS

2.1. Preparation of RCW-fruit juice beverages

The RCW-fruit juice beverages have been prepared from a frozen cow RCW (dairy industry of Lombardy region) and four types of frozen concentrated clear fruit juices without any preservers and colorants addition (G. Mariani & C. S.p.A., Brescia, Italy) according to the process flow diagram described by RIZZOLO and CORTELLINO (2017).

Chemical composition, nitrogen distribution and mineral composition of RCW were reported in Table 1 (MONTI *et al*., 2015).
Before blending, RCW was thawed at 2°C for 24 h and filtered in order to remove the majority of fat, whereas the concentrated clear fruit juices (65 % pectin-free strawberry, 65 % pectin-free blueberry, 70 % pear and 70 % apple) were thawed and diluted to 16.6 % soluble solids content for the preparation of the following beverages: apple-RCW, pear-RCW, strawberry-RCW, blueberry-RCW, apple:blueberry (50:50, v/v)-RCW and apple:blueberry (75:25, v/v)-RCW drinks. Hereafter the two formulations containing a blend of apple and blueberry juices are referred to as RCW-Mix50:50 for apple:blueberry (50:50, v/v)-RCW drink and RCW-Mix75:25 for apple:blueberry (75:25, v/v)-RCW beverage.

Table 1. Chemical composition, nitrogen distribution and mineral composition of RCW. Adapted from MONTI *et al*. 2015.

Chemical composition (g/100 g)				**Mineral composition (mg/kg)**			
pH	6.06	Lactose	2.72	K	1023	P	218.00
Total solids	4.16	Glucose	0.01	Ca	267.00	Mg	158.00
Ash	0.45	Galactose	0.10	Fe	0.48	Cu	0.07
Fat	0.05	Citric acid	0.14	Mn	< 0.01	Se	0.008
Total proteins	0.39	Lactic acid	0.13	Na	881.00	Zn	0.40
Nitrogen distribution							
%	**g/100 g**		**g/100 g**		**%**		
NPN/NT	True protein (NT-NPN)*6.38	Denatured protein (NT-NCN)*6.38	α-la	β-lg	α-la+β-lg	CMP	Peptides
45.9	0.21	0.05	0.02	0.046	37.7	24.0	38.2

NT = total nitrogen; NPN = non-protein-nitrogen; NCN = non-casein-nitrogen; α-la = α-lactalbumin; β-lg = β-lactoglobulin; CMP = casein macropeptide.

Beverages were packed in 125 mL uncolored glass bottles capped with twist-off lid. In order to obtain beverages standardized at about 14 % of soluble solids content, 16.6 % fruit juices and RCW were blended in 80:20 (v/v) ratios separately for every bottle. Six bottles per juice type were divided into two lots and each lot (3 bottles/lot) was separately pasteurized by autoclaving (Ghizzoni, Parma, Italy). Pasteurization temperatures were detected in the autoclave and at the core of the bottle by means of flexible thermocouple probes and monitored with E-Val 2.10 software system; for strawberry, apple and blueberry based beverages, characterized by pH<4.2, the lethal rate F_100^10 value was 11 while for pear beverage, having pH>4.2, an F_100^10=16 value was adopted. After pasteurization bottles were kept for 15 days on open shelves at ambient temperature (20°C); then 50 mL aliquots from every bottle were frozen at −20°C till analysis.
For each beverage, 3 bottles per pasteurization lot (6 replicates/beverage) were analyzed for physical-chemical parameters, (pH, titratable acidity, soluble solids content, color) soon after the 15 days of shelf life at 20°C, and for soluble sugar and organic acid compositions, phenolics, anthocyanins, polymeric color and total antioxidant capacity after overnight thawing at 2°C of the frozen samples. Each bottle was analyzed separately (1 bottle=1 replicate).

2.2. Physical-chemical analyses

The pH values and titratable acidity (TA) were determined with a titroprocessor (model 682, Metrohm AG, Switzerland) by titrating with 0.1 mol/L NaOH to pH=8.2. Color parameters were measured by means of a Spectrophotometer CM-2600 (Minolta, Japan) equipped with a sample holder for 10 mm-plastic cells suited for liquid analysis, using the primary illuminant D65 (CIE, 2006) and 2° observer in the L^*, a^*, b^* color space. Specular component included (SCI) mode was selected and a white calibration tile was used as background. From L^*, a^*, b^* values, chroma (C^*) and hue ($h°$) were computed according to:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h° = \text{arctangent}\ (b^*/a^*) \times 360/\ (2\times 3.14) \quad (2)$$

with $h°$=90° corresponding to the yellow color and $h°$= 360° to the violet one.

2.3. Determination of total phenolic content (TPC) and total anthocyanin pigments (MAP)

Total phenolic content (TPC) was measured spectrophotometrically by the Folin-Ciocalteau assay (SINGLETON and ROSSI, 1965) and was expressed as gallic acid equivalents (mg GAE/100 mL). Total monomeric anthocyanin pigment (MAP) was estimated spectrophotometrically by the pH-differential method (GIUSTI and WROLSTAD, 2001) and was expressed as cyanidin 3-glucoside equivalents (mg C3GE/100 mL). Color density (CD) and percent polymeric color (PPC) were measured according the bisulphite bleaching method described by GIUSTI and WROLSTAD (2001).

2.4. Determination of antioxidant capacity

The antioxidant activity (AntOx) was measured spectrophotometrically by using the stabilized artificial free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by LO SCALZO *et al*. (2004) and was expressed as gallic acid equivalents (mg GAE/100 mL).

2.5. Soluble sugar and organic acid compositions

Soluble sugars were quantified by HPLC (FORNI *et al*., 1992) using a Rezex™ RPM-monosaccharide Pb^{+2} (8%) (300x7.8 mm, Phenomenex) column.
Organic acids were analyzed on a Hypersil Gold (5 μm, 4.6x250 mm, Thermo Scientific) column kept at 30°C with a variable wavelength detector (UV-1575) set at 210 nm, using as mobile phase 0.02 M orthophosphoric acid at the flow rate of 0.7 mL/min.
Data of sugar composition were expressed as g/100 mL of beverage, whereas those of organic acids as mg/100 mL of beverage.

2.6. Sourness Index and Total Sweetness Index

The sour taste of each beverage was estimated by means of Sourness index, taking into account the fact that, within the same pH level, sourness increases with increasing acid concentration (SOWALSKI and NOBLE, 1998).

Hence, Sourness Index was computed according to the following equation:

$$Sourness\ Index = C_{mal} \times a + C_{cit} \times b + C_{fum} \times c + C_{lac} \times d \quad (3)$$

where: C_{mal}, C_{cit}, C_{fum} and C_{lac} are the amounts of malic, citric, fumaric and lactic acids, respectively, expressed in g/100 mL, whereas *a, b, c* and *d* are the estimated sourness units (Table 2) of malic, citric, fumaric and lactic acids, respectively, at the pH value of each beverage.

Table 2. Estimated sourness units of malic acid (*a*), citric acid (*b*), fumaric acid (*c*) and lactic acid (*d*) at the pH value of each beverage used for computation of Sourness Index. The values are taken from the plot estimated sourness in units from ratio scales of MOSKOWITS (1971) vs pH in the 3.0-4.4 range reported by SORTWELL (2005).

	a	*b*	*c*	*d*
Apple	3.2	3.3	1.8	2.5
Pear	2	2	1	1
Strawberry	4.1	3.6	2.5	4
Blueberry	7	5.3	4	8
Mix 50:50	5.3	4.7	3	6
Mix 75:25	5	4.1	2.8	5

Total Sweetness Index (TSI) was estimated by multiplying the amounts of each sugar by its relative sweetness respect to sucrose according to the following equation:

$$TSI = C_{suc} \times 1.00 + C_{glu} \times 0.45 + C_{fru} \times 0.91 + C_{sorb} \times 0.37 + C_{lact} \times 0.22 \quad (4)$$

where: C_{suc}, C_{glu}, C_{fru}, C_{sorb} and C_{lact} are the average amounts of sucrose, glucose, fructose, sorbitol and lactose, respectively, expressed in g/100 mL, multiplied for the relative sweetness values respect to sucrose as determined by MOSKOWITZ (1970) from sweetness power functions related to the weight of solute in solution with the exponent fixed at 1.3.

2.7. Statistical analysis

The Statgraphics v.5.2 (Manugistic Inc., Rockville, MD, USA) software package was used. Data were submitted to one-way analysis of variance (ANOVA) and means were compared by Tukey's test ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1. Physical-chemical analyses

Table 3 shows the physical-chemical parameters of the six types of RCW-fruit juice beverages. As expected, a clear influence of the type of fruit juice used as ingredient in the beverage formulation was found for pH, TA and color parameters. The two 'Yellow' formulations had higher pH and lower TA than the four 'Red' drinks, with the apple-RCW drink having lower pH and higher TA than the pear-RCW ones. Strawberry-RCW beverage had higher pH than all the three formulations based on blueberry juice, and a TA amount intermediate between that of blueberry-RCW drink and the two apple:blueberry-based beverages. Considering the formulations based on blueberry juice, pH increased and TA decreased as the proportion of apple juice added increased (Table 3). Color parameters indicated that 'Yellow' beverages had lighter and more vivid color (higher L^* and C^*) than the four 'Red' beverages. The pear-RCW drink had a yellower (hue value more close to 90°) and lighter (higher L^*) color than apple-RCW drink, while all the 'Red' beverages had a very dark and grayish color ($L^*<25$ and $C^*<2.5$), with blueberry-RCW and RCW-Mix50:50 drinks having lower L^* and C^* than RCW-Mix75:25 beverage. The 'Red' beverages, in addition, differed for hue, which indicated a violet-red color for strawberry-RCW drink ($h \approx +15°$) and a red-violet one for blueberry-RCW beverage ($h \approx -9°$). The blending of blueberry juice with apple juice provoked a shift of hue towards the violet-red color, with the higher change at the higher proportion of apple juice (apple:blueberry 50:50, $h \approx -2°$; apple:blueberry 75:25: $h \approx +5°$). A similar scenario on the relationship between proportion of apple juice added to a fruit juice containing anthocyanins and shift of hue from red violet to violet red has already been reported also for blends of clear apple juice with clear sour cherry juice (NANI *et al*., 1991) and blackcurrant juice (NANI *et al*., 1993).

Table 3. Physical-chemical parameters (TA, titratable acidity; L^* lightness; C^* chroma; $h°$, hue angle) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of *F* ratio.

	pH	TA (meq/100 mL)	L^*	C^*	$h°$
Apple	3.80±0.01 b	6.77±0.03 e	44.4±0.07 b	25.1±0.05 b	76.6±0.1 e
Pear	4.49±0.05 a	3.34±0.05 f	52.2±0.08 a	25.8±0.05 a	85.2±0.0 f
Strawberry	3.61±0 c	15.80±0.14 b	25.2±0.03 c	2.2±0.05 c	15.6±0.4 d
Blueberry	3.19±0.04 e	19.23±0.10 a	24.6±0.05 e	1.4±0.05 d	351.3±1.4 c
Mix 50:50	3.35±0.01 d	13.28±0.04 c	24.5±0.07 e	1.4±0.02 d	357.9±2.3 b
Mix 75:25	3.45±0.02 d	11.02±0.04 d	24.8±0.01 d	2.4±0.18 c	364.7±0.3 a
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

3.2. Sugar and organic acid compositions

Both sugar and organic acid compositions (Tables 4 and 5) were clearly influenced by the ingredients. The sugar and organic acid compositions of the RCW were analyzed according to the methods described in section 2.5 suitable to quantify components from fruit juices. The following data were obtained (mean±standard error, n=3): lactose, 2.08±0.07 g/100 mL; lactic acid, 0.41±0.03 g/100 mL; acetic acid, 0.13±0.002 g/100 mL; citric acid, 0.11±0.005 g/100 mL; fumaric acid, 0.53±0.02 mg/100 mL, whereas glucose and galactose were present in trace levels, below the limit of quantification of the method. In agreement with these data, and taking into account the proportion of RCW used in all the formulations (20 %), in all the beverages it was not possible to quantify galactose and acetic acid from RCW, and, as expected, the amounts of lactose (approx. 416.7±26.4 mg/100 mL) and lactic acid (approx. 66.8±2.9 mg/100 mL) did not differ among formulations (Tables 4 and 5).

Table 4. Amounts of organic acids (mg/100 mL) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

	Malic acid	Lactic acid	Citric acid	Fumaric acid
Apple	483.5±6.8 b	69.0±2.9 a	29.5±0.4 e	0.34±0.01 b
Pear	1,004.0±24.5 a	63.8±2.4 a	28.3±1.7 e	0.41±0.05 b
Strawberry	375.9±9.6 de	66.3±2.2 a	792.6±16.8 b	1.62±0.18 a
Blueberry	351.4±8.3 e	69.9±1.8 a	938.8±9.3 a	1.60±0.36 a
Mix 50:50	425.5±0.8 cd	69.0±3.2 a	424.1±4.5 c	0.41±0.01 b
Mix 75:25	442.3±3.1 bc	63.0±3.4 a	278.4±4.9 d	0.23±0.01 b
P-value	< 0.001	ns	< 0.001	< 0.001

Table 5. Amounts of sugars (mg/100 mL) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

	Sucrose	Glucose	Fructose	Sorbitol	Lactose
Apple	1,219±10 a	3,362±40 c	7,206±76 a	362±8 b	392±15 a
Pear	1,039±12 b	2,941±30 d	5,880±65 d	3,286±40 a	433±12 a
Strawberry	235±11 e	4,484±116 a	6,581±176 bc	247±10 c	402±16 a
Blueberry	4±2 f	4,505±119 a	6,022±168 cd	37±2 d	396±13 a
Mix 50:50	489±16 d	4,184±99 ab	7,016±150 ab	209±4 c	461±15 a
Mix 75:25	717±11 c	3,928±72 b	7,172±127 b	266±8 c	416±13 a
P-value	< 0.001	< 0.001	< 0.001	< 0.001	ns

As for the other organic acids (Table 4) and sugars (Table 5), the amounts and proportions were in agreement with those from the literature for the four fruit species (EISELE and DRAKE, 2005 for apple; SHA *et al.*, 2011 and VIERA ARROYO *et al.*, 2013 for pear; KALLIO *et al*. 2000 for strawberry; RIZZOLO *et al*., 2002 and BETT GARBER *et al*., 2005 for blueberry) and, hence, mirrored the characteristic of the type of fruit juice used in the formulation. Our results on organic acid compositions showed that apple-RCW and pear-RCW beverages had higher malic acid content than strawberry-RCW and blueberry-RCW ones, with pear-RCW showing the highest amount (approx. 1 g/100 mL). Blueberry-RCW beverage had the highest amount, and strawberry-RCW one the second highest amount of citric acid and both these red beverages were characterized by the highest fumaric acid contents (approx. 1.6 mg/100 mL). The blending of blueberry juice with apple juice induced an increase in malic acid and a decrease in citric acid contents, with the major changes observed in the RCW-Mix75:25 drink, but not significant changes in fumaric acid amounts. Considering the sugar composition (Table 5), apple-RCW beverage had the highest amounts of sucrose and fructose, while pear-RCW drink the second highest amount of sucrose, about a tenfold quantity of sorbitol than all the other formulations, and the lowest amounts of glucose and fructose; strawberry-RCW drink was characterized by the highest amount of glucose and about one fifth of sucrose concentration of apple-RCW and pear-RCW formulations. In contrast, blueberry-RCW beverage had very low amounts of sucrose (<10 mg/100 mL) and sorbitol (<40 mg/100 mL) and glucose as much as strawberry-RCW drink. The blending of blueberry juice with apple juice induced an increase of sucrose, fructose and sorbitol and a decrease in glucose, with the major changes observed in the RCW-Mix75:25 drink.

The different organic acid composition coupled to a different pH value and the different sugar composition exerted an influence on sour taste intensity and total sweetness of beverages. In fact it has been reported that, within the same concentration level, sourness intensity increases with decreasing pH, as well as that, within the same pH level, sourness increases with increasing acid concentration (SOWALSKY and NOBLE, 1998); furthermore, DA CONCEICAO NETA *et al*. (2007) found that sour taste intensity was linearly related to the molar concentration of hydrogen ions and to the molar concentration of all organic acid species that have at least one protonated carboxyl group, and, hence, that weaker organic acids were more sour at a given pH than a stronger organic acid because a larger fraction of the carboxyl groups were protonated. On these bases, in order to have a rough estimation of sourness for each RCW-fruit beverage, the Sourness Index, computed according to the Eq. (3) reported in section 2.6, was used (Fig. 1, top). Basing on this index RCW-fruit beverages can be ordered from the most to the less sour as following: blueberry > strawberry = apple:blueberry 50:50 > apple:blueberry 75:25 > pear >apple. As for sugars, in order to have an estimate of the sweetness for each RCW-fruit formulation, the Total Sweetness Index, calculated according to the Eq. (4) reported in section 2.6, was used (Fig. 1, bottom).

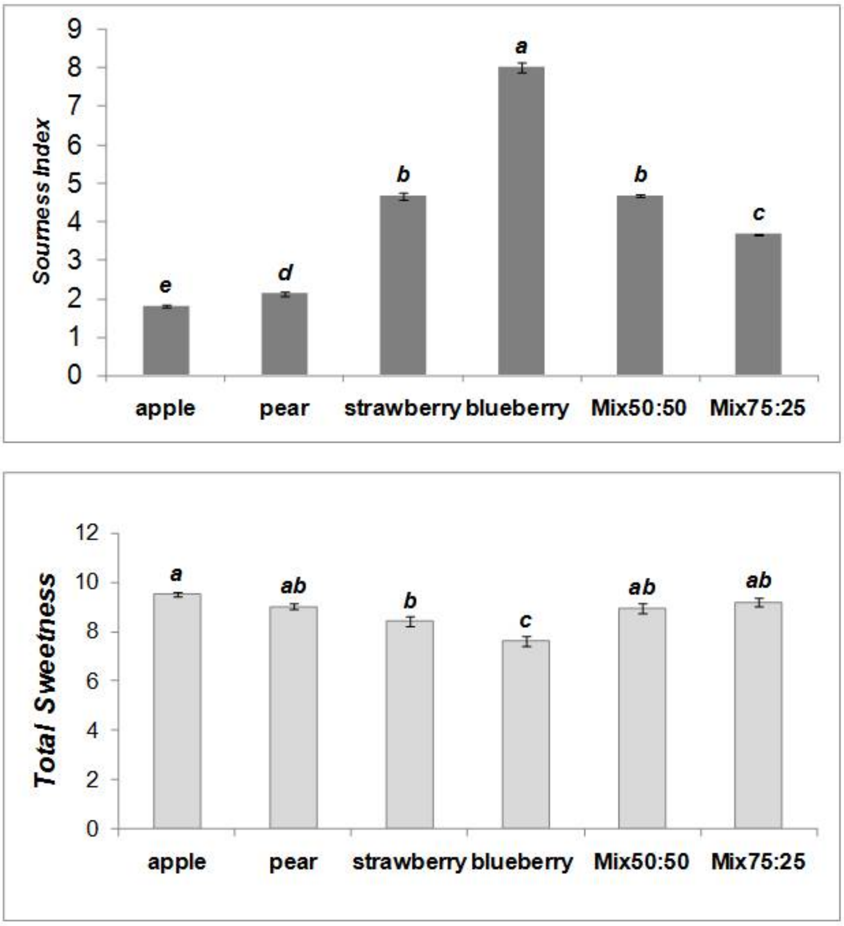

Figure 1. Sourness Index (top) and Total Sweetness Index (bottom) of RCW-fruit juices beverages. Bars refer to standard error (n=6). Sample abbreviations: Mix 50:50, RCW-apple:blueberry (50:50 v/v); Mix 75:25, RCW-apple:blueberry (75:25 v/v). Means having different letters are significantly different (Tukey's test).

Considering the TSI, apple-RCW beverage was characterized by the highest total sweetness, and the blueberry-RCW drink by the lowest; strawberry-RCW had lower TSI than apple-RCW, but higher than blueberry-RCW, while pear-RCW, RCW-Mix50:50 and RCW-Mix75:25 drinks had TSI values not different from apple-RCW and strawberry-RCW ones.
Even if Sourness Index and TSI are an approximation of the more complex scenario which actually occurs, as they do not consider the suppression effect of sugars on sourness intensity (SAVANT and MCDANIEL, 2004), they could have a practical use in evaluating the sensory impact of a product starting from the compositions in the principal organic acids and sugars. In fact, Sourness Index and TSI results are in agreement with the scores of sensory taste pleasantness reported by CORTELLINO *et al*. (2015), which gave to blueberry-RCW beverage the lowest score, being judged too sour and astringent, and to apple-RCW drink the highest.

3.3. Total monomeric anthocyanins and color indices

The mean concentration of total monomeric anthocyanins was highest in blueberry-RCW drink, decreased with blending of blueberry juice with apple juice (approx. −53 % for RCW-Mix50:50; approx. −73 % for RCW-Mix75:25) and was lowest in strawberry-RCW beverage (Table 5). Our results for blueberry-RCW drink are in line with previous reports on anthocyanins content of blueberry juices, which varied according to the cultivar and juice production conditions from 8 to 130 mg/100 g (LEE *et al*., 2002; MÜLLER *et al*. 2012; RIZZOLO *et al*., 2002; ROSSI *et al*., 2003), whereas the MAP content of strawberry-RCW drink was low if compared to the 36.2-38 mg/100 mL range found by ODRIOZOLA-SERRANO *et al*. (2008) for a 7 % clear strawberry juice after heat treatments at 90°C.
The color indices were measured exploiting the ability of bisulphite to form colorless adducts with monomeric anthocyanin compounds but not with the polymeric pigments (GIUSTI and WROLSTAD, 2001) which are formed by polymerization reaction mostly occurring by binding monomeric anthocyanins with other phenolics compounds, such as phenolic acid and condensed tannins (TÜRKYILMAZ and ÖZKAN, 2012). CD (monomeric anthocyanin compounds plus polymeric pigments) of blueberry-RCW drink was twice as much than CD of the other three red formulations, while PC (polymeric pigments) was highest in blueberry-RCW drink and with blending of blueberry juice with apple juice it decreased approx. by 49 % for RCW-Mix50:50 and approx. 61 % for RCW-Mix75:25 beverages. As a consequence, the three formulations based on blueberry juice had PPC values of approx. 50 % independently from the proportion of blueberry juice used in the beverage (Table 6). This value is in line with LEE *et al*. (2002) results on 14-15 % pasteurized blueberry juices, for which PPC ranged from 40.7 to 50.4 %. A different scenario was observed for strawberry-RCW drink; notwithstanding CD was not different from the value observed for the two beverages prepared using blueberry juice blended with apple juice, PPC was 30 % higher than the PPC values found for the three blueberry based formulations. As a matter of fact, RIZZOLO and CORTELLINO (2017) found for the strawberry-RCW beverage already before the pasteurization step an high PPC value (approx. 71 %) coupled to low MAP (approx. 46 mg C3GE/100 mL) and suggested that likely anthocynanins had undergone some degradation already at the factory during the production of the 65 % pectin-free strawberry juice used for the preparation of the drink.
Beverage contained 20% of RCW and consequently very small quantity of total protein (0.08 g/100g), which only partially can be considered as true protein (0.05 g/100g). RCW brought α-lactalbumin, β-lactoglobulin, casein macropeptide and peptides to the drink; these compounds may interact with anthocyanins increasing their stability as reported in the literature.

Anthocyanins are susceptible to chemical degradation and consequently to color fading in the presence of vitamin C. CHUNG *et al*. (2015) demonstrated that the addition of biopolymers significantly enhanced the color stability of purple carrot anthocyanin in model beverage during storage. The best stability was obtained by adding heat denatured whey protein isolate, which through complexation with anthocyanin reduced its degradation due to ascorbic acid. A fluorescence quenching study showed that the anthocyanin formed stronger interactions with the protein through hydrogen bonding than with the ascorbic acid. Moreover CHUNG *et al*. (2017) suggested that also the addition of three amino acid (L-phenylalanine, L-tyrosine and L-tryptophan) and a polypeptide (ε-poly-L-lysine) may prolong the color stability of the same beverage. The most significant improvement was observed for L-tryptophan, which interacted with anthocyanins mainly through hydrogen bonding but also by some hydrophobic interaction.

Table 6. Monomeric anthocyanin pigments (MAP), Color density (CD), polymeric color (PC) and percent polymeric color (PPC) of 'Red' RCW-fruit juice beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

	MAP (mg C3GE/100 mL)	CD	PC	PPC
Strawberry	24.34±4.11 c	5.15±1.20 b	3.76±0.65 b	80.0±6.1 a
Blueberry	119.04±4.28 a	12.01±0.12 a	5.87±0.10 a	48.9±0.6 b
Mix 50:50	56.03±0.98 b	5.86±0.02 b	3.00±0.02 bc	51.2±0.4 b
Mix 75:25	33.22±0.30 c	4.11±0.11 b	2.29±0.05 c	55.7±0.5 b
P-value	< 0.001	< 0.001	< 0.001	< 0.001

3.4. Total phenolic content and antioxidant capacity

Total phenolic content and antioxidant capacity of RCW-fruit based beverages as expected greatly depended on the type of fruit juice used for the preparation of the beverage (Table 7). The mean amount of TPC was highest in blueberry-RCW drink and decreased with blending of blueberry juice with apple juice [−50 % for apple:blueberry (50:50); approx. −52 % for apple:blueberry (75:25)]. Strawberry-RCW beverage had the second highest TPC amounts, which was higher than the values found for both apple:blueberry blends. Strawberry-RCW drink had higher antioxidant capacity than blueberry-RCW beverage, even if the difference between the mean values was very low (approx. 1.8 mg GAE/100 mL). Similarly to what found for TPC, antioxidant capacity of apple:blueberry blends was approx. 50 % (RCW-Mix50:50) and 47 % (RCW-Mix75:25) of that found for blueberry-RCW drink. Apple-RCW and pear-RCW drinks were characterized by the lowest TPC amounts along with the lowest antioxidant capacity. The higher values of antioxidant capacity found for the four 'Red' beverages may be related to the presence of anthocyanins coupled with to higher TPC content compared to the two 'Yellow' drinks, compounds which have demonstrated powerful *in vitro* antioxidant capacity at various tests (TABART *et al*., 2009).
Our results on TPC agree well with those in previous studies on blueberry (CASATI *et al*, 2012; GRANATO *et al*., 2015; RIZZOLO *et al*., 2002), apple (GRANATO *et al*., 2015), pear (SAEEDUDDIN *et al*., 2015) and strawberry (CAO *et al*., 2012; HARTMANN *et al*., 2008) juices, while data of antioxidant capacity cannot be directly compared with those reported in the literature for apple (GRANATO *et al*., 2015; TABART *et al*., 2009), pear (SAEEDUDDIN *et al*., 2015), strawberry (CAO *et al*., 2012; HARTMANN *et al*., 2008;

ODRIOZOLA-SERRANO *et al.*, 2008) and blueberry (GRANATO *et al.*, 2015; CASATI *et al.*, 2012) juices due to either differences in the methodology adopted for the DPPH reaction with the samples or to the use of a different type of test used to estimate the antioxidant capacity of juice samples. The fact that strawberry-RCW beverage showed similar antioxidant capacity to that of blueberry-RCW drink, notwithstanding the lower MAP and TPC amounts, could be due to the higher proportion of anthocyanin polymers (higher value of PPC), whose antioxidant capacity likely compensates for the loss of antioxidant capacity due to monomeric anthocyanin degradation, as well as to the formation of Maillard reaction products in response to thermal treatment which exert antioxidant capacity (YILMAZ and TOLEDO, 2005).

Table 7. Total phenolics content (TPC) and antioxidant capacity (AntOx) of RCW-fruit juices beverages. Data are mean±standard error (n=6). Means within columns having different letters are significantly different (Tukey's test); *P*-value, significance of F ratio.

	TPC (mg GAE/100 mL)	AntOx (mg GAE/100 mL)
Apple	47.3±0.5 d	3.26±0.02 d
Pear	38.5±1.0 d	1.86±0.03 d
Strawberry	216.9±2.5 b	34.03±0.78 a
Blueberry	325.4±3.3 a	32.22±0.42 b
Mix 50:50	162.7±1.8 c	16.20±0.38 c
Mix 75:25	156.0±2.3 c	15.41±0.252 c
P-value	< 0.001	< 0.001

4. CONCLUSIONS

Consumer interest in "healthy" foods and beverages has increased over the last decade, and RCW-fruit juice beverages may be considered novel functional drinks in which the functional status of the product could be modulated by changing the type of fruit juice used in the formulation. Considering the interplay among antioxidant capacity, phytonutrient content and beverage sourness and sweetness indices, it could be concluded that higher quality fruit juice-RCW beverages may be obtained by using apple juice for the 'Yellow' type and the apple:blueberry (50:50) blend for the 'Red' ones. Specifically, apple juice was preferred to the pear one as, being equal for Total Sweetness and Sourness Index, it was richer in total phenolic content and antioxidant capacity. As for the apple:blueberry (50:50) blend, it represented a good compromise having an acceptable Sourness Index along with a remarkable source of phenolic compounds and monomeric anthocyanin pigments.

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PAPER

THE IMPACT OF PARTIAL-FAT SUBSTITUTIONS WITH DOUM (*HYPHAENETHEBAICA*) DREGS ON THE QUALITY CHARACTERISTICS OF BEEF PATTIES

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ABSTRACT

The present investigation aimed to evaluate the impact of partial substitution of kidney fat (10, 20, 30, 40 and 50%) with equal amounts of doum dregs on the quality attributes of beef patties. Doum dregs contain mainly crude fiber (59.6 g/100 g DM) followed by the total carbohydrate content (35.0 g/100 g DM). Water and fat absorption capacities of doum dregs were 2.07 g and 2.51 mL/g of the sample, respectively. Raw and cooked beef patties formulated with different levels of doum dregs had significantly ($p \leq 0.05$) higher moisture, ash, fiber and carbohydrate contents as compared to control samples (Without doum dregs incorporation). Both cooked and uncooked beef patties manufactured with 10% doum dregs had the lowest content of fat 8.02 and 8.08%, respectively. The lowest energy contents were observed for both uncooked (150.60 kcal/100g) and cooked (179.0 kcal/100g) beef patties supplemented with 10% of doum dregs. Reductions in cholesterol content in cooked burgers supplemented with various levels of doum dregs varied from 8.42 to 33.48%. Significant($p \leq 0.05$) improvements in cooking properties were observed for beef patties incorporated with doum dregs. Sensory evaluation results indicate that the highest overall acceptability scores were recorded for those samples formulated with 2, 4 and 6% of doum dregs.

Keywords: doum, fiber, burger, fat, dregs

1. INTRODUCTION

Consumption of meat in developing countries has been constantly growing from a humble average annual per capita consumption of 10 kg in the 1960s to 26 kg in 2000 and it will reach about 37 kg in 2030, according to estimates of Food and Agriculture Organization (FAO, 2007). Beef burgers are among the food that have attractiveness, ready-to-serve foods despite their failure to appear on everybody's plate due to their high fat, trans fatty acids, saturated fatty acids which can lead to obesity, type 2 diabetes and coronary disease (YILMAZ and GEÇGEL, 2007). Studies demonstrated that these diets caused significant elevation in the levels of low-density lipoprotein cholesterol (LDL), which clogs the arteries (ZORAIDA *et al*., 2011). Nowadays there are increased demands for healthier meat products that hold high dietary fiber and low fats. Epidemiologic studies have provided consistent evidence that dietary saturated fat intake has been associated with the risks of cardiovascular disease (MODI *et al*., 2003). Recent researches have been focused on the positive effects of using vegetarian sources to enhance nutritional and quality properties of meat products. (KUMAR and SHARMA, 2004). Meat extenders are non-meat components, which are added into meat products for technical, nutritional and economic reasons (MANSOUR, 2003). Moringa seed flour in beef patties (AL-JUHAIMI *et al*., 2016), wheat flour in buffalo meat burgers (MODI *et al*., 2003), as well as potato flakes in beef patties (ALI *et al*., 2011) have been applied as extenders. Dietary fiber rich meat products are excellent meat substitutes due to their potential nutritional and functional impacts. Dietary fiber intake through meat incorporated with fruits, vegetables and grains is linked with decreases in plasma and LDL cholesterol; decrease the hazard of major dietary problems such as obesity, diabetes, heart diseases and gastrointestinal disorders (SCHNEEMAN, 1999).

The doum palm (*Hyphaenethebaica*) belongs to the family *Palmae* and subfamily *Borassoideae*. Doum fruit, a desert palm native to Egypt, sub- Saharan Africa and West India; is commonly called "African doum palm" or ginger bread palm (DOSUMU *et al*., 2006). Doum fruits contain high levels of protein and minerals. SALIH, 1991 reported that the fruit of doum contains 7.0% ash, 15.0% crude fiber, 0.5% fat, and 3.2% crude protein. Minerals were found to be 0.13%, 0.18%, 0.09% and 3.02% for Ca, Mg, Na and K respectively. Doum powder was used for preparing cake, frozen yoghurt, fermented milk and ice-cream (SELEEM, 2015, ABD EL-RASHID and HASSAN 2005). However, there are, to our knowledge, no sound published data about the use of doum fruit dregs in food products; therefore, the major objective of the current investigation was to evaluate the impact of partial substitution of kidney fat (10, 20, 30, 40 and 50%) with equal amounts of doum dregs on the quality characteristics of beef patties.

2. MATERIALS AND METHODS

Doum palm (*Hyphaenethebaica*) fruits were purchased from the local market in Giza, Egypt, washed with tap water several times, cut into small pieces, and dried in an electric air draught oven (Isotemp Oven, Fisher Scientific) at 50°C for 24 hours. Fresh lean beef meat and kidney fat were obtained from Metro Market, El Haram St., Giza Governorate, Egypt.

2.1. Methods

2.1.1 Preparation of doum dregs

Dried and crushed pieces of doum fruits were ground in an electric grinder (Braun Model 1021), passed through a 150µm mesh sieve. Doum powder was soaked in tap water (1: 20 *w/v*) and kept in a refrigerator at 4°C for 48 hours. The extract was passed through a single layer of muslin cloth to filter out the solid materials. The solid dregs were dried in an electric air draught oven (Isotemp Oven, Fisher Scientific) at 50°C for 24 hours. The dried dregs were packed in clean, dry glass containers and stored at 4°C for further use. Five replicates of doum samples were subjected to chemical analysis

2.1.2 Preparation of beef patties:

The fresh lean beef meat and kidney fat portions were individually ground in meat grinder equipment (Moulinex - ME605131). The ground lean beef (5% lipids), kidney fat (89% lipids), doum fiber and ice flakes have been used for the formulation of beef burgers (Table 1). The control samples contain 65% of lean beef meat and 20% of beef fat. Five levels of fat portions (10, 20, 30, 40 and 50%) were partially replaced by equal amounts of doum dregs. Ground beef meat and the other ingredients were blended together by hand, then ground finally in meat grinder meat with 0.5 cm plate, and formed into beef burgers (100 g weight, 12 mm thickness and 100 mm diameter). Formulated burgers were placed on plastic foam plats, wrapped with 10 microns polyethylene film and kept in freezer at -25°C until further analysis. Five replicates of each beef burger formula were subjected to chemical analysis.

2.1.3 Cooking procedure

Frozen burgers were cooked by using electric grill (Kumtel, Turkey) for 6 min on each side to ensure that the internal temperature of 70±5°C measured at the centre of beef patty using a digital thermometer, model 16454, Pyrex-Accessories Robinson Knife Company, China.

Table 1. Beef patties formulated with various levels of doum fibers

Fat replacement treatment*	Lean beef (g)	Kidney fat (g)	Doum dregs (g)	Ice flakes (g)
Control	65	20	0	10
10%	65	18	2	10
20%	65	16	4	10
30%	65	14	6	10
40%	65	12	8	10
50%	65	10	10	10

*All treatments were formulated with 2 g salt, 1.5 g spices mixture, 1 g sugar, 0.2 g tripolyphosphate, 0.3 g ascorbic acid. Doum fiber was rehydrated with water, doum fiber /water (1:2, w/v).

2.2. Analytical methods

2.2.1 Proximate analysis of doum dregs

The moisture, ash, protein, crude fibre, and fat contents of doum dregs were assessed using the official methods described by AOAC (2005, Methods 930.15, 923.03, 976.05, 962.09 and 920.85, respectively). Total carbohydrate was calculated by difference.

2.2.2 Functional properties of doum dregs

The absorption capacities of water and oil were determined according to the procedures described by SOSULSKI (1962) and SOSULSKI *et al*. (1976), respectively, and the results were expressed as grams of water or milliliters of corn oil bound with one gram of doum dregs flour. Emulsifying and foaming capacities were determined according to the methods of NETO *et al*. (2001) and LAWHON *et al*. (1972), respectively.

2.2.3 Proximate composition and caloric values of beef patties

Moisture (oven drying method) (Methods 930.15), protein (N × 6.25) (Method 920.152), fat (ether extraction with Soxhlet apparatus) (Method 991.36.), ash content (Method 923.03) and crude fibre contents (Method 962.09) were determined using the official methods described by AOAC (2005). Carbohydrate contents were calculated by difference. Total caloric (Kcal/100g sample) were calculated according to MANSOUR and KHALIL (1999), as follow, for fat (9 kcal g^{-1}), protein (4.02 kcal g^{-1}), and carbohydrates (3.87 kcal g^{-1}).

2.2.4 Cholesterol assay

The content of cholesterol was determined according to the previous procedures described by TURHAN *et al*. (2007). Petroleum ether was used to extract the fats from 5 gm of beef burger samples. The petroleum ether was removed by evaporation at 50°C. The extracted fat was weighed and subjected to saponification using aqueous ethanolic KOH solution. The mixture solution was allowed to cool at ambient temperature, and 10 mL of petroleum ether was added. The mixture was shaken vigorously for 1 min. After the layers have separated, the ether layer was transferred into clean test tube, then the ether solvent was evaporated at 50°C. Acetic acid saturated with ferrous sulfate and concentrated sulfuric acid were added to develop the chromophore for colorimetric analysis. The absorbance was then measured at 490 nm against the reagent blank.

2.3. Determination of cooking properties

2.3.1 Cooking yield

Cooking yield percentage was determined by calculating weight differences for samples before and after cooking according to the procedures described by KHALIL (2000).

2.3.2 Fat retention

The fat retention value represents the amount of fat retained in the product after cooking. Fat retention was measured according to the procedures described by KHALIL (2000).

2.3.3 Moisture retention

The moisture retention was measured according to the equation described by KHALIL (2000).

2.4. Sensory evaluation of cooked patties

Sensory evaluation of cooked beef burgers was conducted according to AL-JUHAIMI *et al*. (2016). Each sample of the six formulas was evaluated by ten of trained judges who are belonging to Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. All judges had previous experience in quality attributes. Judges were both male and female in the age range of 25-40 years old. Cooked patties were cut into 2 equal-sized parts and served randomly to each panelist at 50ºC. Three cut of each formulated beef burger samples were served to the panelist. Samples were evaluated in three sessions (each session with two formulas). The panelists were asked to evaluate the appearance, taste, juiciness, flavour and overall acceptability of beef patties using 10-point hedonic scale, where (10 = I like extremely, 1 = I dislike extremely). Cups of drinking water were provided for judges to clean their mouth between samples.

2.5. Statistical analysis

Results were expressed as the average of values±standard deviation (SD). Results were analyzed of variance (ANOVA) ($P \leq 0.05$). Results were analyzed by Excel (Microsoft Office 2007) and SPSS software Version 18.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSIONS

3.1. Proximate composition of doum (*Hyphaenethebaica*) dregs

Table 2 shows the proximate composition of doum (*Hyphaenethebaica)* dregs. Moisture content of dried doum dregs was 6.36%. Moisture content and water activity are key factors affecting the storage, shelf life, and safety of foods (AHMED and ALI, 2015). Low levels of fat and protein (0.39 and 1.69%, respectively) were detected in doum dregs. Doum dregs had adequate amounts of ash 3.32 g/ 100g DM. Doum dregs contain mainly crude fiber (59.6 /100 g DM) followed by the total carbohydrate content (35.0/100 g DM). Doum palm fruit is one of important sources, which supplies human with carbohydrates, fibers, and anti-hypertension compounds (DOSUMU *et al*., 2006). The high level of fiber in doum fruit make it as a potential ingredient for production of bakery products for improving their nutritive value, as well as their contributions to prevent the gastrointestinal problems beside its relevant role as natural anti-cancer agent (COIMBRA and JORGE, 2011).

3.2. Functional properties of doum (*Hyphaenethebaica*) dregs

The investigations of the functional characteristics of raw materials provide an advanced knowledge for their potential use in food products (AHMED and ALI, 2015). Table 2 shows water absorption, oil absorption, emulsifying and foaming capacities of doum (*Hyphaenethebaica*) dregs. Water absorption capacity (WAC) of doum dregs was 2.07 g of H_2O/g of doum dregs. The hydrophilic constituents such as carbohydrate and crude fiber in doum dregs may have contributed to the high water absorption capacity of the dregs.

This finding indicates that the doum dregs can be used as water binding agent in food processing industries. Fat absorption capacity (FAC) of doum dregs was 2.51 mL of oil/g of sample. This finding indicates that doum dregs could be useful in formulation of foods such as sausages and bakery products. Emulsifying activity determines the capacity of substrate to form oil-in-water emulsion. The emulsifying capacity (mL /g) of dried doum dregs was 19.20. Functional characteristics influenced by the ability of dietary fiber to bind with oil and water as well as gel-forming ability. BORDERIAS *et al*. (2005), reported that the addition of dietary fiber agents into fish-based products enhanced the functional properties of these products. Doum dregs have a poor foaming capacity. The foaming capacity (%) of dried doum dregs was 9.10. The low levels of protein in doum dregs accounted for the low foaming capacity of doum dregs. The ability of the flours to form foam depends on the presence of the flexible molecules of protein that may reduce the surface tension of water (SATHE *et al*., 1982).

Table 2. Proximate composition and some functional properties of doum (*Hyphaenethebaica*) dregs (n= 5)[a].

Parameters	Mean±SD	Functional property	Mean±SD
Moisture (%)	6.36±0.95[a]	Water absorption capacity (g of H_2O/g of sample)	2.07±0.11
Fat (%)	0.39±0.02	Fat absorption capacity (mL of oil/g of sample)	2.51±0.16
Protein (%)	1.69±0.15	Emulsifying capacity (mL /g)	19.20±0.96
Ash (%)	3.32±0.31	Foaming capacity (% vol. increase)	9.10±0.87
Crude fiber (%)	59.6±3.08		
Carbohydrates[b] (%)	35.0±2.85		

[a]Values are means±SD of three determinations
[b]By difference.

3.3. Effect of the addition of different levels of hydrated doum dregs on chemical composition of raw and cooked tested beef burgers

The proximate composition of the uncooked and grilled patties as influenced by adding different levels of doum dregs as fat replacer is shown in Table 3. The results indicate that beef patties incorporated with various levels of doum dregs were significantly higher ($P \leq 0.05$) in moisture content than those of control. Moisture content of uncooked beef patties ranged from 60.55 to 67.09%. The lowest ($P \leq 0.05$) content of moisture was recorded for control beef burgers (without doum dregs incorporation). Moisture of uncooked beef patties was gradually and significantly increased with increasing the levels of doum dregs. Beef patty formulated with 10% doum dregs significantly ($P \leq 0.05$) recorded the highest moisture content followed by those formulated with 8% doum dregs. The high content of fiber in doum dregs powder indicates the potentiality of use this powder as a good source of dietary fibre, which plays an important role in increasing water absorption capacity (WAC). The high WAC of fibers encourages their use as a functional ingredient in food formulations, in order to reduce syneresis and dehydration during the storage process, to modify texture and viscosity and to reduce energetic content of food products (BESBES *et al*., 2008). Similar trend was observed for cooked beef patties. In this regard, cooking process caused significant decreases in moisture content of beef patties. During cooking process, the moisture content of beef patties has been lost by evaporation (ALI *et al*., 2011). Moisture content was decreased by cooking process that in turn leads to increase the percentage of protein and dietary fiber in the cooked samples (TURHAN *et al*., 2007). The highest decreases in fat and moisture content were observed for cooked control sample (without doum dregs incorporation), however the lowest reductions of moisture content were observed for those samples incorporated with 10 and 8% of doum dregs. These findings indicate that the incorporation of different levels of doum dregs into beef patties resulted in retention of more moisture during cooking due to a high water-binding capacity of doum fibers. Addition of doum fiber reduces drip and evaporation which resulting in significant increases in the moisture content of cooked patties, these increases in moisture content of beef patties increased significantly ($p \leq 0.05$) as the level of doom dregs inclusion was increased. Incorporation of dietary fiber into meat batters caused significant increases in the viscosity of these products, which was effective in retaining water in the meat products (YUN-SANG *et al*., 2015). Fat content of uncooked beef patties ranged from 8.08 to 19.10%. The highest level of fat was recorded for uncooked control sample (without doum dregs incorporation). Beef patties formulated with various levels of doum dregs had significantly ($P \leq 0.05$) the lower levels of fat than control samples (without doum dregs incorporation). Fat content decreased proportionally with increasing the level of doum dregs used in raw beef patty formulation. Beef burger samples incorporated with 10% and 8% of doum dregs had the lowest ($P \leq 0.05$) content of fat (8.08 and 9.33%, respectively). Similar results were showed by AL-JUHAIMI *et al*. (2016) for low-fat beef patties formulated with different levels of moringa seed flour, and by TURHAN *et al*. (2000) for beef patties incorporated with okara. Cooking process caused marked decreases in fat content, while, the lowest decreases of fat content were observed for beef burgers incorporated with different amounts of doum dregs. These findings are in good agreement with those obtained by MANSOUR and KHALIL (1999) who reported that low-fat patties retained more fat during cooking than higher fat patties. In this regard, KIRCHNER *et al*. (2000) noted that more fat was lost at the 15% fat level than at the 5% fat level in beef burger samples. No significant differences ($p \geq 0.05$) were recorded for protein contents among raw beef burger samples. Cooking process caused significant increases in protein contents of beef burger samples. Loss of moisture during grilling process could leads to concomitant increase in the protein content (BASSEY *et al*, 2014). Crude protein contents for cooked beef burger samples were significantly higher ($p \leq 0.05$) than raw samples (Table 3). However, no statistically significant differences in protein content were observed among the cooked beef burger samples.

Significant increases in fiber and ash content in raw and uncooked beef patties formulated with hydrated doum dregs (Table 3). Fiber content of cooked beef burgers supplemented with 6, 8 and 10% of doum dregs were about 7.25, 8.47 and 8.55 times as high as that in control samples (without doum dregs incorporation).

Statistically the highest ash contents were recorded for cooked samples incorporated with 8 and 10% of doum dregs, however, the lowest value was recorded for control samples (without doum dregs incorporation). This finding could be attributed to the presence of high amounts of fiber and ash in doum dregs. Doum palm fruit (*Hyphaenethebaica*) powder packs a health punch of dietary fiber and minerals (ABD EL-RASHID and HASSAN, 2005). Low amounts of carbohydrates (0.22 -1.0%) were recorded for control beef burger samples and those samples incorporated with different levels of doum dregs (Table 3). With increasing the consumer awareness on food safety systems, and health concerns, there is a rapidly increased demand for the reduction of saturated fats in meat products and its substitution by non-meat substrates, such as dietary fibers, polysaccharides and other non-carbohydrate components. (FUENTES-ZARAGOZA *et al*., 2010). Dietary fibers poss more of advantages such as inhibiting hydrolysis, digestion and absorption in the human small intestine, improving fecal bulk, enhancing colonic fermentation, reducing postprandial blood glucose, and decreasing pre-prandial cholesterol levels (KTARI *et al*., 2014).

Table 3. Chemical compositions of raw and cooked beef patties formulated with different levels of doum dregs (n= 5)[a].

Parameter	Doum fiber level (%)						
	0	2	4	6	8	10	LSD at.05*
Raw beef patties							
Moisture (%)	60.55±0.87[d]	62.09±0.95[cd]	63.48±1.51[bcd]	64.98±1.19[abc]	66.00±2.23[ab]	67.09±1.64[a]	2.62
Fat (%)	19.10±0.66[a]	16.51±0.58[b]	14.10±1.08[c]	11.27±1.06[d]	9.33±0.63[e]	8.08±0.81[e]	1.47
Protein (%)	18.01±0.65[a]	18.12±0.43[a]	18.15±0.86[a]	18.35±0.96[a]	18.61±1.12[a]	18.71±1.01[a]	1.54
Ash (%)	1.86±0.02[d]	1.89±0.04[d]	2.09±0.08[c]	2.35±0.03[b]	2.69±0.04[a]	2.70±0.05[a]	0.08
Crudefiber (%)	0.26±0.02[e]	1.06±0.08[d]	1.68±0.04[c]	2.46±0.10[b]	2.68±0.12[a]	2.71±0.09[a]	0.14
Carbohydrates[b] (%)	0.22±0.01[d]	0.33±0.02[c]	0.50±0.05[b]	0.59±0.08[b]	0.69±0.02[a]	0.71±0.08[a]	0.09
Cooked beef patties							
Moisture (%)	53.80±0.91[c]	54.10±1.58[c]	55.62±1.32[bc]	57.4±2.351[abc]	58.70±2.27[ab]	59.89±1.08[a]	2.98
Fat (%)	17.94±0.91[a]	16.92±0.92[a]	14.23±0.63[b]	11.52±1.23[c]	9.34±0.84[d]	8.02±0.74[d]	1.59
Protein (%)	25.21±0.45[a]	25.31±0.28[a]	25.37±0.38[a]	25.43±0.46[a]	25.54±0.49[a]	25.6±0.61[a]	0.81
Ash (%)	2.21±0.02[b]	2.13±0.05[ab]	2.28±0.08[ab]	2.32±0.08[ab]	2.38±0.11[a]	2.40±0.09[a]	0.15
Crude fiber (%)	0.36±0.07[e]	1.18±0.06[d]	1.83±0.09[c]	2.61±0.14[b]	3.05±0.12[a]	3.08±0.06[a]	0.17
Carbohydrates[b] (%)	0.48±0.02[d]	0.36±0.03[c]	0.67±0.05[b]	0.71±0.05[b]	0.99±0.08[a]	1.00±0.07[a]	0.09

[a]Values are means±SD of three determinations.
Means followed by the same letter are not significantly different (p≤0.05).
[b]By difference.
*Least significant difference at p≤0.05 according to Duncan's multiple-range test.

3.4. Energy content (Kcal) of uncooked and cooked beef burgers incorporated with various levels of doum dregs

Table 4 illustrates the amount of energy of uncooked and cooked beef burgers incorporated with various levels of doum dregs. Generally, Energy content of cooked and uncooked beef patties decreased when fat content decreased or level of doum dregs increased (Table 4). The highest energy content (264.65 and 245.15 kcal/100g, respectively) was recorded for control samples (full fat) in uncooked and cooked patties. Lipids in diet are the source of energy, fat-soluble vitamins and essential fatty acids as well as improve the flavor and texture of food products. On the other hand, fat supplies the body with approximately more than double the calories of protein and carbohydrates (PAPADIMA and BLOUKAS 1999). The lowest energy values were observed for both uncooked (150.60 kcal/100g) and cooked (179.0 kcal/100g) beef patties supplemented with 10% of doum dregs. These findings indicate that incorporation of doum dregs caused significant (p ≤0.05) reductions in energy values. Reduction rates in energy content of uncooked beef burger supplemented with different levels of hydrated doum dregs ranged from 9.15 to 38.56%. While, it varied from 3.49 to 32.36% for cooked patties compared with their control samples. The reduction of caloric energy was associated with the reduction of fat content (MANSOUR, 2003; ALI *et al.*, 2011).

Table 4. Energy content (Kcal) and Cholesterol content (mg/100 g, dry weight basis) of raw and cooked beef patties formulated with different levels of doum dregs (n= 5)[a].

Trait	Doum dregs level (%) 0	2	4	6	8	10	LSD at.05*
Energy content (Kcal)							
Raw beef patties	245.15±046[a]	222.70±0.36[b]	201.79±0.68[c]	177.47±0.81[d]	161.41±0.62[d]	150.60±0.6[f]	1.34
Cooked beef patties	264.65±0.49[a]	255.41±0.43[a]	232.64±0.39[b]	208.64±0.59[c]	190.56±0.49[d]	179.00±0.46[e]	7.59
Cholesterol content (mg/100 g, dry weight basis)							
Raw beef patties	189.76±1.25[a]	170.41±2.05[b]	154.12±1.89[c]	146.91±1.12[d]	145.39±0.93[d]	140.14±2.01[e]	2.85
Cooked beef patties	219.50±3.18[a]	201.00±2.11[b]	183.20±0.97[c]	168.15±0.88[d]	150.02±1.13[e]	146.13±1.07[f]	3.14

[a]Values are means±SD of three determinations.
Means in the same row with different letters are significantly different (p≤0.05).
*Least significant difference at p≤0.05 according to Duncan's multiple-range test.

3.5. Cholesterol content (mg/100 g, dry weight basis) of raw and cooked beef patties formulated with different levels of doum dregs

Table 4 shows the cholesterol content of uncooked and cooked samples. Control samples had significantly higher (P≤0.05) cholesterol content than beef patties formulated with different levels of doum dregs. Cholesterol concentration of uncooked control sample (full fat) was about 1.11, 1.23, 1.29, 1.30 and 1.35 times as high as that in uncooked beef patties formulated with 2,4,6,8 and 10% of doum dregs, respectively. Cholesterol concentrations of beef patties decreased (p ≤ 0.05) significantly with reducing fat levels or increasing the level of doum dregs (Table 4). The lowest cholesterol content (140.14, mg/100 g, dry weight basis) was observed for uncooked beef patties formulated with 10% of doum dregs as fat replacer. MANSOUR and KHALIL (1999) showed that the content of cholesterol of cooked and uncooked samples significantly decreased with addition of wheat fibers. CANDOGAN and KOLSARICI (2003) showed also that reducing fat content in frankfurters from 17.0% to 3.0% resulted in a significant reduction in cholesterol content varied from 50 to 56%. Cooking process caused significant (p ≤ 0.05) increases in cholesterol content of beef patties. Cooked control samples had significantly (p ≤ 0.05) the highest amount of cholesterol (219.50 mg/100 g, dry weight basis). The levels of cholesterol were markedly higher in cooked beef meat samples compared with the fresh beef samples, and these increases may be attributed to the loss of moisture content, which varies depending on the different cooking methods, leading to variation in cholesterol levels (BADIANI *et al.*, 2002; TURHAN *et al.*, 2007). In the respect, the transmission of cholesterol from the adipose tissue to muscle as a result of the cooking process represents a strong explanation for the higher cholesterol content in the cooked samples than fresh. Particularly when the fat found in high amounts in the subcutaneous or intramuscular tissues (BADIANI *et al.*, 2002). Here again, cholesterol content of cooked patties decreased significantly with increasing the level of fat substitutes (doum dregs). MANSOUR and KHALIL (2007) reported that the cholesterol content of cooked beef burger samples markedly decreased with addition of wheat fibers. The concentration of cholesterol of cooked samples which incorporated with various levels of doum dregs were gradually reduced from 201mg/100 g DW in beef burger samples blended with 2% to 146.13 mg/100 g DW in those samples blended with 10% of doum dregs, this means that the decreases in cholesterol content in cooked beef patties formulated with various levels of doum dregs varied from 8.42 to 33.48%. Dietary fibers act as potential therapeutic agents against cardiovascular diseases. They exert this action by acting as lowering agent of hyperlipidemia and hypocholesterolemia (BULLOCK *et al.*, 1995).

3.6. Effect of replacing fat with different levels of doum dregs on some of cooking characteristics of cooked beef patties

The cooking characteristics of beef patties formulated with different levels of doum dregs are shown in Table 5. Cooking yield of formulated beef patties ranged from 68.53 to 75.84%. Beef patties formulated with different levels of doum dregs had significantly higher ($P \leq 0.05$) cooking yield than control samples. The lowest value (68.53%) of cooking yield was observed for control sample (without doum dregs incorporation). This loss in control beef patties might be attributed to the excessive fat separation and water release during cooking process (TURHAN *et al.*, 2007). At the same time, the highest (75.12 and 75.84%) cooking yields were recorded for those samples incorporated with 8 and 10% of doum dregs, respectively. When fat substitution level increased, the yield of cooking increased (Table 5). These increases in cooking yield may be attributed to the ability of doum fibers to hold moisture and fat during cooking process.

Table 5. Effect of replacing fat with different levels of doum dregs on some of cooking characteristics of cooked beef patties (n= 5)[a].

Doum dregs level %	Cooking yield	Moisture retention	Fat retention
Control (0)	68.53±1.33[a]	60.86±1.77[a]	64.36±1.68[b]
2	70.12±1.41[ab]	61.11±1.24[a]	71.86±1.20[a]
4	72.35±1.12[bc]	63.40±1.32[b]	73.01±0.63[a]
6	73.17±0.95[c]	64.66±0.93[bc]	74.79±1.15[a]
8	75.12±1.36[d]	66.80±0.28[c]	75.20±1.30[a]
10	75.84±0.86[d]	67.70±1.08[c]	75.27±1.66[a]
LSD at.05*	2.11	2.12	2.34

[a]Each value in the table is the mean of three replicates and two determinations were conducted for each replicate.
Means in the same column with different letters are significantly different ($p \leq 0.05$).
*Least significant difference at $p \leq 0.05$ according to Duncan's multiple-range test.

The results of moisture retention of beef patties formulated with doum dregs had the same trend of cooking yield. Moisture retention of cooked beef patties varied from 60.86 to 67.70%. Generally, beef patties with doum dregs had relatively higher values of moisture retention compared to control sample (without doum dregs incorporation). The lowest moisture retention (60.86%) was recorded for control sample. The highest moisture retention values (66.80 and 67.70%) were observed for beef patties incorporated with 8 and 10% doum dregs. These findings plainly indicate that the addition of doum dregs into beef burger formulas leads to increase the moisture retention during cooking process, this finding attributed to the capacity of doum dregs to bind more of water (Table 2). Similar findings were recorded by BULLOCK *et al.*, 1995 for low-fat beef patties formulated with modified food starch, by KHALIL, 2000 for beef burger incorporated with mixtures of polydextrose, sugar beet, oat fiber, potato starch, and modified corn starch and by ALI *et al*., 2011 for beef patties formulated with potato flaks.

Fat retention of formulated beef patties ranged from 64.36 to 75.27% (Table 5). The lowest (64.36%) value of fat retention was recorded for control samples (without doum dregs incorporation). Similar finding was observed by TORNBERG *et al*. (1989), who reported that fat was more easily separated from higher fat patties during cooking process. Incorporation of various levels of doum dregs (as fat replacer) into beef patties caused significant ($p \leq 0.05$) increases in fat retention values, these increases were gradually and significantly increased with increasing the level of doum dregs. The highest fat retention values (75.20 and 75.27%, respectively) were recorded for beef patties formulated with 8 and 10% of doum dregs. The addition of dietary fibers causes marked increase in fat retention because of their ability to bind more of moisture and fat in their matrix (WAN *et al.,* 2011). In this regard, TORNBERG *et al*. (1989) reported that the dense meat protein matrix of low-fat ground beef prevented fat migration. Similar results were reported by MANSOUR and KHALIL (1999); TURHAN *et al*. (2007), and WAN ROSLI *et al*. (2011) who used high fiber- substrates such as wheat fibers; hazelnut pellicles and corn silk powder respectively to improve the quality attributes of beef patty formulations.

3.7. Sensory evaluation

Sensory traits for cooked patties are shown in Table 6. Appearance of beef patties formulated with 2, 4 and 6% of doum dregs as fat replacer was substantially higher than those in other formulations and control sample (without doum dregs incorporation). Beef patties with 8 and 10% had significantly ($P < 0.05$) the lowest appearance values (6.20 and 6.10, respectively). Control sample and patties formulated with 2 and 4% doum dregs had significantly the highest value of taste. No significant differences were observed in taste values between control samples and those patties with 2 and 4% doum dregs. Fat plays a good role in foods, it acts as a carrier of flavors and contributes to the strength of food, while the reduction of fat can significantly reduce the overall acceptability, aroma, juiciness and flavor intensity of meat products (KIRCHNER *et al*., 2000).

The taste values decreased gradually with increasing the inclusion percentages of doum dregs to 8 and 10% ($p \leq 0.05$). These decreases may due to the presence of doum flavour. Previous studies have shown significantly lower sensory scores for flavour in beef patties formulated with starch or gums sources (MANSOUR 2003; ALI *et al*., 2011; TURHAN *et al*., 2007).

Table 6. Sensory characteristics of cooked beef patties formulated with different levels of doum dregs (n= 10)[a].

Sensory trait	Doum dregs level (%)						LSD at.05*
	0	2	4	6	8	10	
Appearance	6.30[c]	6.45[b]	7.15[a]	7.20[a]	6.20[d]	6.10[e]	0.07
Taste	7.55[a]	7.66[a]	7.60[a]	7.25[b]	6.50[c]	6.30[d]	0.08
Flavour	6.85[a]	6.90[b]	6.82[c]	6.82[c]	6.80[c]	6.75[d]	0.02
Juiciness	7.87[ab]	7.90[b]	7.93[a]	7.89[ab]	7.88[ab]	7.85[ab]	0.07
Overall acceptability	7.14[d]	7.22[c]	7.37[a]	7.29[b]	6.84[e]	6.75[f]	0.02

[a]Means in a line with different letters are significantly different ($P \leq 0.05$).

*Least significant difference at $p \leq 0.05$ according to Duncan's multiple-range test.

Juiciness values were significantly the higher ($p \leq 0.05$) in beef patties formulated with different levels of doum dregs than control samples (without doum dregs incorporation). Control samples (full fat) had significantly ($p \leq 0.05$) the lowest score (6.87) of juiciness, while, beef patties formulated with 4, 6, 8 and 10% doum dregs had significantly the highest ($p \leq 0.05$) scores were 7.93, 7.92, 7.94 and 7.91, respectively. This finding could be attributed to the ability of doum dregs to hold more of water (Table 3). Beef burgers formulated with white and red beeswings were significantly more tender and juicy than control sample (MANSOUR and KHALIL, 1999).

Beef patties formulated with 2, 4 and 6% of doum dregs were rated higher ($P<0.05$) overall acceptability than control samples (without doum dregs incorporation), while, the lowest scores for acceptability were recorded for those samples formulated with 8 and 10% doum dregs.

4. CONCLUSIONS

The current study shows that addition of different levels of doum dregs (as fat replacer) into beef patties caused marked and significant ($p \leq 0.05$) increases in fiber, ash, cooking yield, moisture and fat retentions of formulated beef patties. The lowest energy contents were observed for both uncooked (150.60 kcal/100g) and cooked (179.0 kcal/100g) beef patties formulated with 10% of doum dregs. The decreases in cholesterol content in cooked beef patties formulated with various levels of doum dregs varied from 8.42 to 33.48%. The sensory evaluation results indicate that the highest scores of overall acceptability were recorded for beef burger samples formulated with 2, 4 and 6% of doum dregs.

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PAPER

SENSORY OPTIMISATION IN NEW FOOD PRODUCT DEVELOPMENT: A CASE STUDY OF POLISH APPLE JUICE

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ABSTRACT

A large variety of juices representing a wide range of sensory profiles can be found on the market. Nevertheless, their manufacturers may not fully recognise the needs and demands of consumers. The aim of the study was to verify a new strategy for consumer product testing using apple juice as an example. The material comprised of 14 apple juices. The sensory analysis was carried out by a team of 130 assessors. The impact of individual physicochemical parameters on the results of the juice sensory assessment was tested with the use of generalised additive models (GAM). The results of the research indicate that the most preferred apple juice is characterised by a balanced sweet and sour taste, low density as well as the colour described by a compromise between the high value of L* and low values of a* and b* parameters and a relatively low price. After the analysis of the map of preferences and its comparison with the results of the sensory assessment, it can be concluded that the products tested do not fully meet the consumer expectations. The research adds new insight into knowledge on new food product development. It shows that by examining the sensory desirability of products available on the market and by employing the GAM analysis the characteristics of the most appreciated product can be determined.

Keywords: new food product development, apple juice, new product, consumer preference

1. INTRODUCTION

Along with the technical and technological progress in the world, consumer needs and expectations are likewise gradually changing. For this reason, from the point of view of food industry companies, it is very important to continuously develop concepts for new products. In this way, they can quickly adapt to new market conditions and meet the needs of their customers. The consumer is more often seen not only as a user of products, but as a decision-maker on the market, and the traditional view of the consumer buying behaviour being associated with the assumptions of economic rationality has been replaced by behavioural theories of choice and preferences (HALAGARDA, 2017; SAGAN 2011).

Rising competition and customer expectations have led juice producers to develop a range of technologies in order to improve production and make their goods more attractive (KUDEŁKA and GŁUSZEK, 2014). As a result, a large variety of juices can be found on the market: raw and not from concentrate, both cloudy and clear, as well as juices from concentrate, or traditional and organic products (KUDEŁKA and GŁUSZEK, 2014). In this respect, products that are commercially available represent a wide range of sensory profiles. Nevertheless, their manufacturers may not fully recognize the needs and demands of consumers (MOSKOWITZ *et al*., 2006).

According to Fuller (2011), the results of the evaluations of the products available on the market should be used as an inspiration when generating ideas for new products that better correspond to the changing needs and expectations of consumers. Cooper (2001) and Lord (2008) state that competitive products are a valuable source of ideas for new products. Based on competitive analysis, the data on the position of the product on the market and characteristics of products and the benefits they offer to customers as well as their weak and strong points can be determined (RUDDER, 2003). This in turn may allow to formulate the necessary criteria to be fulfilled by a newly developed product (COOPER, 2001).

It is, however, important to consider consumer needs at each stage of the product development or improvement process (VAN KLEEF *et al*., 2005; Bogue and Sorenson, 2008). Nevertheless, consumer tests are paramount in their early stages and in the assessment of the product before its commercialisation. Their execution involves significantly lower costs than those that may be incurred in connection with the failure of a new product (MALTZ and KOHLI, 1996). According to Mattsson and Helmersson (2007), the participation of consumers in the initial phase of the new food product development is one of the three factors determining market success.

The unsatisfied needs of consumers create opportunities for the development of new products (LORD, 2008). Fulfilling expectations, especially sensory ones, leads to consumer satisfaction and increases the likelihood of product's marketability (GRUNERT, 2002). The obtained information may also reveal market niches. Products developed to fill these niches have greater chances of success. Moreover, if the consumer reports the demand for a particular food product, the risk of failure significantly decreases (ANDERSEN and MUNKSGAARD, 2009).

The choice of a particular method or a research tool is not a simple task. Despite the fact that the aim is to provide information that can be used to determine the parameters of the product and estimate the level of consumer satisfaction with the new product, the processes of data collection can differ and, accordingly, the respondents can articulate their needs and requirements in different ways. In addition, the purpose behind research is of high importance. The company's goals may involve, for example, an introduction of the product to existing markets or creation of new markets. More reliable research results are achieved when consumers can appeal to their experience with the products already available on the market. The data achieved may provide information about the product benefits that are anticipated by consumers. However, if competitors fully recognize these anticipations, a reliance on such data may result in imitation products being developed (VAN KLEEF *et al*., 2005).

Physicochemical tests and/or descriptive analyses are used to characterise competitive products. Consequently, data on sensory characteristics of the products are compared with their physicochemical parameters, and the degree of their consumer acceptance. (MOSKOWITZ *et al*., 2006; MOSKOWITZ *et al*., 2008).

The aim of the study was to verify a new strategy for consumer product testing using apple juice as an example. To achieve this goal, the following hypothesis was set up: a comparison of the features desired by consumers with the results of physicochemical analyses constitutes valuable input data for the development process of a new product.

2. MATERIALS AND METHODS

2.1. Juice samples

The research material comprised of 14 apple juices, selected from those available on the Polish market, and divided into 3 groups. The first group was formed of five not form concentrate juices that were registered on the List of Traditional Products of the Polish Ministry of Agriculture and Rural Development. The second group comprised of six conventional not from concentrate juices, of which: four were obtained in the pressing process, one via squeezing, whereas the sixth product had no declaration of the manufacturing technology. The last group consisted of three juices made from concentrate and manufactured by leading Polish producers.

For the purpose of sensory analysis and in accordance with the literature guidelines (BARYŁKO-PIKIELNA and MATUSZEWSKA, 2009; ISO 6658:2005), the juice samples were coded using 3 random digits. Detailed data is shown in Table 1.

2.2. Sensory analysis

The desirability analysis of the apple juices was carried out by a team of 130 assessors - all regular consumers of the category. Consumer tests were conducted in a sensory laboratory that was designed in accordance with the ISO 8589:1988 Standard. Evaluations were performed under artificial daylight and at room temperature (controlled between 22 and 24°C) and with the use of recirculation air system. The consumers were given the 50 ml samples of the juices in glasses alongside plain water to prevent carryover of the taste of the former samples. A rest period of at least 30 seconds was scheduled between each sample test. Data were collected through self-administered questionnaires previously explained to consumers. The desirability of colour, consistency, palatability, sweet palatability, sour palatability and the whole product were determined with the use of 9-point hedonic scale and according to the ISO 11136:2014 Standard.

In the first stage the consumers were given the samples for evaluation without knowing the price. Additionally, both the internal (sensory attributes and physicochemical properties) and external (price) factors were taken into account in the assessment of the quality and desirability of the product (MENICHELLI *et al*., 2012). Therefore, in the second stage, the consumers were asked to verify their overall rating when given the price of the product they assessed.

Table 1. The coding and prices of juice samples.

No.	Juice	Price, [PLN/l]	Code
1	NFCJ – fresh, squeezed	10,00	251
2	FCJ	4,24	423
3	FCJ	3,79	513
4	NFCJ – cold pressed	2,65	426
5	FCJ	3,33	754
6	NFCJ – pressed	4,27	896
7	RNFCJ – cold pressed	7,40	712
8	RNFCJ – cold pressed	5,33	381
9	RNFCJ – pressed	6,30	219
10	NFCJ – pressed	5,00	101
11	NFCJ	3,50	411
12	RNFCJ – cold pressed	6,00	129
13	NFCJ – cold pressed	6,10	613
14	RNFCJ – cold pressed	11,77	147

RNFCJ - not-from-concentrate juice, registered on the list of traditional products of the Polish Ministry of Agriculture and Rural Development
NFCJ - not-from-concentrate juice
FCJ- juice from concentrate

2.3. Physicochemical analyses

Four juices from the same production batch that was later used in the consumer assessment were tested. All analyses were performed in triplicate for each juice sample. The analyses covered the following:

2.3.1 Density

The density expressed in grams per 100 mL and converted to kg/L was determined using the Pycnometer Method by measuring the mass of the determined sample volume at 20±0.5°C and equating it to the mass of the same volume of reference liquid (distilled water) 20±0.5°C (PN-EN 1131:1999).

2.3.2 Titratable acidity

Titratable acidity was determined using 25 mL of a sample. The solution was continuously stirred by a magnetic stirrer and titrated against standardized solution of 0.1 mol NaOH to the end point (pH 8.1) using Mettler Toledo MP225 Basic pH/mV/°C meter. The titratable acidity was expressed as mmol H^+/L and calculated according to the following equation:

$$C = \frac{1000 \times V_1 \times c}{V_0}$$

(where, V_0: the volume of the sample in mL; V_1: the volume of NaOH used for sample titration in mL; c: the exact concentration of NaOH in mol/L).
The titratable acidity was also converted to amount of an anhydrous citric acid (gram per 1000 mL) of juice with the use of the following equation:

$$C_{CA} = C \times 0.064$$

(where, C: titratable acidity in mmol H^+/L) (PN-EN 12147:2000).

2.3.3 Total sugars

For the determination of total sugars, 25 mL of the test sample was transferred to a flask of 250 mL. 25 mL of distilled water was added and the sample was deproteinized with Carrez I and II mixture. Then the sample was filtered through a paper filter. 25 mL of filtrate was taken into a 100 mL flask. Subsequently, an inversion was performed with the use of 25 mL of distilled water, 5 mL of concentrated HCl and in the temperature of 68-70°C. The sample was cooled, neutralized and the contents of the flask were filled up to 100 mL with distilled water. The resulting solution was used as a titrant against the mixture of Fehling I and II solutions (5 + 5 mL) with methylene blue as the end-of-reaction indicator. Based on the amount of used solution, the total sugars content in the test sample was determined (PN-90/A-75101/07).

2.3.4 Colour

The colour was mapped by measuring L* a* b* parameters in the CIEL*a*b* system (MCLAREN, 1976) with the use of Minolta CM-3500d spectrophotometer.

2.3.5 Sugars/acids ratio

Sugars/acids ratios were calculated according to the equation:

$$SAR = \frac{S}{A}$$

(where, SAR: sugar acids ratio; S: total sugars; A: titratable acidity expressed as an anhydrous citric acid).

2.4. Statistical analysis

The data obtained from the research were analysed with the use of R 3.2.2. statistical package supplied by R Foundation for Statistical Computing (R CORE TEAM, 2017). The impact of individual physicochemical parameters on the result of the juice sensory assessment was tested with the use of generalized additive models (GAM) (WOOD, 2017). Dimension reduction aimed at the implementation of the juices' preference map was made using the first two principal components. Juices were divided into groups using agglomerative hierarchical clustering (dendrogram) with a complete linkage and Euclidean distance as a measure of the juices similarity. Before the development of the dendrogram, the physicochemical parameters were standardised in order to have a mean of 0 and a standard deviation equal to 1. In this way each of the physicochemical parameters had the same contribution to the Euclidean distance between the two juices. All of the tests were conducted at a significance level of α=0.05.
In order to verify the degree of fulfilment of consumer expectations by traditional and conventional apple juices available on the Polish market, a preference map was developed. Each of the analysed juices was described by 8 parameters (colour parameters: L*, a*, b*,

density, sugar/acid ratio, content of sugars, titratable acidity and price). Due to the difficulties in constructing an 8-dimensional map, it was decided that a two-dimensional one would be developed. 8 parameters were transformed into 2 that best described the tested juices. For this purpose, the Principal component analysis (PCA) was used. Two components that explain most of the variance were selected. Due to the different scales of selected variables, they were all standardised.

3. RESULTS AND DISCUSSION

3.1. The results of sensory and physicochemical tests

Sensory evaluations are used when developing new products and improving the characteristics of the products available on the market. However, due to difficulties arising from the nature of the sensory evaluation they are unfortunately seldom used by small and medium sized food companies (CARBONELL-BARRACHINA, 2007). Instrumental methods certainly provide objective data, but because of sensory analyses more comprehensive knowledge about the characteristics of products perceived by the senses can be provided (MURRAY *et al*., 2001; CARBONELL *et al*., 2008).
The results of the sensory desirability assessment of apple juices are presented in Table 2. They prove that the chosen research material showed variability in respect of consumer preferences of selected apple juice characteristics. The highest ratings for colour were achieved by two juices from concentrate (coded 513 and 754), whereas the lowest ratings by traditional not-from-concentrate juices (coded 381 and 219). Consumers did not have any major objections to the consistency of the juice samples tested. They mostly appreciated the consistency of fresh juice (sample 251), traditional not-from-concentrate juices (sample 712) and juice from concentrate (sample 754). The apple juices samples received mostly high rates for palatability and sour palatability as well. Consumers were highly satisfied with two juices: fresh (coded 251) and from concentrate (coded 423). The lowest rank was given to traditional not-from-concentrate juice (sample 129). Similarly, sweet palatability was highly valued in most of the cases. Again the exception was sample 129. The highest ranks were given to fresh juice (sample 251), juice from concentrate (sample 423) and traditional not-from-concentrate juices (sample 712). Considering overall rating, three juices were most appreciated by consumers: fresh juice (coded 251) and two juices from concentrate (coded 423 and 513). The lowest rating was received by traditional not-from-concentrate juice (coded 129). Considering the price of juice in overall rating two traditional not-from-concentrate juices were barely acceptable by consumers (samples coded 129 and 147). The best ratings were achieved by two juices: fresh (coded 251) and from concentrate (coded 423). The results of overall sensory acceptability ratings are in most of the cases similar to those presented in the literature. Aguiar *et al*. (2012) obtained average ratings for consumer apple juices at the level of 5.5-6.0 points in a 9-point scale. Włodarska *et al*. (2016) received overall acceptance ratings ranging from 4.1.to 6.3 also in a 9-point scale. Lee *et al*. (2016) obtained significantly higher scores of the experienced quality of the coded samples of freshly squeezed apple juices compared to juices pasteurised with the use of different technological methods. Similarly in this study the fresh juice (sample 251) received the highest overall rating, when omitting its price. Therefore, the results of sensory analysis conducted in this study and similar tendencies presented in the literature prove proper selection of the research material.

Table 2. The results of the consumer sensory desirability assessment of 14 apple juices selected for testing.

Juice	Colour	Consistency	Palatability	Sweet palatability	Sour palatability	Overall rating	Overall rating including the price
	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$
251	5.54 (1.69)	6.46 (1.34)	6.92 (1.21)	6.92 (1.00)	6.23 (1.67)	6.77 (0.89)	5.23 (1.62)
423	5.38 (1.33)	5.62 (2.10)	5.92 (1.38)	5.92 (1.49)	6.46 (1.22)	6.08 (1.27)	6.92 (1.21)
513	7.38 (1.39)	5.92 (1.86)	5.54 (1.82)	5.92 (1.64)	5.85 (2.21)	6.00 (1.71)	6.38 (1.15)
426	5.31 (1.32)	5.46 (1.65)	4.85 (2.03)	5.08 (1.98)	4.77 (1.72)	5.15 (1.96)	5.85 (2.11)
754	7.31 (1.77)	6.23 (2.04)	4.46 (2.13)	4.77 (1.89)	5.31 (1.98)	5.31 (2.09)	5.54 (1.78)
896	6.23 (1.58)	5.38 (1.64)	5.38 (1.50)	5.54 (1.50)	5.77 (1.42)	5.85 (1.35)	5.31 (1.26)
712	6.08 (1.49)	6.46 (1.50)	5.23 (2.45)	6.23 (2.42)	5.54 (2.59)	5.77 (2.08)	4.85 (2.41)
381	3.15 (2.18)	5.38 (1.69)	5.38 (1.60)	4.31 (1.59)	4.85 (1.61)	4.69 (1.38)	3.62 (1.60)
219	3.69 (1.73)	5.46 (1.28)	4.00 (2.29)	4.92 (1.86)	4.62 (2.31)	4.08 (1.64)	4.00 (2.04)
101	5.00 (1.47)	6.15 (1.23)	4.77 (1.97)	5.08 (1.21)	4.46 (1.82)	5.15 (1.17)	4.69 (1.38)
411	5.08 (1.90)	6.46 (1.45)	4.00 (2.80)	5.38 (2.17)	4.54 (2.59)	4.62 (2.65)	5.46 (2.76)
129	5.85 (1.46)	4.62 (1.90)	2.31 (1.43)	2.85 (1.51)	3.00 (1.96)	2.62 (1.44)	2.77 (1.76)
613	5.00 (1.75)	5.38 (1.78)	4.15 (1.56)	5.31 (1.49)	4.54 (2.06)	4.54 (1.22)	4.15 (1.10)
147	4.62 (2.10)	5.00 (1.36)	3.92 (1.86)	4.62 (2.24)	4.08 (1.54)	4.08 (1.64)	2.85 (1.61)

S.D. - standard deviation.

Table 3 contains the results of the physicochemical examinations used as a reference for the results of sensory desirability evaluations. They prove the typicality of the products used for the research (AIJN, 2007).

So far, there have only been a handful of studies on the quality of apple juices. RØDBOTTEN *et al*. (2009) and JAROS *et al*. (2009) showed that, as it is in the case of apples (DAILLANT-SPINNLER *et al*., 1996; JAEGER *et al*., 1998), for apple juices sweet and sour palatability are the most important parameters affecting consumer ratings. The sugar content in the product is a measure of the sweet palatability (MAGWAZA and OPARA 2015; BETT-GARBER *et al.,* 2014). The strong correlation between sour palatability and titratable acidity was also proved by various researchers (COROLLARO *et al*., 2014; BONANY *et al*., 2014; VAN DER MERWE *et al*., 2015).

JAROS *et al*. (2009), on the basis of principal component analysis for the instrumental data and Procrustes analysis for sensory data, demonstrated that the key attributes determining the consumer ratings of juices are: sweetness and acidity of the juice, as well as sweet/sour relation, the cloudiness of the juice and its colour.

In this study titratable acidity was chosen as a measure of sour palatability and the total sugar concentration as a measure of sweet palatability. Three juices tested were characterised by the highest total sugars content: two traditional juices (coded 712 and 147) and a pressed not-from-concentrate juice (coded 101). The lowest concentrations of total sugars were detected in two regional not-from-concentrate juices (coded 129 and 219). Two of the juices tested had the highest titratable acidity: juice from concentrate (coded 513) and not from concentrate juice (coded 411). The lowest acidity characterized two not-from-concentrate juices (coded 896 and 426).

The extract to acidity ratio is often used as a measure of the maturity of raw materials and palatability of processed fruits (MAGWAZA and OPARA, 2015). However, due to the importance of acidity and sweetness, in the case of apple juice, confirmed by RØDBOTTEN *et al*. (2009) and JAROS *et al*. (2009), the total sugars to acids expressed as an anhydrous citric acid ratio was chosen as a reference parameter for the overall palatability. The highest value of sugars/acids ratio characterised not-from-concentrate juice (coded 896), whereas the lowest regional not-from-concentrate juice (coded 219), juice from concentrate (coded 513) and not-from-concentrate juice (coded 411).

Table 3. The results of physicochemical examinations, chosen for comparison with the results of sensory analysis, of 14 apple juices selected for testing.

Juice	Colour			Consistency	Palatability	Sweet palatability	Sour palatability
	L*	a*	b*	Density, [kg/Ll]	Sugars/acids ratio	Total sugars, [g/Ll]	Titratable acidity, [mmol H+/Lmval]
	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$	$\bar{\chi}(S.D)$
251	69.49 (0.05)	1.91 (0.04)	26.46 (0.06)	1.043 (0.001)	25.01 (0.15)	95.22 (0.54)	59.5 (0.6)
423	93.98 (0.02)	-1.03 (0.02)	24.64 (0.03)	1.046 (0.001)	23.99 (0.12)	101.31 (0.49)	66.0 (0.0)
513	88.09 (0.05)	2.56 (0.02)	40.84 (0.01)	1.046 (0.001)	18.81 (0.16)	100.495 (0.22)	83.5 (0.4)
426	41.68 (0.30)	12.71 (0.15)	49.37 (0.12)	1.049 (0.001)	42.81 (0.73)	109.596 (0.73)	40.0 (0.4)
754	93.56(0.03)	-0.89 (0.01)	26.74 (0.05)	1.048 (0.001)	22.49 (0.18)	105.091 (0.83)	73.0 (0.6)
896	50.92 (0.22)	11.65 (0.23)	52.42 (0.15)	1.048 (0.001)	57.37 (0.72)	104.65 (0.65)	28.5 (0.8)
712	91.19 (0.13)	-0.59 (0.05)	23.64 (0.09)	1.052 (0.000)	30.09 (0.58)	117.495 (0.50)	61.0 (0.4)
381	58.74 (0.04)	7.20 (0.01)	34.02 (0.04)	1.050 (0.001)	25.49 (0.14)	103.61 (0.24)	63.5 (0.4)
219	55.34 (0.10)	5.64 (0.10)	26.10 (0.38)	1.045 (0.001)	16.76 (0.03)	81.51 (0.15)	76.0 (0.4)
101	58.73 (0.13)	7.79 (0.03)	36.62 (0.06)	1.048 (0.001)	29.01 (0.39)	115.10 (0.33)	62.0 (0.0)
411	58.59 (0.04)	3.96 (0.02)	37.14 (0.01)	1.047 (0.000)	19.08 (0.01)	98.32 (0.57)	80.5 (0.6)
129	87.13 (0.17)	-0.50 (0.05)	29.26 (0.16)	1.105 (0.001)	23.51 (0.27)	77.50 (0.23)	51.5 (0.8)
613	54.91 (0.11)	7.40 (0.10)	32.31 (0.33)	1.049 (0.001)	33.62 (0.08)	101.13 (0.23)	47.0 (0.4)
147	57.34 (0.18)	10.88 (0.05)	44.34 (0.03)	1.112 (0.001)	33.39 (0.21)	118.61 (0.56)	55.5 (0.4)

S.D. - standard deviation.

The density was selected as a measure of juice consistency. The tested apple juice samples showed similar density. The exceptions were two regional not-from-concentrate juices (coded 147 and 129), which had the highest densities.
The L* a* b* parameters mapped the colour of the apple juices tested. The highest value of L* parameter and therefore the highest lightness characterised two juices from concentrate (coded 423 and 754) and one traditional not-from-concentrate juice (coded 712). Sample 426 (not-from-concentrate juice) had the lowest value of this parameter among all of the tested products. This sample on the other hand, had the highest values of the a* parameter (the highest share of green shade). The lowest values of a* parameter characterised juices from concentrate (coded 423 and 754). Samples coded 896 and 426 (not-from-concentrate juices) had the highest values of b* parameter indicating yellow colour saturation.
Due to the fact that the composition of juice aroma is influenced by many chemical substances (mainly esters, C6 alcohols and aldehydes) (STANGL and ZIEGLER, 2014) research concerning this issue was omitted. Nevertheless, an independent project on the subject is planned.

3.2. Comparison of apple juices: similarities

To check the similarity of the analysed samples of juices, a hierarchical cluster analysis was performed. The physicochemical parameters, which constituted a reference point for the results of the desirability evaluations, and the prices of the analysed juices were considered. The results were compared with the results of the sensory assessments (Fig. 1).

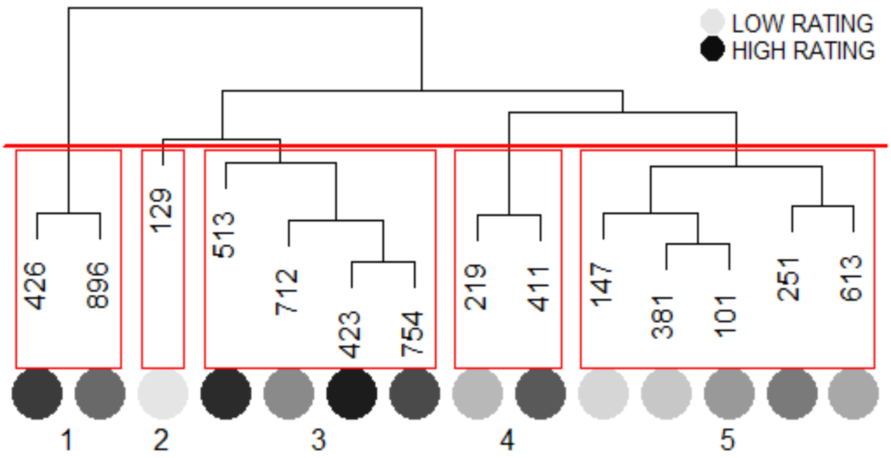

Figure 1. Clusters separated for the analysed samples of apple juice.

On the basis of the dendrogram, five product clusters were distinguished. The juices of clusters: 1 and 3 were characterised by high consumer desirability. Sample 129 shows a similarity to cluster 3 in terms of its physicochemical properties. Despite the preferred characteristics of the product, it received a low score in consumer test. This was a result of the poor palatability. The consumers' comments prove that the product was characterised by a peculiar insipid taste of negative associations, despite a rather good balance between the acid and sweet taste. However, volatile compounds were not analysed in the present study, and therefore, it was impossible to refer consumer opinions to specific physicochemical parameters. The last two clusters (4 and 5) comprised of products of medium to low consumer desirability.

3.3. An attempt to determine the parameters of the most desirable apple juice based on the parameters of juices available on the Polish market

The colour of the product significantly affects its perception. According to Spence (2015), there is a strong relationship between colour and perception of the type and intensity of flavour.

The chart (Fig. 2) presenting the relationship between the value of the colour parameter - L* and the consumer desirability is U-shaped. This means that the most preferred are the extreme values. The rating of the juices increases slightly faster on the right side of the graph. The ideal value of the L* parameter for apple juice determined by the GAM method and based on the analysis of the research material was 93.89. Therefore, it was found that consumers mostly prefer light coloured apple juices. However, the left part of the chart indicates that there is a group of consumers who are in favour of a juice of lower lightness. This was also confirmed by the research of WŁODARSKA *et al*. (2016), in which a segment of consumers who prefer juices with low values o L* colour coordinate was identified.

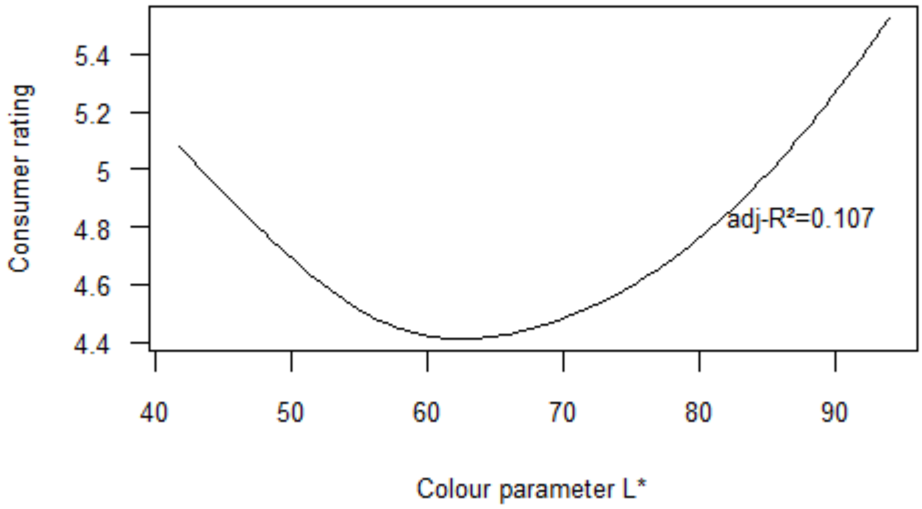

Figure 2. The relationship between the colour parameter L* and consumer desirability of the apple juice colour determined with the use of GAM model.

The relationship between the colour parameter - a* and the consumer desirability is linear (Fig. 3). The lower the value of a* parameter the higher the desirability of the product. Therefore it was found that consumers prefer apple juices of less perceptible greenish shade. The best value of the a* parameter for apple juice determined by the GAM method and based on the analysis of the research material was -1.03.

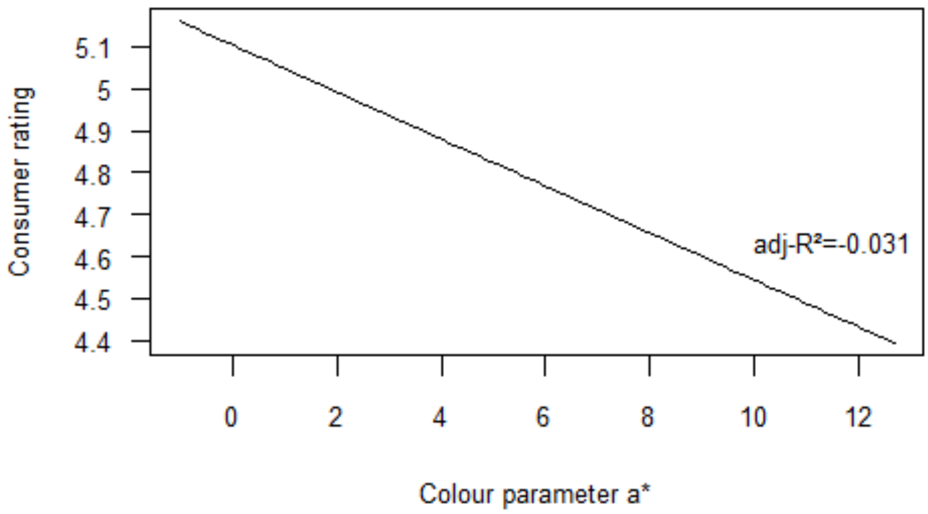

Figure 3. The relationship between the colour parameter a* and consumer desirability of the apple juice colour determined with the use of GAM model

The relationship between the value of the colour parameter b* and consumer desirability of the analysed apple juices is exponential (Fig. 4). The higher the value, the greater the consumer acceptance of the juice. The respondents preferred a more saturated colour, with a more pronounced share of yellow tone. The application of the GAM method made it possible to determine the most desirable value of the parameter b* within the range restricted by the parameters of the research material. It amounted to 52.42. The research results by CZARNOWSKA *et al*. (2014) also confirm that consumers prefer an intensive dark yellow colour of apple juice.

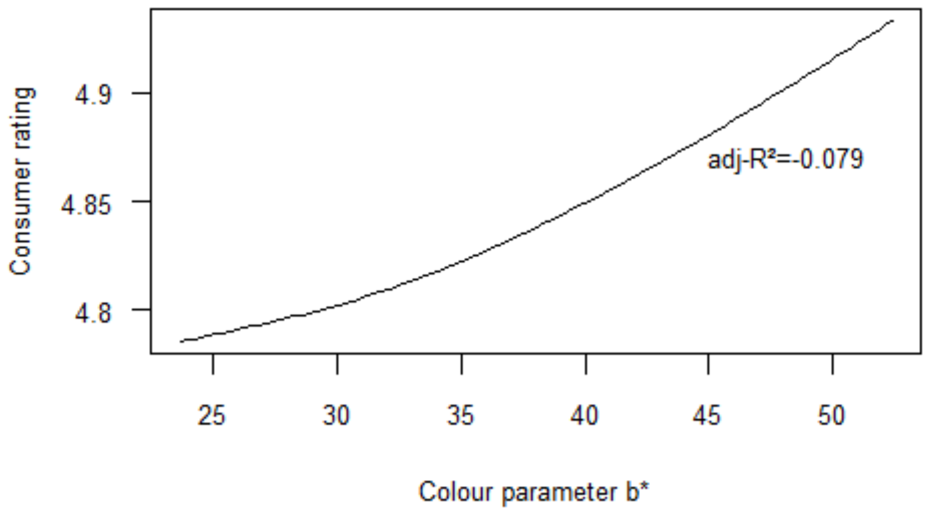

Figure 4. The relationship between the colour parameter b* and consumer desirability of the apple juice colour determined with the use of GAM model

According to the linear chart (Fig. 5), showing the relationship between the density of apple juice and its consumer desirability, the lower the density of apple juice, the more the beverage is favoured by consumers. Application of the GAM method made it possible to determine the most appropriate density - 1.043 kg/L. It was the lowest density among the identified values. Therefore, it can be stated that consumers prefer clear juices. This conclusion is consistent with the data presented in the 2014 report of the European Fruit Juice Association. According to it, 365 out of the 673 million litres of juices drunk by Poles in 2013 were made on the basis of concentrate (ANONYMOUS, 2014). The not-from-concentrate juices are not clarified by Polish producers so as not to lower their nutritional value. The research by CZARNOWSKA *et al*. (2014) also confirms preferences for clear juices. On the other hand, WŁODARSKA *et al*. (2016) recognized consumer segment which showed preference for juices of higher density identified with cloudy juices which are believed to have greater health benefits.

The relationship between the sugar/acid ratio and consumer desirability is linear (Fig. 6). The higher the ratio, limited by the characteristics of the analysed samples, the better the rating the juice received. The most preferred value of sugar/acid ratio was 57.37. JAROS *et al*. (2009) noticed that, when considering cloudy juices, the most preferred products had low sugar/acid ratio. They, however, indicated that there is an optimum of this ratio. Therefore, in the range limited by samples analysed in this study the preferences may be rising with the increasing sugar/acid ratio.

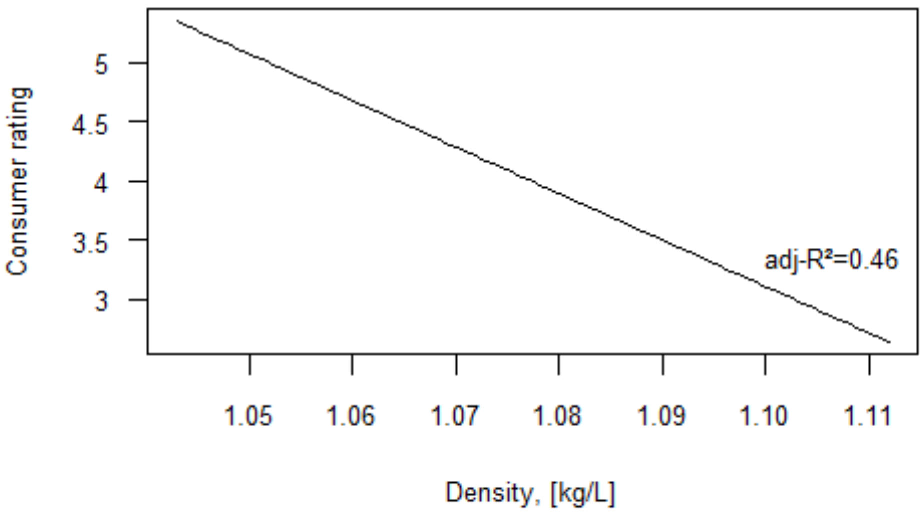

Figure 5. The relationship between density and consumer desirability of the apple juice consistency determined with the use of GAM model

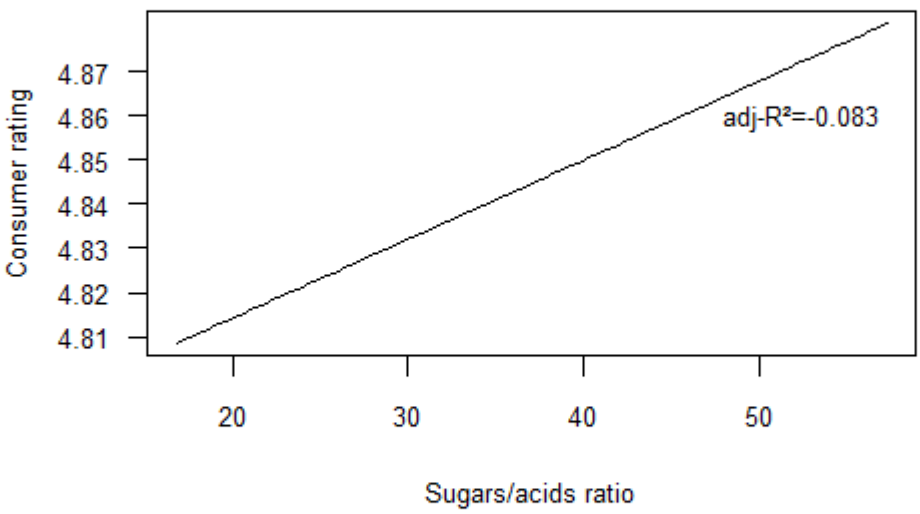

Figure 6. The relationship between sugars/acids ratio and consumer desirability of the apple juice palatability determined with the use of GAM model

According to research by JAROS *et al*. (2009), the majority of the surveyed consumers preferred the juice, which was more acidic and less sweet. However, their research also demonstrated the existence of a large group of consumers who prefer less acidic, sweeter juices. Similarly, RØDBOTTEN *et al*. (2009) showed variability of apple juice acidity preference between analysed consumer segments. This is in accordance with the results of this study, showing that the relationship (Fig. 7) between the titratable acidity and the consumer desirability of apple juice is complex. The ideal acidity for apple juice, determined in this research with the use of the GAM model based on the analysis of the research material, was 83.5 mmol H^+/L.

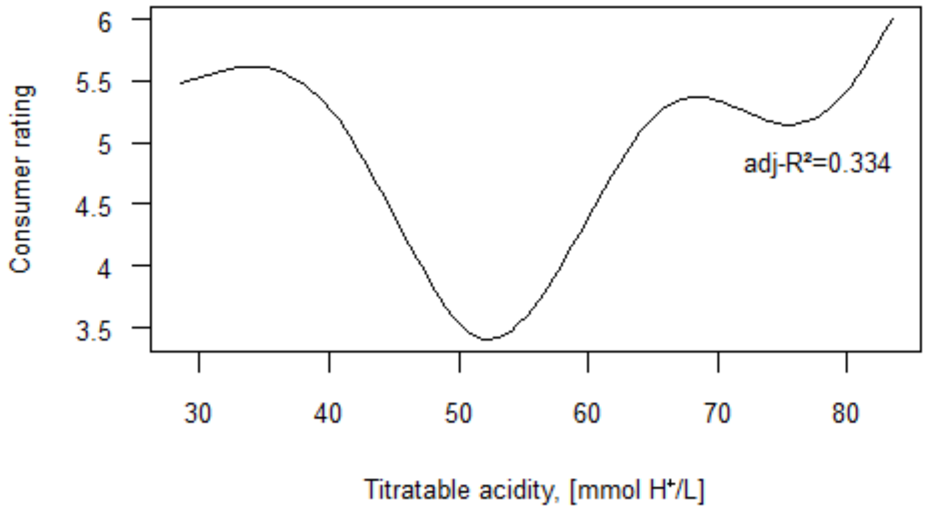

Figure 7. The relationship between titratable acidity and consumer desirability of sour palatability of apple juice determined with the use of GAM model

The chart (Fig. 8) presenting the relationship between the sugar content and the consumer desirability is in the form of an inverted U. The respondents, to a certain degree, prefer the sweet taste of juice. In the present study the limit was the sugar content of 100.83 g/l of juice. This is in accordance with JAROS *et al*. (2009) findings. In their research a correlation between consumer ratings and sweet palatability was found. They noticed that there is a sweetness intensity level limiting acceptance of an apple juice. Similarly, RØDBOTTEN *et al*. (2009), showed consumer preference for apple juices of a high sugar content, noticing that there might be an upper limit of sweetness.

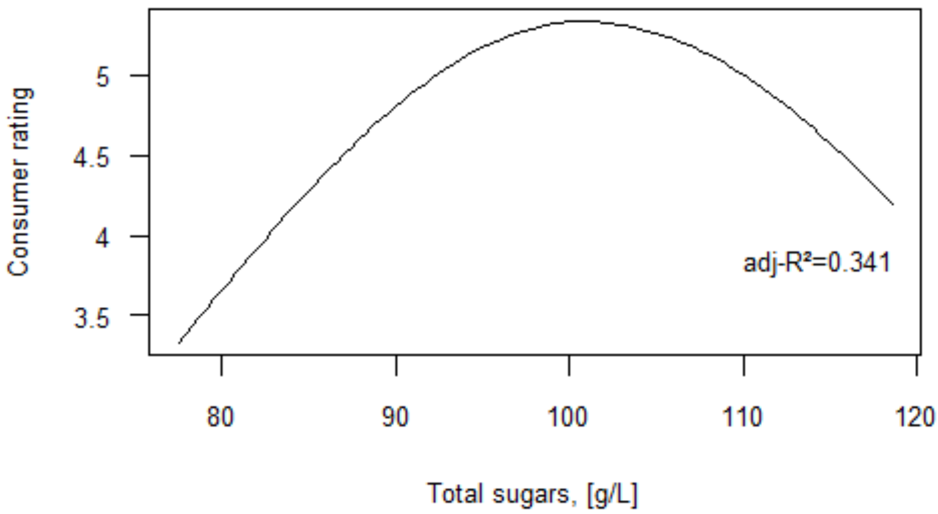

Figure 8. The relationship between sugar content and consumer desirability of sweet palatability of apple juice determined with the use of GAM model.

The relationship between the price of juice and its consumer desirability is non-linear (Fig. 9). Some respondents, most likely, identify the high quality of juice with its high price. However, at a given quality level, below which none of the tested products were found, products with lower prices are generally preferred. These findings are in accordance with the results of SHIRAI (2015) research. On the basis of the GAM analysis, it was determined that most respondents prefer the lowest price of the presented alternatives - 2.65 PLN/l juice.

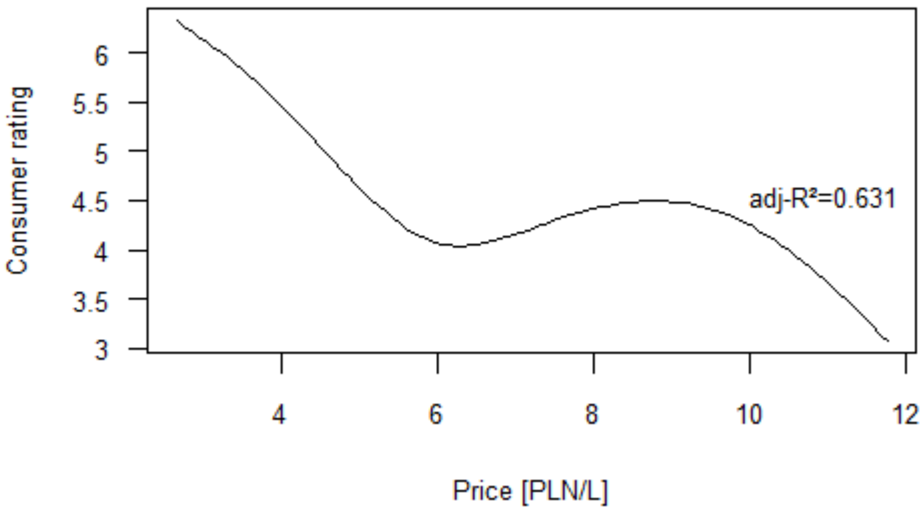

Figure 9. The relationship between the price and consumer desirability of apple juice determined with the use of GAM model.

Based on the results of the GAM analysis on 14 samples of apple juices available on the Polish market it can be concluded that the products most preferred by the respondents should have the following colour parameters: L*=93.98, a*=-1.03, b*=52.42, density of 1.043 kg/L, sugar content equal to 100,827 g/L, titratable acidity of 83.5 mmol H^+/L, sugar/acid ratio equal to 57.37 and the price of 2.65 PLN/L or less.

3.4. The preference map of apple juices available on the Polish market, with consideration of the ideal juice concept

Two principal components determined by PCA explain 66.25% of the variance of the whole data set, thus the obtained 2-dimensional map uses 66.25% of the information contained in the 8 original parameters. The exact patterns of the components were as follows:

- first component:

0.4·L* - 0.48·a* - 0.44·b* - 0.06·density - 0.45·sugar/acid ratio - 0.22· sugar content + 0.4·titratable acidity - 0.04·price

- second component:

0.04·L* - 0.01·a* - 0.12·b* + 0.69·density - 0.08·sugars/acids ratio - 0.03·sugar content - 0.1·titratable acidity + 0.1·price

Based on the determined pattern it can be stated that:

- the high values of the first component result mainly from the high titratable acidity and high values of the L* parameter and low of a* and b* parameters as well as low values of sugars/acids ratio;
- the high values of the second component are primarily related to high density and high price.

The values of the two components, calculated for each tested apple juice and the juice of the parameters most desired by the respondents are shown on the preference map (Fig. 10).

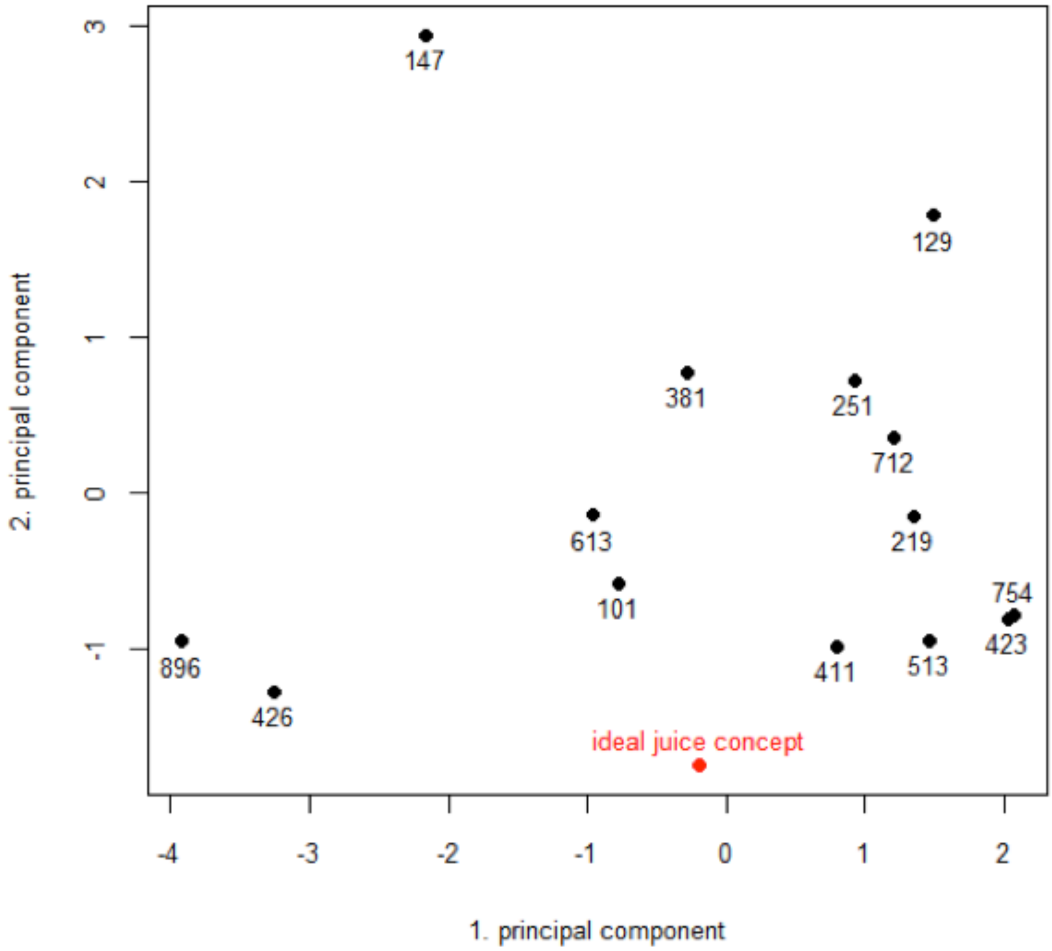

Figure 10. A preference map for the analysed 14 apple juice samples, showing location of ideal juice concept among tested products.

It was determined that the juice with the parameters most preferred by the consumers has:
- a first component being close to 0. The equation determining the first component shows a positive contribution of colour parameter L* and titratable acidity as well as negative contribution of a* and b* colour parameters and sugar/acid ratio. Therefore, the optimal juice parameters are a compromise between parameters of positive and negative contribution.
- low second component, and thus low density and low price.

The significance of the taste of the juice and its price is also confirmed by the results of the survey conducted by UCHEREK (2011). According KRAUS and POPEK (2013), the taste plays a pivotal role in the perception of food products by consumers.

When comparing the preference map with the results of the consumer desirability evaluation it can be concluded that the most appreciated apple juices have a low value of the second main component. Nevertheless, none of the analysed juices has a value of the first principal component at a level close to 0. This leads to the conclusion that the examined products do not fully meet the expectations of consumers. However, the apple juices coded 411, 513 and 101 have the values of the parameters closest to the parameters of ideal juice concept among tested samples. Juices 411 and 513 are characterized by low sugars/acids ratio, average total sugars content and high titratable acidity as well as moderate values of L* colour parameter. Sample 101 has moderate sugars/acids ratio and titratable acidity as well as high total sugars content and high value of L* colour parameter.

4. CONCLUSIONS

The results of the research confirmed the diversity of sensory characteristics of apple juices available on the market in Poland, as well as the typicality of physicochemical parameters of the analysed products. Agglomerative hierarchical clustering allowed to distinguish five groups of products, of which two where highly preferred by consumers. Thanks to GAM analysis dependences between the analysed physicochemical parameters of juices and their consumer ratings were identified. It was shown that: the relationship between the value of the colour parameter - L* and the consumer desirability is U-shaped, the relationships between the colour parameter - a* and the consumer desirability as well as density and the consumer desirability are inversely proportional and linear, the relationship between the value of the colour parameter - b* and consumer desirability is exponential, the relationship between the sugar/acid ratio and consumer desirability is proportional and linear, the relationships between the titratable acidity and the consumer desirability as well as between the price of juice and its consumer desirability are complex, whereas the relationship between the sugar content and the consumer desirability is in the form of an inverted U. On the basis of GAM analysis it was also shown that in the range of variability conditioned by the physicochemical parameters of the research material the ideal juice concept should be characterized by the colour identified by the following coordinates: L*=93.98, a*=-1.03, b*=52.42, density of 1.043 kg/L, sugar content equal to 100,827 g/L, titratable acidity of 83.5 mmol H^+/L, sugar/acid ratio equal to 57.37 and the price of 2.65 PLN/L or less. The results of PCA analysis allowed to indicate that the most preferred apple juice is characterized by a balanced sweet and sour taste, low density and a relatively low price as well as the colour described by the compromise between high value of the L* parameter and low values of the a* and b* parameters. After the analysis of the preference map and its comparison with the results of the sensory desirability assessment, it may be also concluded that the products tested do not fully meet consumer expectations. Therefore, a newly developed product that would meet consumer requirements stands a good chance of being successful on the market.

In this way it was shown that the comparison of the product features desired by consumers with its physicochemical parameters constitutes valuable input data that can be used during the product development process. Therefore, the set hypothesis was positively verified.

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PAPER

NUTRACEUTICAL VALUE OF EDIBLE FLOWERS UPON COLD STORAGE

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ABSTRACT

The attraction and quality of edible flowers correlates with their high perishability. Few studies have evaluated whether edible flowers decay faster than they lose their nutraceutical value. In this experiment, ascorbic acid was negatively affected by cold storage in all the edible flowers investigated, whereas phenolic, flavonoid, and anthocyanin content were affected only in some cases. No decrease in total antioxidant activity was detected in any of the edible flowers at the end of their shelf life. Our dataset highlights that (i) the selection of edible flowers with low moisture content is key in ensuring a longer shelf life, and (ii) more effort should focus on preventing water loss in edible flowers.

Keywords: ascorbic acid, anthocyanins, brightness, edible flowers, nutraceutical value, phenolics

1. INTRODUCTION

The demand for more attractive and high quality foodstuffs is increasing in the West. The appeal of food dishes can be enhanced by edible flowers, which is why they are becoming more and more popular (AQUINO-BOLAÑOS *et al*., 2013).
Edible flowers are mainly purchased by consumers for use in dishes as a garnish or ingredient although more often they are referred to in the literature in terms of their biologically active compounds. Some papers have extensively investigated the chemical composition of many edible flowers (LI *et al*., 2007; GARZON *et al*., 2009; KAISOON *et al*., 2011; GARZON *et al*., 2015; LOIZZO *et al*., 2015), highlighting that they are a substantial source of chemical compounds with a high antioxidant activity (FU and MAO, 2008; GARZON *et al*., 2015).
Phenolic acids, flavonoids, including anthocyanins, have been recognized as the most representative biologically-active compounds found in the petals of fresh edible flowers (MLCEK and ROP, 2011; NAVARRO-GONZALEZ *et al*., 2015). Unlike freshly-marketed edible flowers, in which the profile of bioactive compounds has been extensively investigated (for a review see MLCEK and ROP, 2011), only a few papers have evaluated the stability of edible flower phytochemicals during storage (DAS *et al*., 2010; KAZAZ *et al*., 2010; AQUINO-BOLAÑOS *et al*., 2013; LANDI *et al*., 2015a).
Results are sometimes conflicting. In some cases cold storage has been found to have a negative impact on the nutraceutical value of edible flowers (DAS *et al*., 2010; AQUINO-BOLAÑOS *et al*., 2013), but not in other cases (FRIEDMAN *et al*., 2007).
We investigated the effect of cold storage on various biologically-active compounds namely phenolics, flavonoids, anthocyanins and ascorbic acid in seven edible flowers belonging to five species (*Acmella oleracea* L., *Begonia semperflorens* L. with white, pink, and dark-pink, *Salvia discolor* Kunth, *Tulbaghia cominsii* Vosa, *Tropaeolum majus* L.) with different sizes, shapes and colors (the features of each edible flower are summarized in Table 1). Given that consumers are influenced by the visual appeal of edible flowers, and only high-quality produce encourages repeat purchases, we attempted to establish whether the loss of the visual appeal of edible flowers proceeds faster than the loss of their nutritional value. The overall aim was to address future research aimed at extending the shelf life of edible flowers.

2. MATERIALS AND METHODS

2.1. Chemicals

Methanol (LC/MS grade; > 99.95 % solvent purity), and HCl (ACS reagent, 37%) were purchased from CARLO ERBA Reagents S.r.l., Milan, Italy. All the other reagents were purchased from Sigma-Aldrich S.r.l., Milan, Italy.

2.2. Flower harvest and processing

Flowers of *A. oleracea* (AO), *B. semperflorens* (with white; BS_W, pink; BS_P, and dark-pink petals; BS_{DP}), *S. discolor* (SD), *T. cominsii* (TC), *T. majus* (TM) were kindly provided by CREAT-Chambre d'agriculture des Alpes-Maritimes (Nice, France) (Table 1).

Table 1. Features of the selected edible flowers studied in this work.

Species	Family	Abbreviation	Color	Flower size
Acmella oleracea L.	Asteraceae	AO	yellow	small
Begonia semperflorens L.	Begoniaceae	BS_{DP}	dark pink	medium
Begonia semperflorens L.	Begoniaceae	BS_{P}	pink	medium
Begonia semperflorens L.	Begoniaceae	BS_{W}	white	medium
Salvia discolor Kunth	Lamiaceae	SD	violet	small/ medium
Tulbaghia cominsii Vosa	Amaryllidaceae	TC	light pink	small
Tropaeolum majus L.	Tropaeolaceae	TM	orange	big

Fresh flowers at maturity stage (June, 2014) were harvested early in the morning, transported in refrigerated containers (4 °C) and processed within a few hours in an aseptic laboratory in accordance with KELLEY *et al*. (2003). For each species, some flowers (about 2 g) were finely ground with liquid nitrogen and stored at -80 °C until analysis. These samples represented the first day of storage (t0). The flowers were then randomized in air-tight hinged boxes (500 cm^3, Comital Cofresco, Italy) made from polyethylene terephthalate and stored at 4 °C under light to simulate commercial shelf conditions. Each box contained about 20 g of fresh flowers. Flowers were inspected to ensure there was no visually detectable damage prior to being placed in each container. Samples were collected following the same procedure after 2, 5 and 8 d of storage for biochemical analysis. For the 8-day storage, samples were collected only for edible flowers that would still have been marketable at that time. Before being ground, some of the sample flowers were used for color determination.

2.3. Determination of moisture content

Initial (t0) fresh weight (FW) was evaluated immediately after the preparation of the flower containers. To determine flower dry weight (DW), florets were desiccated in a ventilated oven at 80 °C until constant weight. At each sampling data (2, 5, 8 d), moisture content was evaluated as the difference between FW and DW of the flowers contained in each box, and expressed as percentage moisture content.

2.4. Color determination

For each species, color measurements were performed on five randomly selected flowers (n=5) at different storage times (0, 2, 5, 8 d). The value of each replicate was the mean of three independent spot measurements on each flower's surface evaluated by standard CIE $L^*a^*b^*$ color space coordinates determined by an Ocean Optic HR2000-UV-VIS-NIR spectrometer coupled with a tungsten halogen DH2000 light source (Ocean Optics, USA) as reported in LANDI *et al*. (2015b). Among all colorimetric parameters, L^* represents the lightness of colors (lightness index scale; 0 for black to 100 for white) and is a good parameter for monitoring the development of tissue darkening.

2.5. Postharvest visual quality rating

The visual appeal of flowers was scored on a 9-point scale based on visual observation of the degree of decay, as described by AQUINO-BOLAÑOS *et al*. (2013). For the sake of simplicity, score points were grouped as follows: 9 to 7 = fresh appearance (flower with no defects or the slight beginning of decay; classified with a green triangle in Fig. 1), 6 to 4 = limit of marketability (moderately deteriorating flower with between a quarter or and half the surface area decayed; yellow triangle in Fig. 1), 3 = not suitable for sail (water-soaked, dark and wilted flower with more than half the surface area decayed; red triangle in Fig. 1). The visual quality was assessed by a panel of five people with expertise in browning phenomena and post-harvest loss of quality.

2.6. Total phenolics, flavonoids, and anthocyanins

Extraction of total phenolics and total flavonoids was based on a slight modification of the method reported by DU *et al*. (2009). An aliquot of 100 mg of flower sample was homogenized in 1 mL of ethanol:acetone (7:3, v/v) and shaken overnight at 4 °C. The extract was centrifuged at 1,000 *g* for 15 min at 4 °C and the supernatant was filtered using Minisart filters (pore size 0.45 μm). The filtrate was collected and stored at −20 °C until analysis. Total phenolic content was determined using the Folin-Ciocalteau assay, according to DEWANTO *et al*. (2002), using 10 μL of extract. The absorbance was read at 760 nm and the total phenolic concentration was expressed as gallic acid equivalents (mg GAE g^{-1} DW) using a calibration curve (50-600 μg mL^{-1}).
Content of total flavonoids was determined according to DU *et al*. (2009) with a few modifications. In a 2 mL Eppendorf tube, 100 μL of flower extract were added in 1 mL ethanol 30% (v/v), 45 μL of 50 mM $NaNO_2$, 45 μL of $AlCl_3$ *x* $6H_2O$ 0.3 M. After 5 min at room temperature, 300 μL of 1 M NaOH were added and the mixture absorbance was measured at 506 nm. The content of total flavonoids was expressed as rutin equivalents (mg RE 100 g^{-1} DW) using a calibration curve as a standard (6.25-1000 μg mL^{-1}).
Total anthocyanins were extracted as reported by LANDI *et al*. (2014). Briefly, 100 mg of ground samples were mixed with 1 mL of acidified methanol (1.5% HCl v/v) and shaken overnight at room temperature. The supernatant was filtered using Minisart filters (pore size 0.45 μm); anthocyanin-containing flower extract (50 μL) was added to 950 μL of acidified methanol (1.5% HCl v/v) and the absorbance was read from 408 to 560 nm against a blank. Total anthocyanin content was expressed as the mean value of ABS in the range 408-560 nm per 100 mg^{-1} DW. An Ultrospec 2100 Pro spectrophotometer (GE Healthcare Ltd, Little Chalfont, England) was used for the analyses of total phenolics, flavonoids and anthocyanins, together with all the other spectrophotometric determinations.

2.7. Ascorbic acid determination

Total ascorbate (ASA_{TOT}), reduced ascorbate (ASA), and dehydroascorbate (DHA) were spectrophotometrically determined as described by KAMPFENKEL *et al*. (1995). The assay is based on the reduction of Fe^{3+} to Fe^{2+} by ASA and the spectrophotometric detection of Fe^{2+} complexed with 2,2′-dipyridyl. DHA is calculated as the difference between ASA_{TOT} and ASA, and data were expressed as μg g^{-1} DW.

2.8. DPPH scavenging activity

The antioxidant activity of each sample was determined using a modified version of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging assay, as described by KIM *et al*. (2005). The methanolic flower extract (20 μL) was diluted to 100 μL with 80% aqueous methanol. It was then added to 0.4 mL of 0.1 M Tris-HCl buffer and 0.5 mL of 0.3 mM DPPH in methanol. The solution was mixed thoroughly and incubated in the dark for

20 min at room temperature. The absorbance of the sample mixture ($A_{sample} = A_{sample\ t20'} - A_{sample\ t0}$) was monitored at 517 nm. The absorbance of a control sample ($A_{control} = A_{control\ t20'} - A_{control\ t0}$) containing only methanol/Tris-HCl, and DPPH versus a methanol/Tris-HCl blank was also analyzed. The percentage DPPH free radical scavenging activity was calculated according to the following equation:

$$\%\ \text{DPPH free radical scavenging} = [1-(A_{sample}/A_{control})] \times 100$$

The antioxidant activity was determined by comparing the percentage DPPH free radical scavenging of each sample to a calibration curve prepared with Trolox. Antioxidant activity was expressed as Trolox equivalents (TE; mmol of TE g^{-1} FW) for direct comparison of the free radical scavenging capabilities between all the samples.

2.9. Statistical analysis

Visual quality was assessed by each expert in five randomly selected flowers per species at each sampling time. Reported data for flower moisture and phytochemical contents are the means (±SD) of five independent replicates (*n*=5), where each box was considered as a replicate. Means were compared by one-way ANOVA, following Bartlett's test to assess the homogeneity of variance among samples, considering storage as the variability factor. Percentage values were arcsine transformed prior to the analyses. Means with different letters within species are significantly different after Fisher's least-significant difference test (LSD) for P=0.05. For some comparisons among species (when discussed), one-way ANOVA was applied with species as the variability factor. All statistical analyses were performed using CoStat (CoHortTM Software, Berkeley, CA).

3. RESULTS AND DISCUSSIONS

3.1. Physiological weight loss and visual quality

Values of *L** are indicative of tissue darkening as browning is commonly associated with the oxidation of phenolics and their polymerization into dark brown pigments (MARTIN-DIANA *et al*., 2015; LANDI *et al*., 2015c, REMORINI *et al*., 2015). As expected, in this experiment *L** values decreased in all the EFs under investigation, at least at the end of the storage period (8d, Fig. 1). Flowers of *A. oleracea* and *T. cominsii* were considered to be still marketable after 8 d of storage at 4 °C, whereas all the other species were classified as not suitable for sale at this storage time (Fig. 1).

Differences in the shelf life of edible flowers subjected to cold storage have already been reported (KUO *et al*., 2012; KELLEY *et al*., 2003). Unlike reported by KELLEY *et al*. (2003), five out of the seven edible flowers that we evaluated (including nasturtium, which was one of the edible flowers considered by KELLEY *et al*) had a shorter shelf life at 5 °C (5 d). These differences can be attributable to the different packaging: KELLEY *et al.* (2003) used polyethylene bags, whereas we used polyethylene terephthalate boxes in order to preserve the delicate flowers. Despite the evident differences in terms of shelf life, the main determinants that contribute are less clear from the literature.

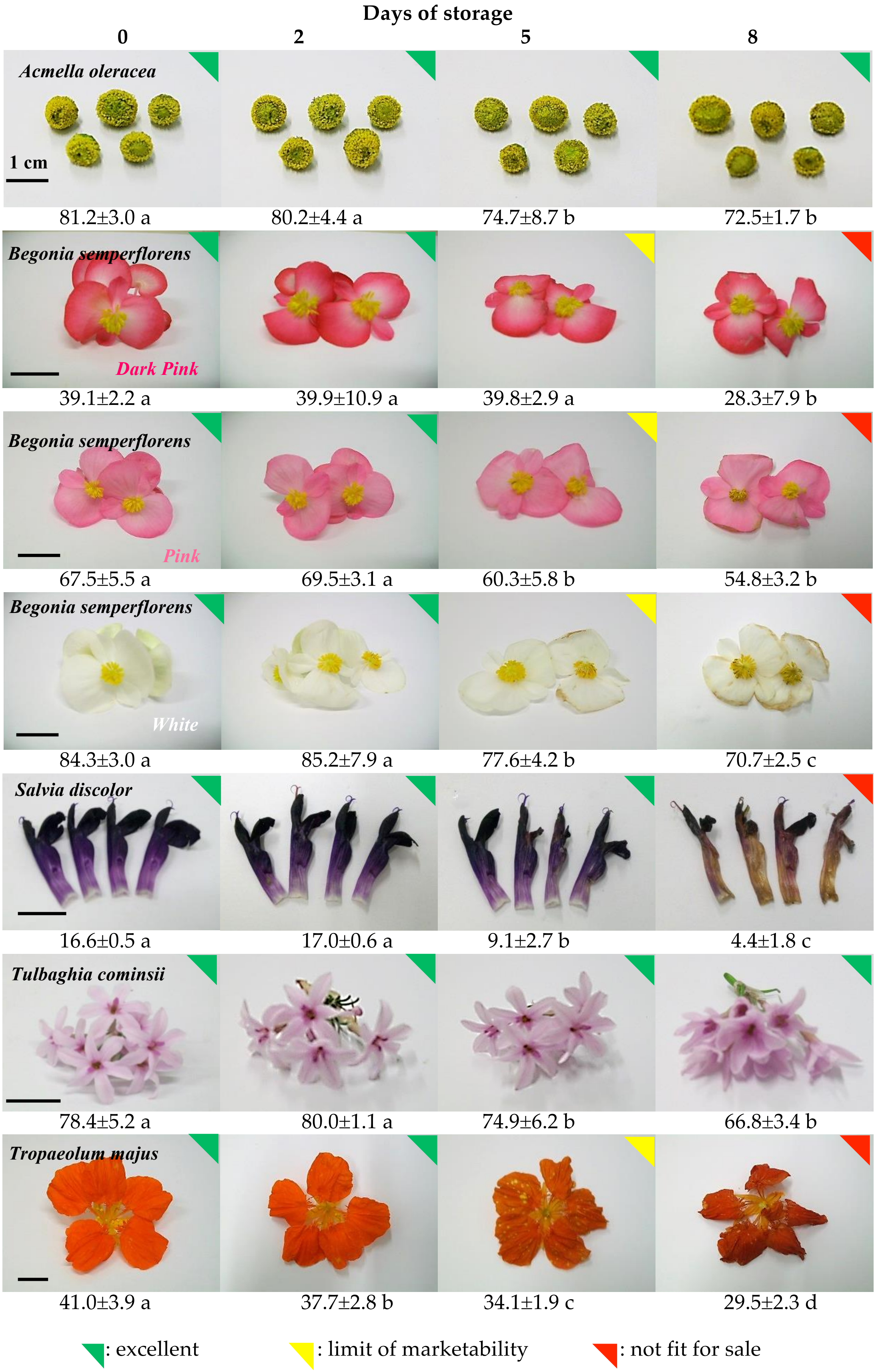

Figure 1. Visual appearance, marketability and lightness (L^*) of edible flowers. Reported results for L^* values are the mean of five replicates (n=5;±SD). Means flanked by different letters are significantly different within each flower species upon storage for P = 0.05 after one-way ANOVA followed by LSD test. Colored triangles at the top right of each figure represent excellent product (green), limit of marketability (yellow), or unsaleable product (red). Bars = 1 cm.

Our data suggest that the constitutive moisture content of edible flowers has the most impact on shelf life. At t0, flowers of *B. semperflorens* with dark-pink, pink and white petals and *T. majus* had a significantly higher ($p<0.001$) moisture % (which averaged 97.34% in *B. semperflorens* with dark-pink, pink and white petals and *T. majus*) than *A. oleracea* and *T. cominsiii* (87.82 and 88.19%, respectively), and had a 5-d *versus* a 8-d shelf life for *A. oleracea* and *T. cominsiii* (Table 2 and Fig. 1). The hypothesis of reduced dehydration related to longer flower shelf life is also in agreement with KOU *et al*. (2012).
This hypothesis does not seem applicable to *S. discolor* flowers which had a simultaneously lower moisture content than *B. semperflorens* with dark-pink, pink and white petals and *T. majus*, but only 5 d of marketability. In this species, the reduced marketability seems mainly related to the loss of anthocyanin content (Table 3) as *S. discolor* was the only edible flower in which anthocyanin decreased during storage.
Although not measured in our experiments, the high perishability of edible flowers might be connected to their respiration rate and the production of ethylene, as is the case with other horticultural commodities (KADER and SALTVEIT, 2003). However, KOU *et al*. (2012) demonstrated that after 7 d of storage the decay index of carnation increased to a similar extent and irrespectively of the use of modified atmosphere packaging and/or 1-methylcyclopropene, which is a commonly used ethylene inhibitor. FRIEDMAN *et al*. (2007) also found that *T. majus* flower quality was not related to CO_2 or to ethylene levels inside the packaging in a short-term storage period. VILLALTA *et al*. (2004) found that the respiratory rate of yellow summer blossom remained relative constant and low during the 8-d storage period at 5 °C.

Table 2. Moisture content of edible flowers of *Acmella oleracea* L. (AO), *Begonia semperflorens* L. (with white; BS_W, pink; BS_P, and dark-pink petals; BS_{DP}), *Salvia discolor* Kunth (SD), *Tulbaghia cominsii* Vosa (TC), *Tropaeolum majus* L. (TM) during storage.

Species	Storage (d) 0	2	5	8
	Moisture content (%)			
AO	87.82±1.74 a	87.08±2.03 a	79.15±5.39 b	61.44±3.41 c
BS_{DP}	97.33±0.39 a	83.63±1.95 b	69.15±3.22 c	47.23±4.66 d
BS_P	97.41±0.03 a	92.89±1.08 b	82.87±1.80 c	61.67±3.18 d
BS_W	97.38±0.35 a	89.21±1.26 b	74.13±3.95 c	47.85±0.12 d
SD	83.09±0.57 a	80.11±1.48 b	73.41±2.60 c	57.25±1.65 d
TC	88.19±0.08 a	86.43±1.55 a	83.22±3.40 b	77.56±3.67 c
TM	97.27±0.54 a	91.66±4.10 b	80.31±6.45 c	45.03±7.24 b

Data represent the mean±SD (n=5). Means flanked by different letters are significantly different within each flower species upon storage for P = 0.05 after one-way ANOVA followed by LSD test.

This evidence weakens the hypothesis that the flower's respiration rate is the key factor in extending an edible flower's shelf life, at least for short-term storage. On the other hand, controlling the respiration rate seems to be more important for longer storage periods (at least two weeks; KUO *et al*., 2012).

3.2. Bioactive compounds and antioxidant activity

Phenols (including phenolic acid, flavonoids and anthocyanins) are currently the target of numerous studies since their intake has been associated with the decreased risk of cancer, cardiovascular diseases and neurodegenerative disorders (SALEM *et al*., 2011). In our study, constitutive levels of total phenolics ranged from 10.02 mg GAE g^{-1} DW in *A. oleracea* to 194.33 in *T. majus* (Table 3).

Table 3. Phytochemical content of edible flowers of *Acmella oleracea* L. (AO), *Begonia semperflorens* L. (with white; BS_W, pink; BS_P, and dark-pink petals; BS_{DP}), *Salvia discolor* Kunth (SD), *Tulbaghia cominsii* Vosa (TC), *Tropaeolum majus* L. (TM) during storage.

Species	Storage (d)	Total phenols (mg GAE g^{-1} DW)	Total flavonoids (mg RE g^{-1} DW)	Total anthocyanins (ABS*b* 100 mg^{-1} DW)	DPPH (mmol TE g^{-1} DW)
AO	0	10.02±2.93 c	4.85±1.05 a	ND	26.25±2.58 b
	2	15.00±0.35 a	6.17±1.39 a	ND	32.04±1.39 a
	5	12.62±1.59 b	4.94±1.06 a	ND	30.77±4.16 a
	8	11.83±0.18 bc	4.66±0.09 a	ND	30.73±1.27 a
BS_{DP}	0	64.21±1.56 b	37.81±3.63 a	2.20±0.37 a	96.21±8.80 a
	2	77.78±4.70 a	32.77±2.23 b	1.92±0.64 a	90.42±12.03 a
	5	63.85±3.86 b	29.11±0.43 c	1.94±0.47 a	89.37±8.14 a
	8	-	-	-	-
BS_P	0	51.72±4.30 c	41.79±13.87 a	0.84±0.16 a	74.71±4.87 c
	2	94.21±8.80 a	36.50±3.10 ab	0.76±0.21 a	94.53±0.52 a
	5	84.54±11.3 b	27.43±0.97 b	0.65±0.15 a	83.80±5.81 b
	8	-	-	-	-
BS_W	0	77.77±9.50 b	32.01±6.50 a	ND	67.94±17.34 b
	2	95.27±1.02 a	36.09±1.53 a	ND	93.10±8.47 a
	5	69.29±6.01 b	28.37±4.60 a	ND	63.40±9.25 b
	8	-	-	ND	-
SD	0	26.76±0.92 a	11.35±1.59 a	0.27±0.03 a	32.62±0.41 a
	2	19.29±1.53 b	9.60±1.45 a	0.23±0.03 ab	31.37±3.47 a
	5	19.20±3.05 b	9.76±1.22 a	0.19±0.06 b	29.17±0.15 a
	8	-	-	-	-
TC	0	30.51±2.16 a	3.02±0.24 a	0.09±0.03 a	44.85±0.36 a
	2	28.55±0.47 b	2.74±1.01 a	0.10±0.04 a	47.59±1.53 a
	5	28.13±1.28 b	3.11±0.58 a	0.13±0.01 a	46.79±2.10 a
	8	25.1±1.02 c	3.51±0.78 a	0.13±0.01 a	47.40±0.78 a
TM	0	194.33±16.50 a	28.34±3.70 a	10.10±3.34 a	142.13±22.16 c
	2	140.51±9.98 c	28.91±0.50 a	9.81±1.70 a	156.33±4.00 b
	5	163.50±2.33 b	31.55±1.41 a	11.22±1.27 a	181.02±1.40 a
	8	-	-	-	-

Data represent the mean±SD (n=5). Means flanked by different letters are significantly different within each flower species upon storage for P = 0.05 after one way ANOVA followed by LSD test. ABS, absorbance; DPPH, total antioxidant activity evaluated by 2,2-diphenyl-1-picrylhydrazyl radical; d, days; DW, dry weight; GAE, gallic acid equivalents; ND, not detectable; RE, rutin equivalent; TE, Trolox equivalents. *b*, Mean value of absorbance in the range 408-560 nm.

These values are common in flower species, as also testified by other studies (ROP *et al*., 2012; LI *et al*., 2014).
In many cases, the total phenol values in all the edible flowers studied here were abundantly higher than those reported for other vegetables and fruits, which are usually considered as good sources of phenols (KÄHKÖNEN *et al*., 1999). Edible flower species could thus represent an interesting source of phenolic compounds, despite the small amount usually consumed compared to other fruits and vegetables. Interestingly, the levels of total phenols found in *T. majus* flowers are two and a half times higher than those reported for blueberry genotypes, which are usually classified as some of the richest sources of phenols, in particular due to their high level of anthocyanins (CASTREJÓN *et al*. 2008).
Overall, we found that 8 d of cold storage affected the concentration of all the phenolic bioactive compounds evaluated here only in some cases (Table 3). A reduction in total phenols was recorded only in *S. discolor*, *T. cominsii*, and *T. majus* flowers. Total flavonoids decreased only in pigmented *B. semperflorens*, whereas the loss of anthocyanins was observed only in *S. discolor*. Results regarding the effect of storage on phenolic compounds are scarce and conflicting. For example, AQUINO-BOLAÑOS *et al*. (2013) found a reduction in total phenols in yellow summer squash flowers during postharvest storage. FRIEDMAN *et al*. (2007) found no difference in anthocyanin content in *B. semperflorens* flowers and LANDI *et al*. (2015a) found no significant changes in total phenols in sage flowers during storage. We are not aware of any other work focused on the variation in phenolic content and profile in edible flowers upon storage.
Ascorbic acid is a well-known key antioxidant in plants and is an essential vitamin for humans. Unlike the polyphenols mentioned above, the concentration of ASA_{TOT} decreased significantly during storage (at least at the end of the marketability stage) in almost all the edible flowers under investigation (*A. oleracea*, *B. semperflorens* with dark-pink and white petals, *T. cominsii*, and *T. majus*) (Table 4). Notably, in *B. semperflorens* with pink petals and *S. discolor*, whose level of ASA_{TOT} was unchanged, we found an increased level of the oxidized form of ascorbate (decremented ratio ASA/ASA_{TOT}). Thus, storage may have negatively affected the level of ASA (which is the biological active form of ascorbic acid) in all the edible flowers under investigation. A reduction in ascorbate levels in edible flowers has also been reported by DAS *et al*. (2010) and by AQUINO-BOLAÑOS *et al*. (2013) under cold storage. AQUINO-BOLAÑOS *et al*. (2013) attributed the loss of ascorbate to the loss of cell integrity and compartmentalization, which expose ascorbic acid to oxygen, which, in turn, decreases the reducing power of this key antioxidant.
The biological activities of phenolic compounds and ascorbate seem to be related to their strong antioxidant capacity *in vitro*, as also reported for many edible flower-derived compounds (KAISOON *et al*., 2011; SALEM *et al*., 2011; LI *et al*., 2014; GARZON *et al*., 2015; LOIZZO *et al*., 2015). Interestingly, the total antioxidant activity of some edible flowers, including some of the flowers tested in our investigation (i.e., *S. discolor* and *T. cominsii*), is even higher than that of many blueberries varieties (*V. corymbosum* L.), which is one of the richest reported antioxidants (GIOVANNELLI and BURATTI, 2009).
The total antioxidant activity of edible flower extracts did not decrease in any of the edible flowers upon storage (Table 3). Conversely, in others (i.e., *A. oleracea*, *B. semperflorens* with pink petals, and *T. majus*) the total antioxidant activity was also found to increase during the storage. Our findings are in agreement with FRIEDMAN *et al*. (2007), who reported that 7-8 d of cold storage (2-5 °C) did not reduce the antioxidant activity of *B. semperflorens* flowers.
The stability of the antioxidant capacity of edible flowers seems principally related to the relative stability of the total phenol content given that many researchers have found a strong linear relation between total phenol content and the antioxidant activity of edible flowers ($R^2 > 0.93$) (LI *et al*., 2014; NAVARRO-GONZALEZ *et al*., 2015).

Table 4. Ascorbic acid content of edible flowers of *Acmella oleracea* L. (AO), *Begonia semperflorens* L. (with white; BS_W, pink; BS_P, and dark-pink petals; BS_{DP}), *Salvia discolor* Kunth (SD), *Tulbaghia cominsii* Vosa (TC), *Tropaeolum majus* L. (TM) during storage.

	Storage (d)			
Species	**0**	**2**	**5**	**8**
		ASA_{TOT} (mg g^{-1} DW)		
AO	2.51±0.12 b	3.05±0.12 a	2.40±0.53 b	1.04±0.09 c
BS_{DP}	16.87±3.63 a	16.06±3.12 a	11.53±2.14 b	-
BS_P	16.90±3.63 a	17.52±2.06 a	16.38±1.93 a	-
BS_W	5.13±0.59 a	5.76±0.51 a	2.31±0.46 b	-
SD	8.13±2.00 a	8.18±2.66 a	6.75±1.45 a	-
TC	12.84±0.12 a	8.07±1.29 b	7.43±2.67 c	8.80±1.12 b
TM	89.25±15.88 a	68.75±14.10 b	47.01±1.33 c	-
		ASA/ASA_{TOT}		
AO	0.45±0.01 a	0.41±0.05 a	0.47±0.04 a	0.50±0.03 a
BS_{DP}	0.74±0.01 a	0.57±0.01 b	0.77±0.06 a	-
BS_P	0.46±0.02 a	0.39±0.04 a	0.39±0.02 b	-
BS_W	0.53±0.08 a	0.46±0.15 a	0.58±0.13 a	-
SD	0.73±0.10 a	0.54±0.12 b	0.58±0.13 b	-
TC	0.86±0.03 a	0.89±0.01 a	0.87±0.01 a	0.75±0.12 b
TM	0.62±0.10 a	0.58±0.18 a	0.64±0.02 a	-

Data represent the mean±SD (*n*=5). Means flanked by different letters are significantly different within each flower species upon storage for P = 0.05 after one-way ANOVA followed by LSD test. ASA, ascorbic acid (reduced form); ASA_{TOT} total ascorbate (sum of oxidized and reduced form); DW, dry weight.

Our data also highlight a good correlation between total phenol content and total antioxidant activity, although we found a lower R^2 than that mentioned above ($R^2 = 0.655$). This determination coefficient is however in agreement with that reported by LI *et al*. (2009) ($R^2 = 0.664$) and TAI *et al*. (2011) ($R^2 = 0.652$) in flowers of peony and *Sophora viciifolia*, respectively. It suggests that, despite the main role of phenolics as antioxidants, other antioxidant compounds contribute significantly to the flower antioxidant activity. This would also justify the reduction in total phenolic content found in *S. discolor*, *T. cominsii*, and *T. majus* associated with unchanged (though increased in *T. majus*) levels of their total antioxidant activity. In our experiments, the contribution of ascorbic acid to the total antioxidant activity seems less significant than total phenolics, given that ascorbic acid levels were negatively affected by the storage, while the total antioxidant activity was not.

4. CONCLUSIONS

Our data suggest that the loss of visual appeal of most edible flowers proceeds faster than the loss of their bioactive compound content. Only ascorbic acid was found to be highly susceptible to the storage process. The content of its reduced form (ASA) decreased upon storage in all the edible flowers under examination before the end of their shelf life. On the other hand, phenolic moieties were less affected by the storage. The total antioxidant activity of all the edible flowers evaluated here was stable under cold storage up to the end of their shelf life. This suggests that edible flower decay is directly related to their constitutive water content, thus (i) the selection of edible flowers with low moisture is a key factor in ensuring their longer marketability; (ii) more efforts should focus on the processes and technologies aimed at preserving (or delaying) edible flowers from water loss, such as the use of boxes or bags made from appropriate plastic material and/or appropriately modified atmospheres, in order to extend the shelf life of edible flowers.

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PAPER

VOLATILE COMPOUNDS AND SENSORY PROPERTIES OF COLESLAW MIX PACKAGED IN MODIFIED ATMOSPHERE

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ABSTRACT

The effect of modified atmosphere and film microperforation on the aroma of a coleslaw mix stored for 12 days at 4°C was detected. Samples were packaged in air and modified atmosphere (5/10/85, 70/30/0% $O_2/CO_2/N_2$) and sealed with the film with or without microperforations. The application of microperforated film makes it possible to maintain a desirable aroma for 12 days. Use of the unmicroperforated film resulted to increase in allyl isothiocyanate and dimethyl trisulfide concentration. A strong correlation was observed between the following aroma attributes and volatile compounds: sharp - allyl isothiocyanate (R^2=0.80), dimethyl trisulfide (R^2=0.83); and carrot – *p*-cymene (R^2=0.71).

Keywords: aroma, cabbage, carrot, minimally processed, modified atmosphere

1. INTRODUCTION

In minimal processing, the modified atmosphere packaging (MAP) is frequently used to extend the shelf life of product. In the case of fruits and vegetables, the recommended solution is to expose them to the atmosphere containing 1-5% oxygen and 5-10% carbon dioxide (balanced with nitrogen). A new trend is packaging in superatmospheric oxygen atmospheres (RADZIEJEWSKA-KUBZDELA and CZACZYK, 2016). From literature, it was observed that an increased oxygen content in the atmosphere may inhibit enzymatic discoloration, prevent anaerobic fermentation reaction, influence aerobic and anaerobic microbial growth, reduce decay of the fresh vegetable and also prevent odour losses (DAY, 1996). The studies of CLIFFE-BYRNES *et al*. (2003), and RADZIEJEWSKA-KUBZDELA and BIEGAŃSKA-MARECIK (2009) indicated that aroma is one of the most important factors determining the quality of minimally processed cabbage. Available literature lacks data concerning both the profile of aroma compounds in coleslaw mix and the effect of packaging conditions on the aroma of this product. Research is limited to minimally processed broccoli, carrot, York cabbage or lettuce (CLIFFE-BYRNES *et al.*, 2007; DEZA-DURAND and PETERSEN, 2014; JACOBSSON *et al.*, 2004; LONCHAMP *et al.*, 2009). The profile of aroma compounds may be a significant determinant of quality for such products.

During storage in modified atmosphere, the respiration rate of tissue may lead to a decreased O_2 content and an increase in the CO_2 level inside the package if the gas composition and the permeability of the film is insufficient. This change may result in the formation of fermentative metabolites (such as ethanol, ethyl, acetate, acetaldehyde, methyl acetate and acetone) occurring during anaerobic respiration. Aroma of product from the *Brassicaceae* family is also determined by sulfurous compounds. During the loss of intracellular compartmentalization, enzymes can react with substrates causing strong off-odors. There are mainly products of S-methyl-L-cysteine and S-methylcysteine sulfoxide degradation by the action of cysteine sulfoxide lyase (methanethiol, dimethyl disulfide and dimethyl trisulfide) as well as degradation products of glucosinolates formed as a result of myrosinase activity (AKPOLAT and BARRINGERB, 2015; JANG *et al.*, 2015). Aroma of coleslaw mixes may also be influenced by terpenes originating both from carrot roots and cabbage (VUORINEN *et al.*, 2004; YAHYAA *et al.*, 2015). KJELDSEN *et al.* (2003) reported that during cold storage of carrot, an increase in the contents of terpene compounds to moderate amounts (10-30 ppm) was connected with the carrot aroma, whereas at >35-40 ppm, terpenes produced a harsh and burning turpentine-like flavor. Correlation between aroma and volatile compounds is dependent on the composition of product. Relationship between cabbage and carrot in coleslaw mix was not tested. A better understanding of these relations is needed to facilitate the development of strategies in order to prevent off-odor formation in package.

Our previous studies indicate that the application of modified atmosphere composed of 70/30/0% $O_2/CO_2/N_2$ in packaging of a coleslaw mix with a microperforated barrier film resulted in a good sensory and microbiological quality during the 12 days of storage (RADZIEJEWSKA-KUBZDELA and CZACZYK, 2016). For this reason, an analysis of the profile of volatiles in the coleslaw mix was conducted using the above-mentioned composition in the atmosphere, while for comparison, samples packaged in air and in the atmosphere of 5/10/85% $O_2/CO_2/N_2$ were also tested. In the case of an atmosphere with a 70% oxygen content, a barrier film was also applied in product packaging, as it is frequently recommended for superatmospheric oxygen atmospheres in packaging products of plant origin.

The aim of this study was to determine the effect of modified atmosphere, and film microperforation on the aroma of a coleslaw mix stored for 12 days at 4°C. Correlations between sensory aroma attributes and contents of volatile compounds in the atmosphere composition were also investigated.

2. MATERIALS AND METHODS

2.1. Materials

Coleslaw mix was produced by mixing shredded white cabbage cv. Galaxy and carrot cv. Perfekcja. The raw materials came from a farm located in western Poland. Prior to technological process, white cabbage and carrot were stored for 1 week at 1°C (this step is not necessary).

2.2. Technological process

White cabbages and carrots were washed in tap water. Thereafter, the outer leaves of white cabbage were removed and the heads were cored using a sharp knife. Carrots were hand-peeled. The vegetables were washed in tap water and dried with absorbent paper. After drying, the vegetables were shredded mechanically, a Nagema HU-1 device (Dresden, Germany) was used for cabbage, while a Robot Coupe CL 50 Ultra device (Vincennes, France) was used for carrot. Shredded cabbage and carrot were mixed at a ratio of 80/20 (w/w). The anti-microbial treatment involved dipping coleslaw for 5 min with agitation in 5 g/L ascorbic acid and 5 g/L citric acid solution. The adherent water after pretreatments was removed using a manual vegetable spinner (Zepter, Viersen, Germany). After that, the shredded material was weighed out (160 g white head cabbage and 40 g carrot) and placed on 205 x 160 x 60 mm polypropylene trays with an oxygen transmission rate of 7-8 $cm^3/m^2/24$ h. The selected atmosphere concentrations % of $O_2/CO_2/N_2$: 5/10/85 and 70/30/0, and air atmosphere were introduced into the packages before thermally sealed with a gas packaging device gas mixer (Witt-Gasetechnik, Witten, Germany), Multivac T 200 packaging machine (Wolfertschwenden, Germany). The trays were sealed with an Opalen HB 55 packaging film with oxygen permeability of 35 $cm^3/m^2/24$ h * atm at 23°C and 85% RH (according to data provided by the film manufacturer) (Bemis, Soignies, Belgium). The film was then microperforated. The microperforations had been made by the Multivac system (Wolfertschwenden, Germany) using one cylinder with 10 needles of 70 µm in diameter (10 microopenings in the film, sealing the tray – 333 holes/m^2 of the film). In the case of modified atmosphere composed of 70/30/0% $O_2/CO_2/N_2$, the trays were also sealed with the same film but without microperforations.

2.3. Gas composition

Contents of oxygen and carbon dioxide inside the packages were determined using an OXYBABY gas analyzer by Witt-Gasetechnik (Witten, Germany). The results were reported as means of three experimental determinations for separated sample ((RADZIEJEWSKA-KUBZDELA and CZACZYK, 2016).

2.4. Analysis of volatile compounds

The volatile compounds were analyzed after 1, 6, 9 and 12 days of storage at 4°C using solid phase microextraction (SPME). A SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was used to collect volatile compounds within each package. To facilitate the headspace-SPME analysis in a semi-quantitative way deuterated (d8) naphthalene (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) was used as an internal standard (IS). As addition of the internal standard to sampled coleslaw would result in non-uniform distribution of standard, IS adsorption on the fiber was used before sample extraction option. For this purpose, before each coleslaw sampling, SPME fiber was exposed for 5 minutes to IS solution (10 μg/mL) in silicon oil. The solute (10 ml) was placed in a 20 ml headspace vial capped with silicon rubber/PTFE membrane. Both IS sampling and subsequent coleslaw sampling was performed at room temperature (20°C ± 1°C). The high concentration of IS in the solution compared to fiber capacity allowed for multiple extraction of IS from a single vial. The fiber was exposed to a headspace of the sample for 30 min and after extraction time, the fiber was transferred immediately to an injection port of a gas chromatograph and desorbed for 5 min at 250°C in a splitless mode. Compound identification was performed using an Agilent 7890A gas chromatograph coupled to a 5975C TAD single quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA) with a DB-5MS column (25 m $\times$0.200 mm$\times$0.33 μm, Agilent Technologies, Santa Clara, CA). The carrier gas was helium at a flow rate of 0.8 ml min^{-1}, while oven temperature was 40°C for 1 min, followed by an increase of 8°C min^{-1} to 220°C and 20 °C min^{-1} to 280°C. Mass spectra were recorded in an electron impact mode (70 eV) in a scan range of m/z 33-333. The mass spectra of volatile compounds were identified tentatively by comparison with spectra of the NIST 05 mass spectral library. Volatile peaks (Total Ion Current) were compared to that of IS and the results provided in ng/g of fresh weight of coleslaw. No correction factors were used.

2.5. Sensory evaluation of aroma

Quantitative descriptive analysis was used to characterize the aroma of coleslaw mixes packaged in air and modified atmosphere during 12 days of storage at 4 °C. The panel consisted of 10 members (all employed at the Poznan University of Life Sciences) who were trained in evaluation according to ISO 8586-1. Sensory attributes were selected from literature and from orientation sessions, in accordance with ISO 11035. A total of 8 descriptors were established as the final list. Panelists assessed all descriptive attributes on a 10 cm unstructured line. The results from linear scale were converted into numerical values for data analysis. The 0 value indicated the lowest value intensity and 9 – the highest.

2.6. Statistical Analysis

The two-way variance analysis (ANOVA) and Fisher's least significant difference (LSD) were performed and Pearson's correlation coefficients between aroma attributes and contents of volatile compounds were calculated. Statistically significant differences were reported at P = 0.05. Principal component analysis (PCA) and partial least-squares (PLS) regression analysis were performed using the Statistica version 9.1 computer software (StatSoft Inc., Tulsa, USA).

3. RESULTS AND DISCUSSION

3.1. O_2 and CO_2 contents

The results concerning changes in O_2 and CO_2 contents in stored samples are presented in Table 1. After 9 days to the end of the assumed storage time, the oxygen content in samples packaged with microperforated film was uniform and remained at 11.2% to 11.7% In the case of samples packaged in the atmosphere containing 70/30/0% $O_2/CO_2/N_2$ in the film with no microperforation a superatmospheric oxygen level was maintained throughout storage and after 12 days it was 44.1%.

In samples packaged in an atmosphere with a 30% content of CO_2, sealed with microperforated film, a significantly (P=0.05) higher level of this gas lasted till day 6 of storage. After 9 days of storage, CO_2 contents was observed (from 11.3% to 12.1%) in all tested salad mixes packaged in microperforated film equalization. In turn, in samples packaged in modified atmosphere (70/30/0% $O_2/CO_2/N_2$) and in film with no microperforation the carbon dioxide content is significantly (P=0.05) increased during storage and after 12 days amounted to 46.8%.

Table 1. O_2 and CO_2 contents in coleslaw mix stored for 12 days at 4°C.

Content of gas (%)	Storage time (days)	air with film microperforation		5/10/85 % $O_2/CO_2/N_2$ with film microperforation		70/30/0 % $O_2/CO_2/N_2$ with film microperforation		70/30/0 % $O_2/CO_2/N_2$ without film microperforation	
O_2	1	15.3±0.9	e	8.8±0.9	a	43.1±0.2	f	60,1±0.7	i
	6	12.9±1.0	cd	11.9±0.5	bc	13.9±0.9	d	51.7±0.8	h
	9	11.2±0.2	b	11.7±0.5	bc	11.2±0.6	b	48.3±1.4	g
	12	11.4±0.4	b	11.5±0.6	b	11.5±0.8	b	44.1±1.2	f
CO_2	1	5.8±0.5	a	10.5±0.6	bc	20.2±1.2	e	30.2±0.4	f
	6	9.2±0.2	b	11.8±0.6	c	14.9±2.1	d	33.5±0.7	g
	9	11.5±0.4	c	11.8±1.1	c	11.3±1.2	c	44.3±1.9	h
	12	11.7±0.8	c	12.1±1.3	c	11.7±0.8	c	46.8±1.0	i

Mean ± standard deviation (n=3) for parameter with different letters are significantly different at P = 0.05.

Contents of oxygen and carbon dioxide in the atmosphere inside the packaging are influenced both by film permeability and intensity of tissue respiration processes (RADZIEJEWSKA-KUBZDELA and CZACZYK, 2015). The composition of the atmosphere inside the packaging at the application of the barrier film with no microperforation is influenced first of all by respiration processes. A study by JACXSENS *et al.* (2001) showed that packaging of grated celeriac, mushroom slices and shredded chicory endive in a modified atmosphere with a 95% oxygen content in barrier film caused a considerable reduction in oxygen content in the atmosphere of the packaging (to as little as 19.9%, 6.8%, 12.0%, respectively) and an increase in carbon dioxide levels (45.5% for grated celeriac and 47.5% for mushroom slices). In the case of the coleslaw mix, the application of superatmospheric oxygen atmosphere in combination with an elevated content of CO_2 seems to be a factor inhibiting the intensity of respiration processes in finely comminuted tissue. In the tested samples after 12 days of storage the content of CO_2 was approximately 17% higher than after packaging. The effect of the reduced respiration intensity in butterhead lettuce at the application of a superatmospheric oxygen atmosphere with an elevated (10-20%) content of carbon dioxide was reported by ESCALONA *et al.* (2006).

3.2. Volatile compounds

The Figs. 1, 2, 3 and 4 presented the content of volatile compounds in stored coleslaw mixes. Volatile compounds identified in the coleslaw mix included monoterpenes, sesquiterpene, sulfur compounds and alcohol.

The presence of sulfur compounds was recorded only in samples packaged in a modified atmosphere of 70/30/0% $O_2/CO_2/N_2$ in the film with no microperforation. Allyl isothiocyanate was detected after 6, 9 and 12 days of storage, while dimethyl trisulfide was detected after 12 days of storage. The content of allyl isothiocyanate significantly (P=0.05) increased during storage. From literature, it was observed that these compounds are responsible for the sharp, sulfury aroma, which in the case of a minimally processed product may be considered undesirable (AKPOLAT and BARRINGERB, 2015). Allyl isothiocyanate is formed as a result of enzymatic (myrosinase) degradation of sinigrin. The accumulation of this compound in the above-mentioned samples may result both from the lack of film microperforation and the high concentration of carbon dioxide inside the packaging. Studies conducted by RADZIEJEWSKA-KUBZDELA and CZACZYK (2015) showed that a high level of CO_2 in the atmosphere may damage cell membrane integrity. In turn, the formation of dimethyl trisulfide is most probably connected with the degradation of S-methylcysteine sulfoxide, under the influence of cysteine sulfoxide lyase (JONES *et al*., 2004).

Monoterpenes found in the coleslaw mix may originate both from carrot and cabbage. A study by VUORINEN *et al.* (2004) showed that such compounds as α-thujene, α-pinene, β-pinene, sabinene, limonene and γ-terpinene are released as a result of break in continuity of the cabbage tissue. KJELDSEN *et al.* (2003) and CLIFFE-BYRNES *et al.* (2007) indicated the presence of these compounds also in the aroma profile of carrot. Moreover, in that raw material, they identified camphene, myrcene, α-phellandrene, α-terpinene, p-cymene, β-phellandrene, ocimene and terpinollene and indicated the presence of sesquiterpenes, of which only β-caryophyllene was identified in the coleslaw mix. However, the profile of volatile compounds determined by KJELDSEN *et al.* (2003), and YAHYAA *et al.* (2015) in carrot and cabbage was much broader.

A derivative of the octadecadienoic pathway, 3-hexen-1-ol, was identified only in salads packaged in the atmosphere with a superatmospheric oxygen content after 1 day of storage (Fig. 1). This compound is emitted after mechanical tissue damage and it is labelled as the green leaf aroma.

After 1-day storage, salads packaged in air contained camphene, myrcene and β-phellandrene. In turn, in all the tested samples α-phellandrene and α-terpinene were detected. In samples sealed with microperforated film α-thujene, sabinene and β-pinene were detected up till day 9 of storage. However, during storage, a significant (P=0.05) decrease in their contents was recorded. In samples packaged in the film with no microperforation the depletion of α-thujene and sabinene was observed after 6 and 9 days of storage, respectively, while β-pinene was present till the end of the assumed storage period. In the case of the latter compound its content was found to increase significantly (P=0.05) during storage. Ocimene, γ-terpinene and terpinolene were found in all the samples only after 1 day of storage.

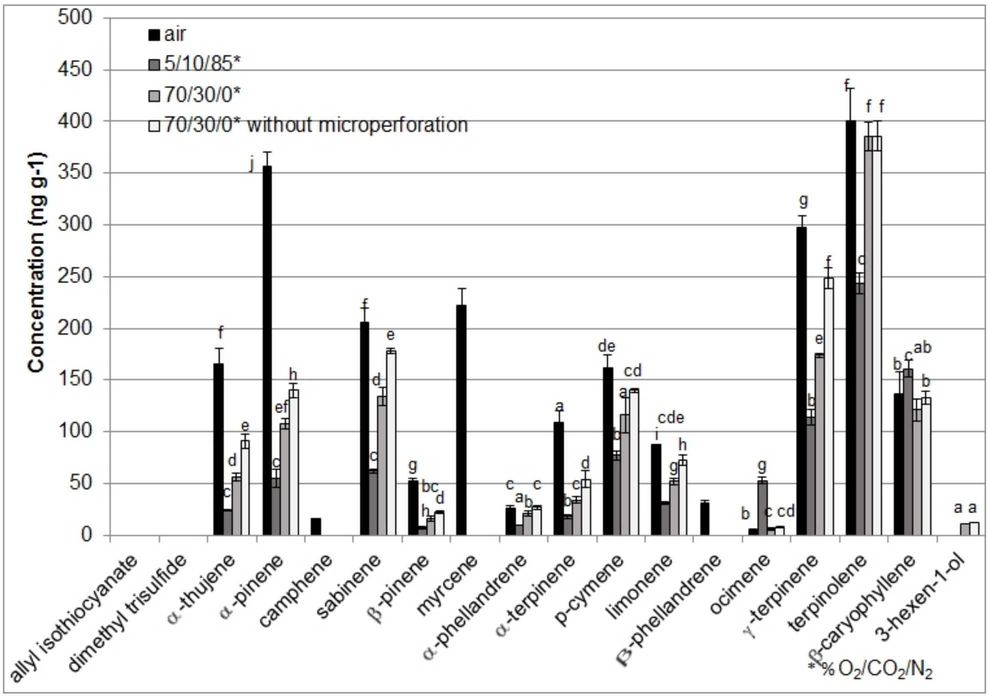

Figure 1. Volatile compounds in coleslaw mix after 1 day storage (means (n=3) and standard deviations, different lowercase letters are significantly different (*P*=0.05).

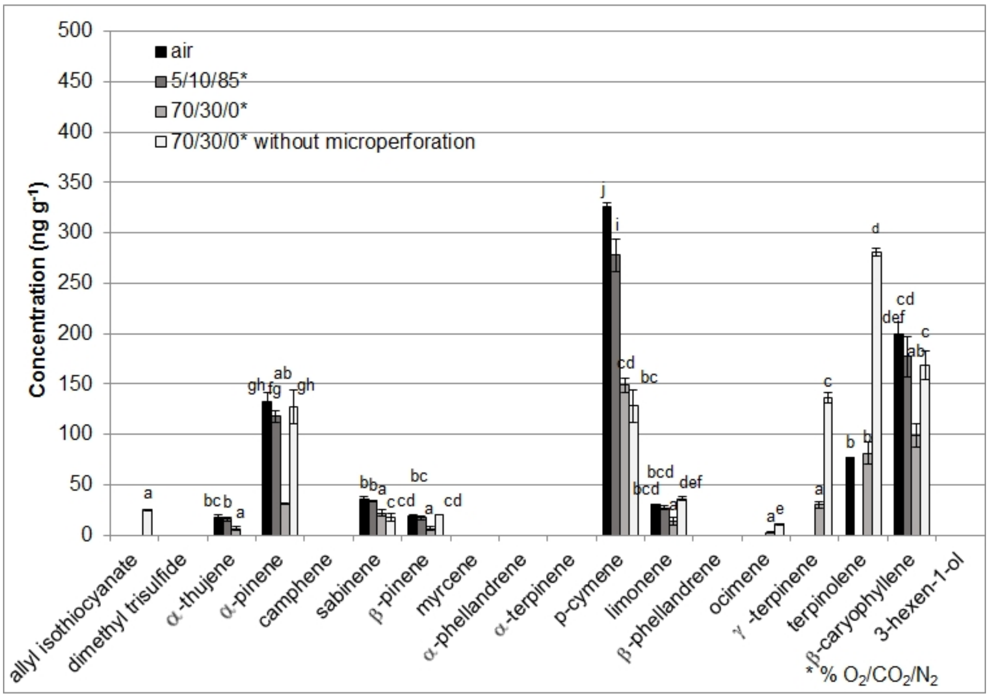

Figure 2. Volatile compounds in coleslaw mix after 6 days storage (means (n=3) and standard deviations, different lowercase letters are significantly different (*P*=0.05).

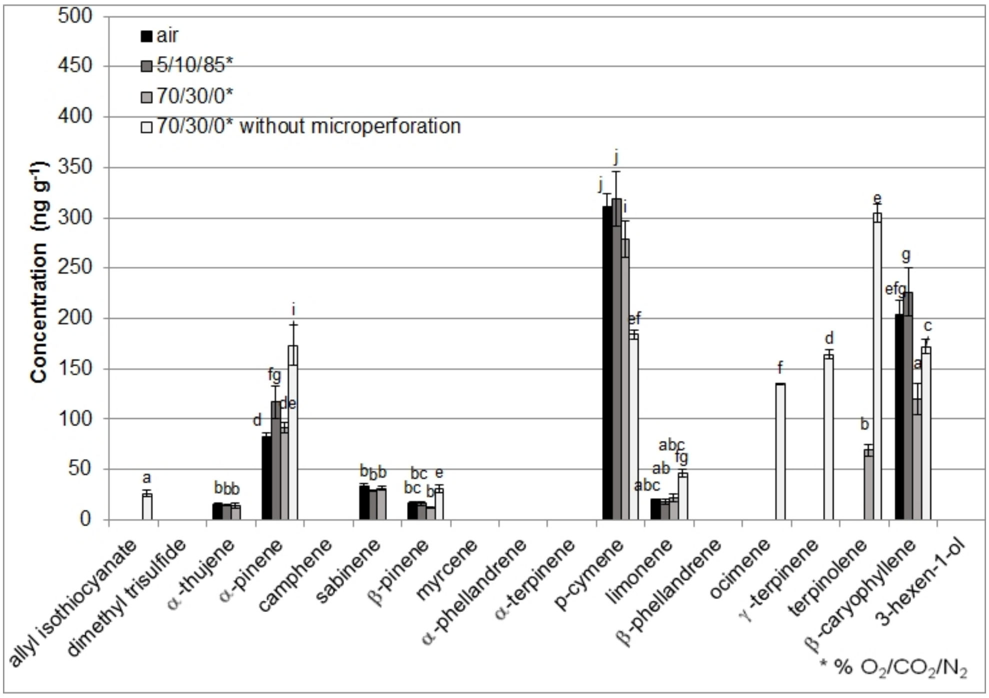

Figure 3. Volatile compounds in coleslaw mix after 9 days storage (means (n=3) and standard deviations, different lowercase letters are significantly different (*P*=0.05).

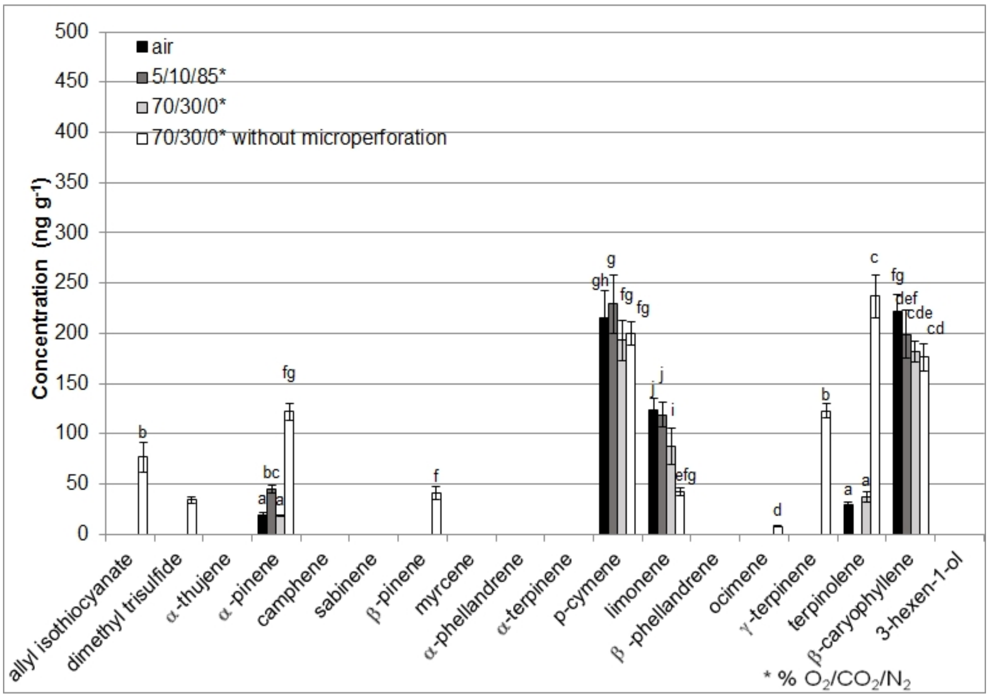

Figure 4. Volatile compounds in coleslaw mix after 12 days storage (means (n=3) and standard deviations, different lowercase letters are significantly different (*P*=0.05).

Terpinolene remained present in salads packaged in air and in the modified atmosphere of 70/30/0% $O_2/CO_2/N_2$ up to day 6 of storage. After 9 and 12 days, it was detected only in samples packaged in superatmospheric oxygen atmosphere, while its content was significantly (P=0.05) greater in salad sealed with film and with no microperforation. Ocimene and γ-terpinene after 6 days were still found in samples packaged in the atmosphere of 70/30/0% $O_2/CO_2/N_2$, while after 9 and 12 days, they were detected only in samples sealed with film without microperforation. Content of ocimene in that sample after 12-day storage was identical to that after 1 day of storage, whereas in the case of γ-terpinene and terpinolene, it was significantly (P=0.05) lower. Throughout the entire storage period α-pinene, p-cymene, limonene and β-caryophyllene were recorded in all the tested samples. Their contents were also dominant (Figs. 1, 2, 3, 4). In carrot, KJELDSEN *et al.* (2003) among the main mono- and sesquiterpenes also indicated the presence of α-pinene, limonene, p-cymene and β-caryophyllene, as well as sabinene, β-myrcene, γ-terpinene and α-humulene, (E)- and (Z)-γ-bisabolene. During storage in the tested samples, a significant (P=0.05) decrease in the level of α-pinene was observed, except for the sample packaged in the atmosphere of 5/10/85% $O_2/CO_2/N_2$. After 12 days of storage, the highest content of α-pinene was recorded in the sample packaged using film with no microperforation. In the case of limonene, its contents were found to initially decrease, followed by an increase. After 12 days of storage, the content of limonene in samples packaged with microperforated film was significantly (P=0.05) greater than after 1 day, while in the sample sealed using film with no microperforation it was lower. Limonene is a possible oxidation product of chlorophyll in leafy green vegetables, thus its greater concentration in samples packaged in microperforated film may be connected with the depletion of chlorophyll in cabbage leaves (LONCHAMP *et al.*, 2009). In the case of p-cymene and β-caryophyllene, their contents were found to increase during storage. After 12 days the content of these compounds in tested samples did not vary significantly (P=0.05) (Fig. 1, 2, 3, 4). An increase in contents of mono- and sequiterpenes during cold storage of carrot was also observed by KJELDSEN *et al.* (2003). The terpenoids may be synthesized in response to physiological stress. β-caryophyllene may be produced by the mevalonate pathway (LONCHAMP *et al.*, 2009) and monoterpenes by the methylerythritol phosphate pathway (BOUVIER *et al.*, 2005).

3.3. Sensory analysis

Table 2 illustrated the sensory attributes for stored coleslaw determined by quantitative descriptive analysis.

After 1 day of storage in all the examined samples, aroma attributes defined as carrot and cabbage predominated. After 6 days of storage, a significant (P=0.05) deterioration of aroma was observed in samples packaged in film with no microperforation. It was related with an increased intensity of perceived aroma attributes defined as sour and off-odor and a decrease in the intensity of the cabbage aroma. In all the tested samples, an aroma defined as terpene appeared and the green aroma disappeared. After 9 days, a further significant (P=0.05) deterioration of aroma was observed in the sample sealed with film without microperforation, which could have been caused mainly by the appearance of the sharp aroma and a greater intensity of terpene aroma. The intensity of cabbage aroma attributes significantly (P=0.05) decreased in salads packaged in modified atmosphere using microperforated film. After 12 days of storage, a further significant (P=0.05) increase in the intensity of sharp aroma was recorded in coleslaw mixes sealed with film with no microperforation. In those samples in comparison with other samples a greater intensity of sour, terpene and off-odor aroma was recorded at a significantly (P=0.05) lesser intensity of cabbage aroma. In salads packaged in microperforated film, the aroma defined as earth and off-odor was reported. In the case of samples packaged in modified atmosphere a significantly (P=0.05) greater intensity of aroma defined as sour was also found.

Table 2. Aroma profile of coleslaw mix stored for 12 days at 4°C.

Aroma descriptive attributes	Storage time (days)	air with film microperforation		5/10/85 % O_2/ CO_2/ N_2 with film microperforation		70/30/0 % O_2/ CO_2/ N_2 with film microperforation		70/30/0 % O_2/ CO_2/ N_2 without microperforation	
sharp	1	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	6	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	9	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	5.3±0.2	b
	12	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	8.0±0.4	c
carrot	1	5.7±0.3	ab	5.2±0.2	a	6.5±0.4	cd	5.9±0.3	b
	6	8.1±0.4	e	7.2±0.4	d	6.3±0.4	bc	5.9±0.2	b
	9	8.3±0.5	e	8.0±0.4	e	6.9±0.4	cd	6.7±0.2	cd
	12	7.3±0.5	d	7.1±0.3	d	6.3±0.4	bc	7.0±0.2	d
sour	1	3.3±0.2	de	3.0±0.3	cd	2.8±0.2	c	4.2±0.3	e
	6	2.2±0.2	b	0.0±0.0	a	2.3±0.2	b	5.8±0.2	g
	9	0.0±0.0	a	3.2±0.4	de	3.4±0.2	d	5.9±0.2	g
	12	0.0±0.0	a	3.9±0.4	e	2.8±0.1	c	5.3±0.2	f
green	1	2.2±0.2	b	2.4±0.3	c	2.5±0.3	c	0.0±0.0	a
	6	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	9	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	12	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
earth	1	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	6	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	9	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	12	1.3±0.1	c	1.0±0.1	b	1.1±0.2	c	0.0±0.0	a
terpene	1	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	6	1.3±0.2	c	1.0±0.1	bc	1.1±0.3	bc	1.2±0.2	bc
	9	1.3±0.2	c	1.0±0.2	bc	1.1±0.3	bc	2.7±0.1	d
	12	0.9±0.2	b	1.3±0.3	c	1.8±0.3	d	4.2±0.1	d
off-odour	1	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a
	6	0.0±0.0	a	0.0±0.0	a	0.0±0.0	a	4.3±0.2	d
	9	0.0±0.0	a	0.0±0.0	a	1.2±0.2	b	4.2±0.3	d
	12	2.2±0.2	c	1.2±0.2	b	1.0±0.2	b	4.3±0.3	d
cabbage	1	6.2±0.4	e	6.3±0.5	e	5.9±0.3	de	8.1±0.5	f
	6	5.3±0.3	c	4.9±0.4	c	5.4±0.3	cd	2.9±0.3	b
	9	2.8±0.3	b	2.9±0.2	b	2.8±0.3	b	3.2±0.3	b
	12	3.2±0.2	b	3.0±0.2	b	3.1±0.3	b	1.8±0.3	a

Mean ± standard deviation (n=3) for parameter with different letters are significantly different at P = 0.05.

In the tested samples, a strong correlation at $P \leq 0.05$ was observed between the following aroma attributes and volatile compounds: sharp - allyl isothiocyanate (R^2=0.80), sharp - dimethyl trisulfide (R^2=0.83) and carrot – *p*-cymene (R^2=0.71). Applying the Partial Least Squares techniques, we determined the contribution of volatile compounds to each attribute. In these models, the aroma attributes were *Y* variables, while the concentration measured by GC–MS were *X* variables. For each aroma attribute, a proper PLS regression model was established and interpreted using R^2 coefficient. The importance of each volatile for the sensory attributes was determined by variable importance for the projection (VIP) values (Table 3). The R^2 value were satisfactory for sharp, carror, terpene, off-odour and cabbage. Allyl isothiocyanate and dimethyl trisulfide was the most important contributor to sharp and p-cymene for carrot. This confirms dependencies determined by Pearson's correlation coefficients. Additionally, the dependency between terpene, off-odour, cabbage attributes and volatile compounds was determined (Table 3). Allyl isothiocyanate, dimethyl trisulfide, sabinene and α-phellandrene were the most important contributor to terpene and ocimene for off-odour. Sabinene and α-phellandrene, 3-hexen-1-ol, α-terpinene, allyl isothiocyanate and γ-terpinen were the most important contributor to cabbage. Allyl isothiocyanate is also determined as pungent in Flavor net. KJELDSEN *et al.* (2003) divided terpene compounds present in carrot into carrot (*α*-pinene, *β*-pinene, sabinene, *α*-phellandrene, myrcene, *p*-cymene), sweet, citrus, fruity (limonene, *γ*-terpinene, terpinolene) and terpene-like, spicy, woody aroma compounds (*β*-caryophyllene). In the tested product only a dependence between *p*-cymene and carrot aroma was observed. Such a correlation may also be indicated by the very low levels of the odor threshold (13 ppb) for *p*-cymene in comparison with *α*-pinene (1000 ppb), *β*-pinene (140 ppb) and sabinene (75 ppb) (KJELDSEN *et al.*, 2003). Moreover, the concentration of *p*-cymene in the tested samples was significantly (P=0.05) greater than in the case of other above-mentioned monoterpenes, thus indicating their greater odor activity value. In the case of terpene aroma, sabinene, *α*-phellandrene are determined as turpentine in Flavor net. Sabinene and *γ*-terpinene were also detected as a result of break in continuity of the cabbage tissue as reported by VUORINEN *et al.* (2004)

Table 3. R^2 for PLS regression model and VIPs affected the aroma attributes.

Attributes	R^2	Volatiles	VIP
sharp	0.91	allyl isothiocyanate	2.457
		dimethyl trisulfide	2.240
carrot	0.81	p-cymene	2.133
sour	0.54	-	-
green	0.47	-	-
earth	0.45	-	-
terpene	0.84	allyl isothiocyanate	1.870
		dimethyl trisulfide	1.643
		sabinene	1.352
		α-phellandrene	1.237
off-odour	0.77	allyl isothiocyanate	2.207
		dimethyl trisulfide	1.396
		sabinene	1.356
		ocimene	1.289
		α-phellandrene	1.093
cabbage	0.77	sabinene	1.480
		α-phellandrene	1.462
		3-hexen-1-ol	1.316
		α-terpinene	1.190
		allyl isothiocyanate	1.166
		γ-terpinene	1.038

3.4. PCA

Based on the correlation matrix, 2 principal components were identified, explaining 63% total variability for which correlations with input variables are presented in Fig. 5.

The PC1 was positively correlated with contents of α-thujene, α-pinene, camphene, sabinene, myrcene, 3-hexen-1-ol, α-phellandrene, α-terpinene, limonene, β-phellandrene, γ-terpinene, terpinolene as well as aroma attributes, that is green and cabbage, while it was negatively correlated with β-caryophyllene, p-cymene, carrot and earth aroma. The PC2 was negatively correlated with contents of oxygen and carbon dioxide, allyl isothiocyanate, dimethyl trisulfide, β-pinene, ocimene and aroma attributes, that is sharp, sour, terpene and off-odour (Fig. 5).

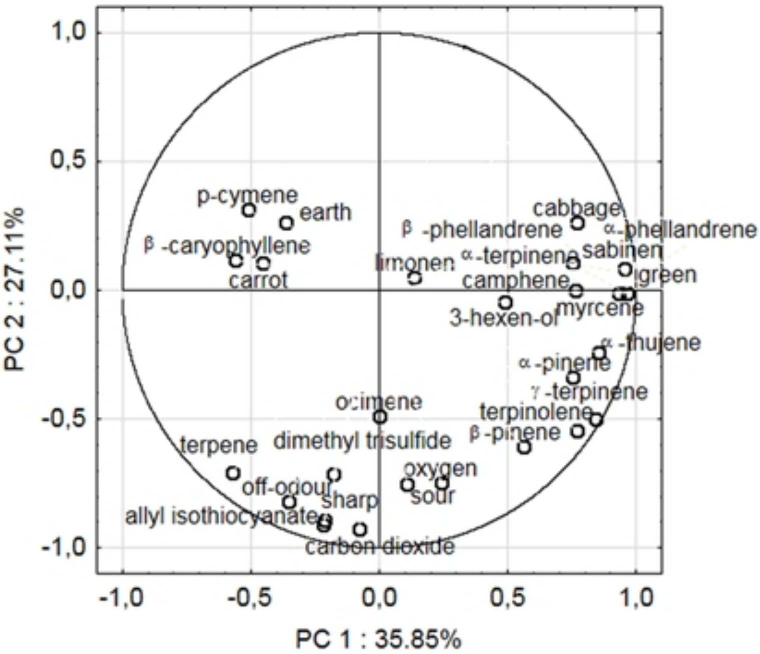

Figure 5. PCA of volatile compounds, aroma attributes and content O_2 and CO_2.

Figure 6 presents values of PC1 and PC2 for sample maps. Among the tested samples, the lowest PC2 values were found for salads packaged in film with no microperforation after 6, 9 and 12 days of storage. This was connected with the greatest contents of oxygen, carbon dioxide, allyl isothiocyanate, dimethyl trisulfide, ocimene and β-pinene inside the packaging and aroma defined as sharp, sour, terpene and off-odor. After 12-days of storage the other samples had negative PC1 values, which indicates high contents of β-caryophyllene and p-cymene and aroma defined as carrot and to a limited extent earth aroma.

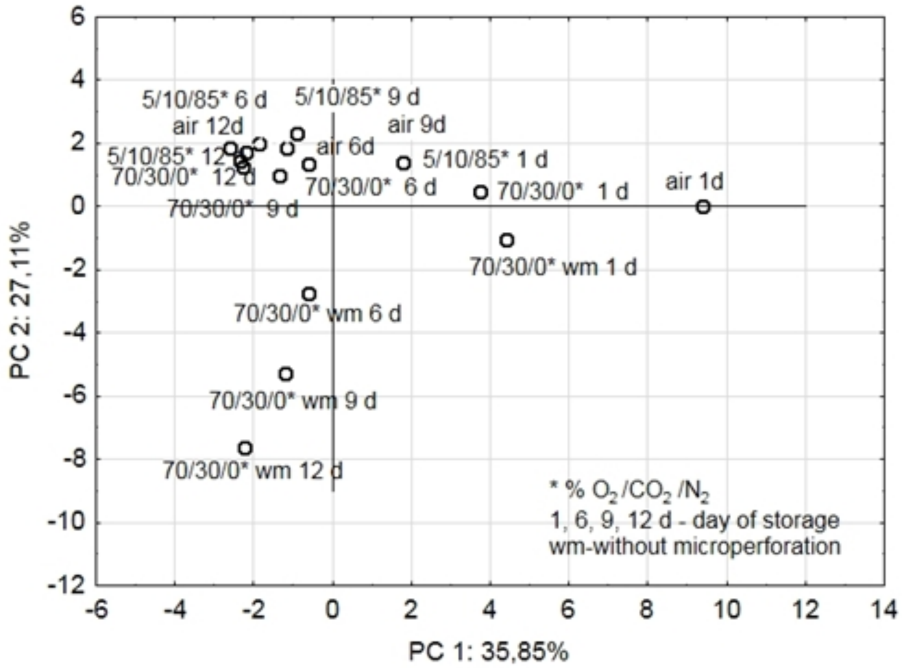

Figure 6. Sample map.

4. CONCLUSIONS

The application of microperforated film in packaging of coleslaw mix makes it possible to maintain the desirable product aroma for 12 days of storage at 4 °C. It also guarantees maintenance of aerobic conditions and does not contribute to the accumulation of CO_2 inside the packaging. Although it may be assumed that in those samples we observed the degradation of chlorophyll found in comminuted cabbage leaves, it may indicate a significantly (P=0.05) greater concentration of limonene than in samples packaged in film with no microperforation. The broadest profile of aroma compounds during storage was found in samples sealed in the atmosphere of 70/30/0% $O_2/CO_2/N_2$. In the case of packaging the coleslaw mix in the superatmospheric oxygen atmosphere using film with no microperforation, the significant (P=0.05) deterioration of aroma in the sensory analysis was the effect of allyl isothiocyanate and dimethyl trisulfide accumulation inside the packaging. Partial least squares regression analysis determined the quantitative contributions of volatile compounds to sharp, carrot, terpene, off-odour and cabbage in coleslaw mix aroma.

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PAPER

THE ECO-EFFICIENCY OF THE DAIRY CHEESE CHAIN: AN ITALIAN CASE STUDY

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ABSTRACT

The eco-efficiency of mozzarella cheese production was investigated in two dairy chains that differ in liquid whey recycling, with whey recycling (B) and without whey recycling (A), in cow diets. The total eco-efficiency (total GVA/total GWP) for 1 kg of mozzarella cheese ranged from € 0.19 (B) to € 0.16 per kg CO_2-eq (A). The cheese-making phase of each diet accounted for about 3% of GWP total emissions. The mozzarella cheese making phase had the highest eco-efficiency ratio, while the milk production phase showed the lowest economic value and the highest impact. Findings suggest improvements in reducing the environmental burden of the primary phase while increasing its economic value.

Keywords: carbon footprint, cheese whey recycling, eco-efficiency ratio; economic value added, mozzarella cheese production

1. INTRODUCTION

Food supply chains are increasingly associated with environmental impacts, and this has brought global attention to the sustainability of the agri-food systems (FANTOZZI *et al*., 2015).

Dairy products have a great impact, especially in terms of resource depletion and greenhouse gas emissions (GONZÁLEZ-GARCÍA *et al*., 2013). Furthermore, the dairy industry is considered responsible for a significant impact due to the characteristics of its wastewaters and effluents (MIRABELLA *et al*., 2014). Solid waste treatment and wastewater treatment along the dairy chain affect several environmental indicators. Cheese whey is the main pollutant generated from cheese production that can cause several environmental impacts (PRAZERES *et al*., 2012). Thus, cheese whey cannot be discharged directly into the environment without appropriate treatment. According to some authors (SUCCI *et al*., 1986), apart from potential environmental benefits, liquid whey is also an interesting animal diet ingredient from an economic point of view, especially when distances from the cheese industry are short and costs of handling and transportation are high.

In the framework of a circular economy approach, the reuse of whey in dairy cows' diet may minimize resources use and waste production from cheese making. In this regard, the European Commission has recently adopted an action plan on the circular economy - where the value of products, materials, and resources is maintained in the economy as long as possible, and the generation of waste is minimized- to develop a sustainable and competitive economy with low carbon content and efficient resource use.

Assessing the environmental performance of dairy chains can reduce their impacts and improve the efficiency of resource use (MU *et al*., 2017).

Life cycle assessment (LCA) is a methodology widely used to investigate the environmental impact of food production. SALA *et al*. (2017) underlined the importance of the environmental and socio-economic impacts associated with the food supply chains and indicated life cycle thinking and assessment as key elements in identifying more sustainable solutions for global food challenges. Furthermore, NOTARNICOLA *et al*. (2015) deepened the issue of LCA in the agri-food sector with case studies, methodological issues and best practices.

Existing literature reports several studies that addressed different topics related to the LCA of cheese production. KIM *et al*. (2013) conducted a US-based LCA to determine the environmental impacts of cheddar, mozzarella cheese and dry whey from cradle-to-grave. GONZÁLEZ-GARCÍA *et al*. (2013) studied the life cycle of mature cheese production in Portugal from a cradle-to-gate perspective and identified the environmental hotspots. PALMIERI *et al*. (2017) applied an LCA approach to assess the impacts of mozzarella cheese production and evaluate the contribution of different strategies in a traditional dairy chain.

Global warming potential is one of the most studied impacts of dairy products. ROTZ (2018) reviewed the models for evaluating GHG emission from dairy farms —along a continuum from relatively simple models for single GHG emission sources to very detailed simulations over the whole farm production system— and concluded that LCA is a comprehensive method for quantifying and evaluating the different sources of emissions over the full cycle. COLOMBINI *et al*. (2015) applied an LCA cradle-to-farm-gate to assess the global warming potential of milk production in three forage systems scenarios and lactating cow diets. HAWKINS *et al*. (2015) estimated how the formulation of the ration and the associated land allocation decisions, contribute to reductions in GHG emissions of the intensive dairy production systems in Ontario. VAN MIDDELAAR *et al*. (2013) studied the environmental effect of replacing grass silage with maize silage in a feeding strategy and applied a life cycle assessment to predict GHG emissions at chain level. Finally, FINNEGAN *et al*. (2015) measured the global warming potential associated with the processing of raw milk into 11 dairy products in the Republic of Ireland following a cradle-to-processing factory gate boundary.

A general result from literature suggested that raw milk production is the most impactful phase along the chain due to feed production and animal emissions.

Few studies dealt specifically with the environmental impact of mozzarella cheese production. Two studies investigated the impact of American and Canadian mozzarella cheese production (KIM *et al*., 2013; VERGÉ *et al*., 2013) by considering several impact categories. Concerning the Italian mozzarella product, a study (DALLA RIVA *et al*., 2017) investigated a cradle-to-processing-gate LCA of two types of mozzarella (the traditional one produced from raw milk, and the mozzarella obtained from curd) focusing mainly on transformation and consumption of mozzarella cheese, also dealing with different environmental impacts. A study by PALMIERI *et al*. (2017) focused on several impact categories of both farm and factory phases based on some study cases of the mozzarella production in Italy. HELMES *et al*. (2016) assessed the carbon footprint of an Italian mozzarella facility dealing with the sensitivity of LCA results according to different allocation choices. Finally, FALCONE *et al*. (2017) applied the LCA approach to assess the environmental effect of a shelf life extension technique in the lacto fermented Italian mozzarella cheese production.

Under a wider sustainable perspective, the assessment of a dairy product should be extended beyond environmental impacts by considering its profitability and economic performance. Recent studies started focused on the economic and environmental assessment of dairy products by using different approaches and focusing on minimising costs and/or on maximising profits.

SOTERIADES *et al*. (2016) proposed to combine the LCA approach with the Data Envelopment Analysis (DEA) method in order to holistically assess dairy farm eco-efficiency by maximising output per unit of environmental impacts.

KIRILOVA and VAKLIEVA-BANCHEVA (2017) designed an optimal "green" portfolio for curd production in Bulgaria to demonstrate the role of the environmental impacts - measured in terms of wastewater and CO_2 emissions- within a profit maximization function that includes the costs of the above impacts. MURPHY *et al*. (2017) compared male dairy calf-to-beef production systems based on different animal performance and applied economic profitability and GHG emissions models to highlight the best performing system per each perspective. HAWKINS *et al*. (2015) used an optimization model of ration formulation to determine how specific GHG targets can be reached while maximising net returns to an intensive dairy farming system.

WETTEMANN and LATACZ-LOHMANN (2017) estimated the potential costs and GHG emissions savings for a sample of 216 dairy farms in northern Germany using an input-oriented Data Envelopment Analysis and showed that cost and GHG emission reductions are complementary across a wide range. An economic approach focused on costs is also followed by HUYSVELD *et al*. (2017) that analysed a sample of 103 specialized dairy farms in Flanders (Belgium) and showed potential simultaneous savings in costs and overall natural resource demand (up to 48%). FALCONE *et al*. (2017) applied a Life Cycle Assessment and Life Cycle Costing methods in order to assess the environmental and economic impacts of innovations in the Lacto-fermented mozzarella cheese production in Calabria region. Finally, HESSLE *et al*. (2017) studied different production scenarios of the dairy chain in Sweden by performing a Life Cycle method to assess the best environmental performance and by quantifying the costs in the primary production of dairy and beef to find out the most cost-efficient production models.

Another approach that integrates economic and environmental assessment is based on the eco-efficiency ratio (SALING, 2016). Eco-efficiency is defined as economic efficiency combined with environmental benefits and deals with three main goals: the reduction of resource consumption, the reduction of environmental impacts, and the increase of product value. The concept of eco-efficiency has been applied to several agricultural products to estimate the value added per kg of GHG emitted into the atmosphere for each system studied. In the dairy sector, BASSET-MENS *et al*. (2009) applied an eco-efficiency analysis of milk production in Flanders. MEUL *et al*. (2007) studied the eco-efficiency of milk production in some Flemish dairy farms, but the authors intended *eco*-efficiency in terms of *ecologic* and not economic terms and measured an indicator based on nitrogen and energy use efficiency.
To the best of our knowledge, few studies considered the eco-efficiency of the dairy chain. A study measured the economic performance of the cheese production chain by calculating the gross value added (GVA) of stages along the chain (VAN MIDDELAAR *et al*., 2011). Another study (SANJUAN *et al*.. 2011) measured the economic added value and the net income of Mahon-Menorca cheese production under different scenarios regarding technical and cleaner production criteria. However, that study included the assessment of the cheese production phase and excluded the milk production phase. A different approach to eco-efficiency was applied in a study that related the environmental performances with the economic efficiency in the use of dairy farms inputs (IRIBARREN *et al*., 2011).
This study aims to contribute to the literature on the environmental and economic performances of the mozzarella cheese production by measuring its eco-efficiency ratio based on an Italian case study. The study answers the question, "how much value is added per kg of GHG emitted to the atmosphere?". Firstly, the environmental and economic assessments were implemented; subsequently, the two perspectives were combined within an eco-efficiency analysis. In an earlier study (PALMIERI *et al*., 2017) an environmental analysis was performed according to a global approach. The present study goes further by focusing on the carbon footprint assessment and adding the analysis of the economic performance of mozzarella cheese production.

2. MATERIALS AND METHODS

2.1. The environmental assessment

2.1.1 Goal and scope definition

The main purpose of the study was to calculate the eco-efficiency ratio of mozzarella cheese production based on raw milk produced following different feeding strategies. The environmental impact of the dairy cheese chain was based on GHG emissions, and the economic performances considered the GVA of the dairy cheese chain. The value added per GHG emission of one kg of mozzarella cheese produced was finally measured.
The carbon footprint (CF), an important index of the climate change impacts of food production within the whole supply chain (ROMA *et al*., 2015), was measured by an Attributional Life Cycle Assessment methodology (BAITZ, 2017; ISO 14040, 2006; ISO 14044, 2006). The CF of 1 kg of mozzarella cheese is defined as the sum of all GHGs emitted along the production cycle (RÖÖS *et al*., 2014). GWP is expressed in CO_2 equivalent (CO_2-eq) using weights of 1,28 and 265 for carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), respectively (assuming 100 years lifespan; IPCC, 2015).

Furthermore, an economic analysis considered the added economic value of the dairy cheese chain as the difference between total revenues and total costs for intermediate consumption (VAN MIDDELAAR *et al*., 2011). Intermediate consumption costs measure the value of goods and services consumed, including raw materials, services, and other operating expenses, other than fixed assets. The GVA does not include labor costs, depreciation, nor interest loan payment; when considering the depreciation of fixed capital, a net value added is obtained. The GVA indicator was chosen because it is frequently used to measure the economic sustainability of agricultural systems (VAN MIDDELAAR *et al*., 2011). The final goal of jointly assessing the environmental and economic performances in the case study was pursued by measuring the eco-efficiency ratio (GVA/GWP) of mozzarella cheese production based on milk produced following different feeding strategies.

2.1.2 Functional unit and system boundary

The functional unit (FU) of the environmental and economic analysis was expressed per 1 kg of mozzarella cheese produced from 8.11 L of cow milk. The LCA system boundary (Fig. 1) refers to the first two phases of a dairy chain, namely the dairy farming and the cheese-making phases.

The boundary considers: the dairy farm - including the agricultural processes of feedstuffs and the whole life cycle of cows -; and the cheese factory -including all the activities that take place for the mozzarella cheese making, from the milk reception to the mozzarella production and the whole liquid whey disposal (the wastewater treatment plant or recycled into the cow diets).

Two dairy diets that differ in the usage/non-usage of liquid whey were assessed. In relation to the different disposal of the liquid whey, along with the two diets (A and B diets), two different chains are considered. In A chain, the whole amount of liquid cheese whey is mixed with the wastewater effluent from the mill and delivered to a municipal wastewater treatment plant (Fig. 1). In the B chain, the whole amount of liquid whey produced at cheese-making level is delivered to the farm where it is used, after microbial stabilization, in animal feeding as partial substitute of drinking water.

The physical allocation method was used in the baseline scenario to share the environmental burden between milk and meat at the farm level, while the environmental burden of the mozzarella production was totally allocated to curd (GONZÁLEZ-GARCÍA *et al*., 2013). The percentages of physical allocation at case farm level were 88% to milk and 12% to meat (as live weight cow and calf) (IDF, 2015). The manure/slurry allocation was not necessary because farmyard manure was recycled as fertilizer in the feed cultivation.

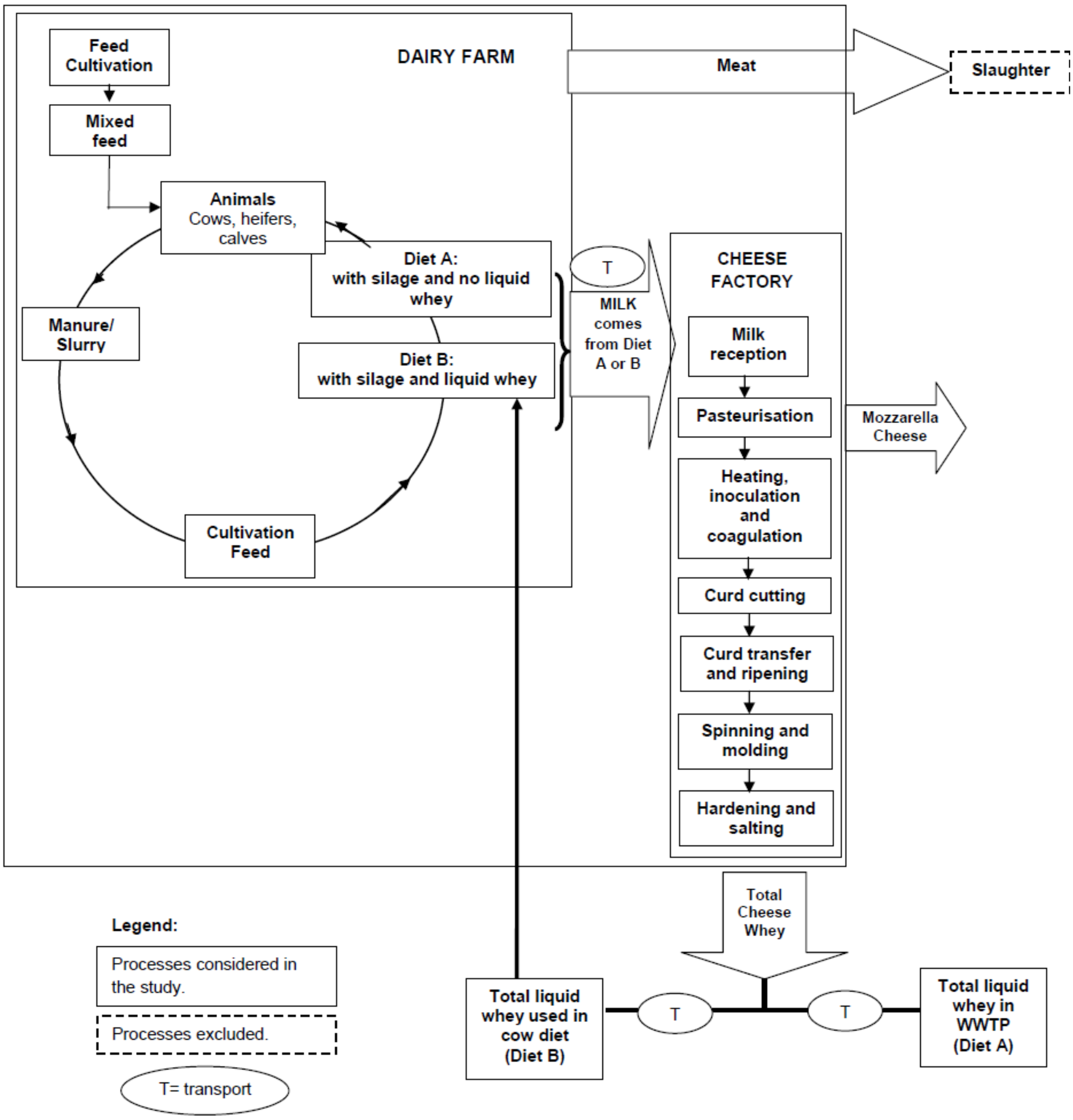

Figure 1. System boundaries: dairy farm and cheese factory. Abbreviations: See Table 1.

Table 1. List of Abbreviations and Acronyms.

ALCA	Attributional Life Cycle Assessment
CF	Carbon Footprint
CO_2	Carbon dioxide
CU	Cereal Unit allocation method
CH_4	Methane
FPCM	Fat and Protein Corrected Milk
FU	Functional Unit
GVA	Gross Value Added
GHG	Greenhouse Gas Emissions
GWP	Global Warming Potential
IPCC	Intergovernmental Panel on Climate
LCA	Life Cycle Assessment
N_2O	Nitrous Oxide
WWTP	Wastewater treatment plant

2.1.3 Life cycle inventory

Data for the life cycle inventory analysis partly comes from the INLATTE Project (Tables 2-4) and were collected through a questionnaire drawn according to the guidelines for the application of LCA to food and agricultural products (NERI, 2009). Secondary data (Table 5) were taken from both the ECOINVENT database v. 3.0 (WEIDEMA *et al*., 2013) and literature (FRANCHINI and NERI, 2004; NERI and BORSARI, 2005; KIM *et al*., 2013).
Primary data were collected from two firms (a dairy farm and a cheese factory) located in Molise region (IT). Data from the case farm reported the milk quantity and quality, the Italian Friesian cow rations and water consumption, and the manure/slurry produced. The case farm experimented two different dietary strategies: a diet including ensiled forages and no liquid whey usage (A diet) and a diet including both silages and liquid whey (B diet). Data reported in Table 2 summarise the management of animals in the case farm. For the present study, 36 lactating cows were divided into two groups of 18 cows each which were homogeneous and comparable in terms of milk yield and days of lactation and parity. The average fat and protein corrected milk (FPCM) yield has been calculated on a 305 days basis for each experimental group and used in the LCA study. The FPCM yield was calculated according to FINNEGAN *et al*. (2015). Table 3 shows the composition of the diets. In this regard, it is worth noting that feedstuffs were offered as total mixed rations, except for the microbiologically stabilized liquid cheese whey offered to B diet cows as partial substitute of drinking water. Water consumption in B diet was, therefore, lower than that in the A diet.
Primary data from the cheese factory have been recorded throughout the experiment and summarised in Table 4. Mozzarella cheese for fresh consumption traditionally obtained directly and solely from liquid milk is the dairy product considered in the study.

Table 2. Case farm characteristics.

Case farm data		
Cow breed		Holstein Friesian
Number of lactating cows		36
Number of dry cows		9
Dairy replacement calves and heifers, n.		32
Number of calves (male)		18
Days of production/year (lactating cows)		305
Males raised as beef cattle, age (days)		Calves: 20
Milk production	**Diets**	
Milk yield – FPCM (kg/per yr)	A	8,332
	B	8,039
% Fat	A	4.03
	B	3.99
% True Protein	A	3.68
	B	3.60

Table 3. Water consumption and characteristics of diets on a dry matter (DM) basis.

	Diets	
Calves diet	A	B
Water consumption (L/day)	10	10
Liquid whey (kg/day)	-	-
Total DM intake (kg/ day)	1.96	1.96
Heifers diet	A	B
Water consumption (L/day)	35	25
Liquid whey (kg/day)	-	0.57
Total DM intake (kg/ day)	4.53	5.10
Lactating cow diet	A	B
Water consumption (L/day)	80	50
Liquid whey (kg/day)	-	1.48
Total DM intake (kg/ day)	20.06	21.54
Dry cow diet	A	B
Water consumption (L/day)	40	40
Liquid whey (kg/day)	-	-
Total DM intake (kg/ day)	13.08	13.08

When real data were not available, inventory data were collected from literature and ECOINVENT database (v. 3.0) (WEIDEMA *et al*., 2013), as reported in Table 5. Emissions considered in the study were drawn from literature (Table 6). Data for the raw milk and whey transportation and for the wastewater treatment plant for whey disposal came from ECOINVENT database.

Table 4. Cheese factory data.

Products data	
kg of mozzarella produced by 8.11 L of milk	1
kg of whey produced by 1 kg of mozzarella	0,89
Fat in mozzarella (g/kg of product)	185
Protein in mozzarella (g/kg of product)	154
Fat in whey (g/kg of product)	2
Protein in whey (g/kg of product)	7
Resources consumption	
Electricity consumption (kWh/ kg of mozzarella)	0,20
Heat consumption (MJ/kg of mozzarella)	0,11
Water consumption (L/kg of mozzarella)	18,08

Data source: INLATTE Project.

Table 5. Secondary data considered in the study.

	Source
Feed cultivation and processing	
Barley	
Maize	ECOINVENT DATABASE (v. 3.0)
Meadow hay	
Milk powdered	FRANCHINI and NERI (2004); ECOINVENT DATABASE (v. 3.0)
Mixed feed	
Mineral feed	
Sugar beet pulp	ECOINVENT DATABASE (v. 3.0)
Soybean meal 44%	
Triticale silage	
Mozzarella production	
Milk reception	ECOINVENT DATABASE (v. 3.0);
Pasteurisation	FRANCHINI and NERI (2004); ECOINVENT DATABASE (v. 3.0)
Heating, inoculation and coagulation	
Curd cutting	
Curd transfer and ripening	ECOINVENT DATABASE (v. 3.0)
Spinning and molding	
Hardening and salting	
Raw milk transportation	ECOINVENT DATABASE (v. 3.0) for diesel track of 16 t capacity. Real distance from the dairy farm to the factory 10 km
Wastewater treatment	ECOINVENT DATABASE (v. 3.0); moderately large municipal wastewater treatment plant with a three-stage process (mechanical, biological and chemical)

Table 6. Emissions considered in the study.

Emissions	Source
Enteric and animal housing emissions	
CH_4 emissions and the ammonia emissions	BATTINI *et al.* (2016); EMEP/EEA (2009)
Nitrous oxide (N_2O) emissions from animal housing	Not considered according to BATTINI *et al.* (2016)
Storage emissions	
Emissions of methane (CH_4) and nitrous oxide (N_2O)	DALLA RIVA *et al.* (2014); IPCC (2006) (Tier 2); using ISPRA (2008) methods
Ammonia (NH_3) emissions due to manure/slurry storage	FALCONI *et al.* (2011) using ISPRA (2008) method
Nitrogen oxides (NOx) emissions	BATTINI *et al.* (2016) using the factor by IPCC (2006)
Emissions related to manure/slurry spreading	
N_2O, NH_3, NOx and nitrate leaching	BATTINI *et al.* (2016) using IPCC (2006)
The P leaching run-off emissions	BATTINI *et al.* (2016)
Emission factor of Potassium, Copper and Zinc	NERI and BORSARI (2005)

2.2. Economic assessment and eco-efficiency ratio of the dairy chain

The eco-efficiency indicator is based on data from both environmental and economic accounting systems. The higher the indicator value, the higher the economic performance per unit of environmental burden. Since ecological and economic data need to be derived

from the same data set (MULLER *et al*., 2015), we collected information based on the annual budget of the considered dairy farm and the cheese factory.
The economic data for both stages, milk production and mozzarella cheese making, are shown in Table 7. The B dairy chain had lower total costs than A chain due to both the elimination of treatment costs of whey in the WWTP and saved transportation costs of whey from the cheese factory to the dairy farm. The factory and the farm agreed to equally share the costs of both whey transportation (from the cheese factory to the dairy farm) and whey management at firm's level. Finally, the lower costs of B chain were due to the reduction of water consumption in the diet.
The eco-efficiency analysis was applied to the two stages of mozzarella cheese production (i.e., milk production and mozzarella cheese-making phases). The eco-efficiency of each stage was computed by dividing its economic value added by its ecological impact (VAN MIDDELAAR *et al*., 2011).

Table 7. Cheese factory and dairy farm economic data.

Economic data	Units	Cheese factory (€/kg of mozzarella)		Dairy Farm (€/8,11 L of milk)	
		A chain	B chain	A chain	B chain
Gross revenue	€/kg	6.10	6.10	4.00	4.00
Variable and fixed costs	€/kg	5.10	4.90	3.44	3.37
Economic value added	€/kg	1.00	1.20	0.56	0.63

Source: Data came from the dairy farm and cheese factory case studies.

2.3. Sensitivity analysis: allocation method and variability of GVA

The choice of the allocation procedure for agricultural co-products may affect the results of LCA study as discussed in FLYSJO *et al*. (2012) and HELMES *et al*. (2016). Both studies compared the dry matter and the economic allocation methods for assessing the impact of dairy industry and underlined the need for testing results against different approaches.
For this reason, a sensitivity analysis for environmental impacts was performed by changing the allocation method of milk according to a cereal unit (CU) method (BRANKATSCHK and FINKBEINER, 2014). This sensitivity analysis involved only the case farm level, as in many reported studies (FANTIN *et al*., 2012; GONZÁLEZ-GARCÍA *et al*., 2013; KIM *et al*., 2013; VAN MIDDELAAR *et al*., 2011), because milk production is more impactful than cheese-making. The CU allocation method is based on the metabolizable energy content of product and co-product for feed purpose so that it allows considering agricultural products and co-products used in different sectors. The environmental burden was allocated 86.6% to milk, 6.8% to live-weight dairy cow and 6.6% to live-weight fattening male calf (BRANKATSCHK and FINKBEINER, 2014).
Furthermore, if the economic dataset was based on the annual reports of the dairy farm and the cheese factory -and therefore are real and accurate-, a further sensitivity analysis was performed to estimate the effect a ±10% change of GVA of the two stages for each dairy chain.

3. RESULTS AND DISCUSSION

3.1. The carbon footprint of 1 kg of mozzarella: baseline allocation

Results of the environmental impact of 1 kg of mozzarella cheese showed that raw milk production was the most impactful phase along the considered supply chain, irrespective of the diet followed at the farm level (Fig. 2).

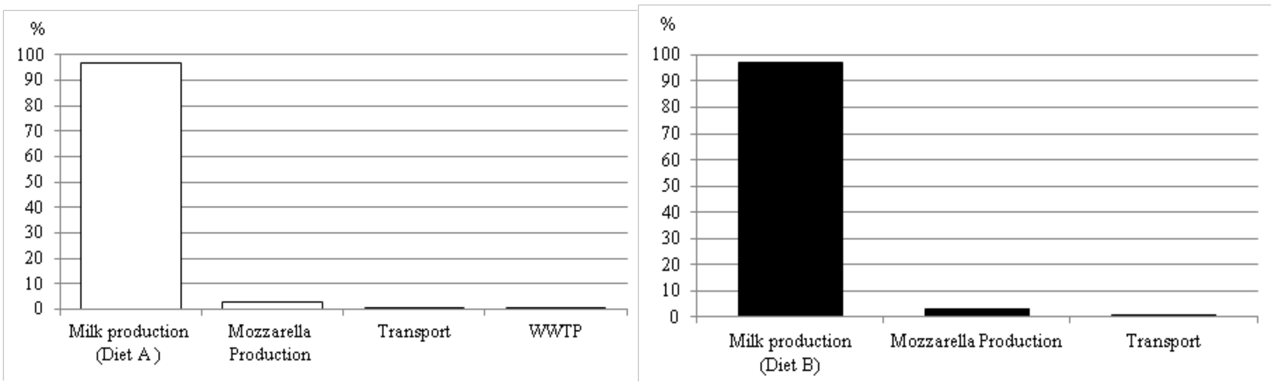

Figure 2. Carbon footprint of 1 kg of mozzarella cheese in A supply chain (on the left side) and B chain (on the right side): milk and mozzarella production (physical allocation).
Note: Transport refers both to the milk delivered to the dairy factory (supply chain A and B) and to the liquid whey delivered to the dairy factory (B supply chain) or the wastewater treatment plant (WWTP; A supply chain).

Milk production was the most critical phase along the dairy chain, with contributions of 96% (A diet) and 97% (B diet) of the global warming potential (GWP). The high contribution of milk production phase to the environmental impact of the mozzarella dairy chain observed is consistent with the study of DALLA RIVA *et al*. (2017), even considering the farm gate-to grave perspective followed by the authors. A similar conclusion was in the study of FINNEGAN *et al*. (2015) that, although was based on different cheese product and fluid milk, showed that milk production contributes to GWP within 81% - 97% range (depending on the amount of raw milk per kg of the six cheese products considered in the study). The remainder contribution being mainly due to the processing phase.

The environmental impacts of milk production phase were due to emissions of both methane from the enteric fermentation process and dinitrogen monoxide and carbon dioxide from manure management and spreading, confirming the study of GONZÁLEZ-GARCÍA *et al*. (2013) which referred to the cheese chain in Portugal. Methane from enteric fermentation and manure management was also the main GHG emission source in other studies dealing with cheese (KIM *et al*., 2013) and milk production (VIDA and TEDESCO, 2017). In the studies of VAN MIDDELAAR *et al*. (2011) and SANTOS *et al*. (2016), the enteric fermentation was the main emission source affecting GWP. According to VAN MIDDELAAR *et al*. (2011), the stage that contributed most to total global warming potential along the production chain of Dutch semi-hard cheese was on-farm milk production (65%), mainly due to enteric fermentation. In a study by SANTOS *et al*. (2016) about the cheese production in a small-sized dairy industry in Brazil, the contributions of the raw milk production ranged from 70 to 98% depending on the different midpoint impact categories.

The cheese-making phase of each diet accounted for about 3% of GWP total emissions. Mozzarella production phase showed impacts due to carbon dioxide from heat consumption during the cheese making process. This result confirms VAN MIDDELAAR *et al*. (2011) findings that measured the contribution of semi-hard cheese-making and packaging phases in about 3% - 4% of GWP emissions, each. Even in the study of HELMES *et al*. (2016), the contribution from the processing step of mozzarella production was quite limited compared to raw milk and transport impacts.
Furthermore, in our study, impacts of transportation of both milk —from dairy farm to cheese factory— and whey, either from factory to the wastewater plant or from factory to the dairy farm- were negligible due to the close distance between the locations of the two firms involved, the farm and the factory. A similar result was reported in the study of FINNEGAN *et al*. (2015) where liquid milk transportation contributed for less than 0.5%, whichever dairy products considered in the assessment. The relative burden of the wastewater treatment (in A diet) along the whole dairy chain was also considered insignificant.
Comparing impacts between the chains, results based on a cradle-to-processing-gate boundary showed that the B dairy chain had a CF 1% higher than the A chain per unit of product. The carbon footprint of mozzarella cheese in A chain was 9.65 kg CO_2-eq/kg mozzarella cheese, while it was 9.81 kg CO_2-eq /kg mozzarella cheese in B chain. The B dairy chain, although with the liquid whey usage, appeared to be a slightly worse solution due to a lower milk yield (8,039 kg FPCM) compared with A chain (8,332 kg), confirming that the environmental impact increases at decreasing milk yields (NEMECEK *et al*., 2011).
Study findings were similar to those reported in KIM *et al*. (2013) where the carbon footprint of US mozzarella cheese was 9.30 kg CO_2-eq/kg. Furthermore, the results of our study are consistent with the study of HELMES *et al*. (2016), even if these authors considered different scenarios (mozzarella with ricotta or mozzarella with whey powder) from that of the present study. According to SANTOS *et al*. (2016), GWP emissions of cheese production were 14.44 kg CO_2-eq/kg of product, while in VERGÉ *et al*. (2013) the carbon footprint of Canadian dairy products was significantly lower than the one assessed in this analysis. However, both studies cannot be directly compared to the present findings due to several differences related to the final cheese products, to the production process and different methodological choices.
In our study, GWP emissions of mozzarella cheese-making phase were 0.32 kg CO_2-eq with A diet and 0.29 with B diet. These findings are quite in line with the study of FINNEGAN *et al*. (2015) that calculated the GWP emission of six groups of dairy products (not mozzarella cheese) and showed that GWP emissions from the dairy processing phase ranged 0.11-2.5 kg CO_2-eq/kg according to the different groups of studied products.
In conclusion, despite different environmental assessment methods used in literature, the milk production is the process that mostly contributed to the environmental impact. Improvement alternatives at the dairy-farm level are therefore required, and they involve many aspects, among which is the use of fertilizers for feedstuffs cultivation. In this regard, KOESLING *et al*. (2017) assessed the variations in nitrogen utilisation of conventional and organic dairy farms in Norway. These researchers concluded that, for both a dairy farm and system area, N-surpluses increased with increasing use of fertilizer N per hectare, biological N-fixation, and imported concentrates and roughages, while they decreased with higher production per area. PAGANI *et al*. (2016) investigated direct and indirect energy inputs in a sample of dairy farms -either grain-based, forage-based or organic- and demonstrated that potential reduction in the overall energy input could be achieved by shifting to organic farming, switching to forage-based farming, and by promoting reduced use of fertilizers. Both studies highlighted the importance of good agronomy that utilizes available nitrogen and reduces energy inputs properly.

Other studies focused on improvements in the composition of dairy ration to mitigate the environmental impact. HAWKINS *et al*. (2015) suggested that feeding decisions have important implications for GHG emissions from intensive dairy production due to the wide variation in emissions from alternative crops that can be used in the ration. PATRA *et al*. (2011) reviewed several potential methane mitigation options such as animal interventions (i.e., number and productivity of animals or genetic selection), dietary interventions, suppression of rumen methanogens, and new potential technologies, by underlying areas worthy of investigation for CH4 mitigation and improvements most likely to be adopted by farmers. Finally, WHITE (2016) proposed a farm-scale diet optimization model to reduce land use, water use, and GHG emissions within dairy production systems and assessed how improved energy and protein use efficiency reduces the environmental impacts of dairy production systems.
Finally, improvements in the environmental profile of cheese production should also be directed at the dairy factory level, mainly due to a high-energy consumption of machinery used during the production process. However, according to VAN MIDDELAAR *et al*. (2013), mitigation strategies may be case-specific and must consider the level of the analysis –at animal, farm and chain level-.
To achieve a sustainable mozzarella cheese production chain, not only its environmental impact must be considered and minimized, but also the economic value that is added along the chain.

3.2. The eco-efficiency of the dairy chain

The total eco-efficiency (total GVA/total GWP) of 1 kg of mozzarella cheese accounted for € 0.19 per kg CO_2-eq in the B supply chain and € 0.16 per kg CO_2-eq in the A supply chain (Table 8). Findings showed that dairy chain in case of B diet had a better eco-efficiency ratio per unit of GHG emitted to the atmosphere.

Table 8. Carbon footprint and gross value added (GVA) per functional unit (FU=1 kg mozzarella cheese), and eco-efficiency of the two stages in the dairy chain (Physical allocation).

Stage	GWP ($kg\ CO_2$-eq/FU)		Economic Performance GVA/FU (€)		Eco-efficiency Total GVA/ total GWP	
	A chain	B chain	A chain	B chain	A chain	B chain
Milk production	9.33	9.52	0.56	0.63	0.06	0.07
Mozzarella cheese- making	0.32	0.29	1.00	1.20	3.12	4.13
Total	9.65	9.81	1.56	1.83	0.16	0.19

Under the economic viewpoint, the B dairy chain had lower total costs than the A chain due to: 1) the elimination of treatment costs of whey in the WWTP at cheese factory level; 2) the reduction of water consumption due to whey usage in B diet; 3) finally, to lower transportation costs.
The total value added for 1 kg of mozzarella cheese was € 1.56 for the A dairy chain and € 1.83 for the B chain. When considering the distribution of total GVA along the chain, milk production accounted for a lower economic weigh (36 % in A chain and 34 % in B chain) compared to the value contribution of the cheese making process. For the above reasons, mozzarella cheese making had the highest eco-efficiency ratio for each dairy chain (€ 3.12

in A chain and € 4.13 in B chain) and added the highest economic value per unit of environmental impact.
The average GVA per 1 kg of fat and protein correct milk (FPCM) for the milk production phase was € 0.56 (per 8.11 kg FPCM to produce 1 kg of mozzarella) for the A dairy chain and € 0.63 per (8.11 kg FPCM to produce 1 kg of mozzarella) for the B chain.
Our results were consistent with the VAN MIDDELAAR *et al*. (2011) study that calculated the economic performances of a cheese chain as defined in this study (*i.e.* gross value added per environmental impact of stages along a production chain) and showed that the milk production contributed 34% to the total GVA of mozzarella cheese production. Furthermore, the economic performance of mozzarella production phase accounted for € 1.00 for the A chain and for € 1.20 in the B supply chain, confirming the VAN MIDDELAAR *et al*. (2011) results that showed a GVA of € 1.04 for the cheese-making phase. The above differences, while negligible, were likely due to both different local markets, products, and manufacturing costs and prices.
For this reason, two sensitivity analyses were carried out to test eco-efficiency results against changes in the economic indicator and to test environmental results against an allocation method different from the one applied in the baseline analysis.

3.3. Sensitivity analysis results

Results of the sensitivity analysis confirm previous results about the eco-efficiency of mozzarella cheese production.
The first sensitivity analysis (Table 9) showed that results from the CU allocation were lower than results achieved through a physical allocation for each dairy chain, but the differences in the value of the carbon footprint were negligible (around 1% for each chain). Furthermore, comparing findings based on CU allocation for the two dairy chains, results were consistent with those presented in Fig. 2 based on the physical allocation method (data are available on request). The B dairy chain confirmed its lower environmental performance.

Table 9. Sensitivity results of the Carbon footprint to the allocation method (Physical and CU allocation).

Stage	**GWP** (kg CO_2-eq/FU)			
	Physical allocation		**CU allocation**	
	A chain	**B chain**	**A chain**	**B chain**
Milk production	9.33	9.52	9.18	9.37
Mozzarella cheese- making	0.32	0.29	0.32	0.29
Total	9.65	9.81	9.50	9.66

FU=1 kg mozzarella cheese

The second sensitivity analysis (Table 10) was performed to estimate the effect of ±10% change of GVA for each stage, for each dairy chain and each allocation method on the eco-efficiency ratio. Compared with the baseline scenario, the ±10% change of GVA modified the eco-efficiency scores in the range ±0.04 €/kg CO_2-eq, (e.g., from a score of 0.14 to 0.18 and from a score of 0.16 to 0.20 €/kg CO_2-eq, respectively in the A and B chains under the physical allocation method). Finally, findings showed higher eco-efficiency values with a CU allocation than a physical allocation method. Even in this case, results reported small changes in the absolute values of the eco-efficiency per 1 kg of mozzarella cheese and showed that the best-performing dairy chains did not change. Therefore, the dairy chain in case of B diet had the best eco-efficiency ratio per unit of GHG emitted to the atmosphere. From the two sensitivity analysis, it is possible to affirm that study results are not very much influenced by the choice between the two considered allocation methods, nor by the change in the economic value added.

Table 10. Sensitivity results of the Economic performance (±10% change of GVA) and of the Eco-efficiency scores in the two dairy chains (Physical *versus* CU allocation and ±10% change of GVA).

Change	Stage	Economic* performance GVA/FU (€)		Eco-efficiency* scores GWA/GWP (€/kg CO_2-eq)			
				Physical allocation		CU allocation	
		A chain	B chain	A chain	B chain	A chain	B chain
+10% of GVA	Milk production	0.62	0.69	0.06	0.07	0.07	0.08
	Mozzarella cheese-making	1.10	1.32	3.44	4.55	3.44	4.55
	Total	1.72	2.01	0.18	0.20	0.19	0.21
Baseline scenario	Milk production	0.56	0.63	0.06	0.07	0.06	0.07
	Mozzarella cheese-making	1.00	1.20	3.12	4.13	3.13	4.14
	Total	1.56	1.83	0.16	0.19	0.17	0.20
- 10% of GVA	Milk production	0.50	0.57	0.05	0.06	0.05	0.06
	Mozzarella cheese-making	0.90	1.08	2.81	3.72	2.81	3.72
	Total	1.40	1.65	0.14	0.16	0.15	0.17

*The different allocation method (Physical or CU allocation) does not imply any variation in the economic performance (GVA), while it influences the environmental assessment (GWP, as reported in Table 9) and the eco-efficiency results (because the eco-efficiency is the ratio between Total GVA/total GWP).

4. CONCLUSIONS

In this paper, the eco-efficiency ratio of mozzarella cheese production is assessed in an Italian case study according to the handmade cheese making system considering two different diets at the farm level, including or not including liquid cheese whey in cows' diet.

From an environmental point of view, one of the main findings of the study was that the primary phase had the highest impact within the mozzarella cheese supply chain.

For the phases along the dairy chain, the mozzarella cheese making had the highest eco-efficiency ratio for each dairy chain and produced the highest economic value per unit of environmental impact. The milk production phase added the lowest value of total GVA in both dairy chains while showing the highest environmental impact in GHG terms.

To reduce the environmental impact of the dairy chain and the wastage of a mozzarella cheese co-product, we assessed the carbon footprint of two dairy chains changing the diet composition at case farm level and using the liquid whey in cows' diet. The study hypothesis was that the use of the by-product of mozzarella cheese production within the local dairy chain would provide benefits under both environmental and economic perspectives. From the environmental point of view, the B supply chain with the whey showed an environmental performance per unit of mozzarella cheese lower than that of the A chain, although in a negligible measure, due to the effect of the milk yield in the primary phase. However, when considering the economic assessment of the two diets, the comparison of the eco-efficiency indicator evidenced a better performance of the B chain whose value per unit of impact was higher thanks to the liquid whey recycling.

Study findings lead to certain conclusions on the need of improving both sides of sustainability. On the economic side, improvements are needed in the market mechanisms to set costs and revenues that increase the value added along the dairy chain, mainly at the farm level. Under an environmental perspective, based on the carbon footprint assessment, improvements in the milk production should provide practices and alternatives that can further reduce the primary phase emissions up to the limit allowed by the ruminant physiology. Finally, the circularity in nature and economic cycles should be further analysed to improve the performances of both sides of sustainability. By recycling the liquid whey and strengthening the relation between dairy farms and cheese factories at a local level, some economic benefits (the cost of whey transportation and the disposal costs of liquid whey) emerged, while the environmental burden of whey treatment is avoided.

The best scenario satisfying both environmental and economic goals would realise a reduction in costs related to efficiency improvements in the usage of natural resources and dairy chain by-products, and a lower environmental burden associated with production processes. Concerning the revenues, the best scenario would be related to the attainment of a price premium for the environmental performances of the dairy products. For example by leveraging on marketing tools, such as environmental standards, labels, and environmental product declarations.

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PAPER

CALCIUM CARBONATE EFFECT ON ALKYL ESTERS AND ENZYMATIC ACTIVITIES DURING OLIVE PROCESSING

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ABSTRACT

The effect of coadjuvants during olive oil processing on the oxidative enzymes and the content of fatty acid alkyl esters (FAAE) has been investigated. Two Italian olive cultivars, at different ripening degree, were processed immediately after harvesting or after 5 and 12 days of storage. The results highlighted a general decrease of FAAE and a significant increase in the PPO and POD activities due to the coadjuvant use. The increased oxidases activity could lead to a reduction of oils phenolic compounds.

Keywords: fatty acid alkyl esters, extra virgin olive oil, oxidases, olive processing, technological coadjuvant

1. INTRODUCTION

Over the years, the goals of the oil industry have gradually changed. After the introduction of the centrifugal decanters, which made the oil extraction process continuous and led to cost decreases, solving the issues linked to the traditional production, the newest aim was to ensure the highest quality of virgin olive oils (VOO) obtained. In fact, suddenly appeared that oils had lower content of phenolic compounds compared to those obtained with the traditional method (pressure), because of the olive paste leaching by the added water, an essential step in order to ensure satisfactory extraction yields by centrifugation (RANALLI and ANGEROSA, 1996). It is common knowledge that phenolic compounds, besides affecting VOO sensory notes (bitter and pungent notes are directly related to the total phenolic content) are the main responsible of the health benefits associated to the VOO consumption (MARTÍN-PELÁEZ *et al*., 2013). Furthermore, often happened that the olive pomace after the separation step is too much wet, with moisture content even higher than 60%, and thus not appreciated by the pomace oil factories (SÁNCHEZ MORAL *et al*., 2006). Usually oil producers overcome this drawback submitting olive pomace to a second centrifugation step by means of three-phase decanter (CAPONIO *et al*., 2015; PASQUALONE *et al*., 2016). This allows even to recover an additional amount of olive oil called "ripasso" rising, however, the overall costs of production. Moreover, according to the Council Regulation 1513/2001 (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2001), that oil has to be classified as "crude olive-pomace oil".

Nowadays, innovative decanters, such as those equipped with variable Dn system, are available on the market. Respect to older machines, these decanters allow real-time setting of several working parameters as a function of the raw material characteristics (maturity degree), as well as of the expected virgin olive oil quality, e.g. in terms of phenolic compounds (SQUEO *et al*., 2017a). Currently, the focus was moved towards the optimisation of the process efficiency (that is maximise the extraction yields) especially during processing of the so-called "difficult pastes" (CERT *et al*., 1996; UCEDA *et al*., 2006; CAPONIO *et al*., 2014), without jeopardising VOO quality. Different approaches were tested to solve such an issue and, besides working on the malaxation parameters (time-temperature), numerous researches were recently carried out regarding the use of physic processing aids (not forbidden by the European laws). Among them, micronized natural talc (MNT) and calcium carbonate have shown a significant positive effect on the extraction process efficiency while the influence on the chemical and organoleptic features of the VOO was not univocally pointed out (BEN BRAHIM *et al*., 2015; CAPONIO *et al*., 2016). Besides, calcium carbonate is less expansive and does not involve any health risk for oil-mill operators than the use of MNT (ESPÍNOLA *et al*., 2009). The employment of technological coadjuvants was even proposed in order to avoiding the need of a second centrifugation step (CAPONIO *et al*., 2015).

The European Commission (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2011), aiming at the protection of the highest VOO quality and in order to prevent illegal mixtures with low-quality oils, have introduced the determination of fatty acids alkyl esters (FAAE) as a quality parameter for the extra virgin olive oils (EVOO) classification. Alkyl esters originate from the esterification of fatty acids and low molecular weight alcohols, methanol and ethanol, arising from the progressive pectin degradation during the olive ripening and from the bad and/or prolonged storage of drupes, respectively (BIEDERMANN *et al*., 2008; JABEUR *et al*., 2015; BELTRAN *et al*., 2016). That is, FAAE content in EVOO is strongly linked to the quality of the raw material and is considered a clear marker of the sanitary state and/or of the handling procedures of the olive fruits before processing. Moreover, due to the high stability, these compounds were even proposed as an efficient tool for discovering mixture with mild deodorised olive oils (PÉREZ-CAMINO *et al*., 2008). Afterwards, the focus was moved towards the content of the fatty acids ethyl esters (FAEE) only (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2013).

In this framework, greater attention would be given to the correlation between the use of coadjuvants in olive oil mill and alkyl esters in the obtained oils. The only study present in literature (SQUEO *et al*., 2017b) reports that the use of calcium carbonate on Coratina cv. olives processed immediately after harvesting led to a general reduction of FAAE compared to the untreated samples, evidencing a higher susceptibility of methyl esters (FAME) than ethyl esters. Furthermore, previous papers have reported as its use led to a decrease in the oil phenolic content (SQUEO *et al*., 2016; TAMBORRINO *et al*., 2017) but, despite the great relevance of phenolics, still today this side effect is not studied and understood. As far as we know, no information are available about the effect of the coadjuvants on the most important olive enzymatic activities and, in particular, polyphenol oxidase (PPO) and peroxidase (POD) which are responsible of the oxidation of phenolic compounds in the first stages of extraction and during the malaxation step (GARCÍA-RODRÍGUEZ *et al*., 2011).

Hence, the aim of this research was to assess the influence of calcium carbonate on the FAAE content and enzymatic activities involved in the oxidation of the phenolic compounds, in order to reach a deeper knowledge about the possible side effects of such coadjuvant during olive processing. In particular, two Italian olive oil cultivars, having different maturation degree, were considered, both processed immediately after harvesting and after 5 and 12 days of storage, with the addition of calcium carbonate at different level and particle size.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Calcium carbonate ($CaCO_3$) was kindly furnished by Omya Spa (Milan, Italy). Two different mean particle sizes were considered: 2.7 μm (Calcipur®2) and 5.7 μm (Calcipur®5). All the reagents used for the analytical determination were for analytical purpose or HPLC and GC grade.

2.2. Sampling

Olives of Coratina and Nociara cultivars having the following features respectively were used for the experiment: pigmentation index (defined as in SQUEO *et al*., 2016) 2.32 and 4.40, respectively; moisture content 52.92% and 47.15%; total oil content 21.20% and 19.66% (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 1991).

For each cultivar a homogenous lot of olives were used, which was further divided in 18 batches of about 30 kg each. Five trials were carried out on olives processed immediately after harvesting: one, the control (C), without $CaCO_3$ addition and the others by means of coadjuvant addition (two different particle sizes: 2.7 μm and 5.7 μm) at two different percentages of use (2% and 4% respect to the olives weight), as follows:

- without calcium carbonate addition (C, control),
- with 2% of Calcipur®2 (Ca2-2%),
- with 4% of Calcipur®2 (Ca2-4%),
- with 2% of Calcipur®5 (Ca5-2%),

- with 4% of Calcipur®5 (Ca5-4%).

The remaining batches were stored in reticular plastic bins for 5 and 12 days (T5 and T12) and processed without any treatment (control, C) or by the use of 2% of $CaCO_3$ (2.7 µm) as follows:

- without calcium carbonate addition (C_{T5} and C_{T12}, control),
- with 2% of Calcipur®2 ($Ca2_{T5}$-2% and $Ca2_{T12}$-2%).

When expected, calcium carbonate was added at the beginning of the malaxation phase. Oil extraction was performed by means of a small industrial plant (Oliomio Mini 50, Mori-Tem S.r.l., Tavarnelle Val di Pesa, Florence, Italy) of a maximum capacity of 30 kg h^{-1}. After crushing by a fixed hammer crusher, olives paste was malaxed at 20±1°C for 20 min and sent to a 2-phase decanter for the oil separation. Two oil samples were collected for each extraction. The samples were then further finished by centrifugation (SL 16R model, Thermo Scientific, Waltham, MA, USA) at 8,867 × *g*, 5 min, 4°C. Finished oils were poured in 100 mL dark glass bottles, leaving an head-space of about 1 cm, hermetically sealed, and stored at room temperature (18-20°C) until were analyzed. All the trials were repeated twice.

2.3. Routine analyses

The determination of free fatty acids, peroxide value, spectrophotometric extinctions at 232 and 270 nm, and fatty acid composition were carried out as reported by the Official Journal of the European Communities (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 1991).

2.4. Determination of FAAE

The analyses of the methyl and ethyl esters of fatty acids were carried out according to the official method (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2011). The gas chromatographic system was made up of a 7890B Agilent Technologies (Santa Clara, CA, USA) gas-chromatograph equipped with a flame ionization detector (FID). The column used was a capillary fused silica DB-5HT (length 15 m, i.d. 0.32 mm, film thickness 0.10 µm). The operating conditions were as follows: oven temperature, 80°C for 1 min and then increased from 20°C min^{-1} to 140°C, then increased from 5°C min^{-1} to 335°C and maintained for 20 min. The detector temperature was 350°C. Helium was used as the carrier gas, with a flow through the column of 2 mL min^{-1} in on-column mode. Each sample was analysed twice.

2.5. Enzymatic activity assessment

Ten g olive pastes were homogenized with 150 mL cold acetone (-20°C) in a Waring Blender homogenizer at highest speed for 30 s. Powder extract was filtered under vacuum, then further extracted twice with 20 mL of cold acetone, finally washed with diethyl ether, dried at room temperature and finally stored at -20°C (GARCÍA-RODRÍGUEZ *et al.*, 2011).
Enzymatic extracts of polyphenoloxidase (PPO, EC 1.14.18.1) and peroxidase (POD, EC 1.11.1.7) were prepared according to (PERES *et al.*, 2016): 0.4 g acetone powder were resuspended in 5 mL extraction buffer (0.05 M potassium phosphate, 1 M KCl, pH 6.2) and 2% polyvinilpyrrolidone (PVP) and stirred for 30 min at 400 rpm at 4°C; the suspension was centrifuged at 15,777 × *g* for 30 min at 4°C and filtered (0.45 µm).
For both enzymatic assays the reaction medium consisted of 50 mM sodium phosphate buffer pH 6.2, containing 0.5 mL of filtered crude extracts (above described) in a final volume of 2.5 mL. PPO activity was evaluated using catechol (30 mM) as substrate,

following the increase in absorbance at 420 nm, during 1 min. One unit of PPO was defined as the quantity of enzyme that causes the absorbance variation of 0.001 min^{-1} mL^{-1}, at 25°C; results were expressed as U g^{-1} FW (fresh weight). POD activity was evaluated using guaiacol (30 mM) and H_2O_2 (4 mM) as substrates. In particular, guaiacol was incubated for 5 min at 25°C, then H_2O_2 was added and the absorbance at 470 nm was measured after 2 min. One unit of POD was defined as the consumption of μmol of guaiacol min^{-1} mL^{-1}, at 25°C. Results were expressed as U g^{-1} FW (fresh weight) (PERES *et al*., 2016).

2.6. Total phenolic content

For the extraction of phenols, 1 g of oil was dissolved in 1 mL of hexane and 5 mL of methanol/water (70:30, v/v). The mixture was vortexed for 10 min and centrifuged at 6000 rpm for 10 min at 4°C (SL 16R Centrifuge, Thermo Fisher Scientific Inc., Waltham, MA, USA). The methanolic phase was recovered, centrifuged again at 9000 rpm for 5 min at 4°C, and finally filtered through 0.45mm pores filters. The quantification of the phenolic compounds was carried out by means of the Folin-Ciocalteau method. Briefly, 100μL of extract were mixed with 100μL of Folin-Ciocalteu reagent. After 4 min, 800μL of Na_2CO_3 solution 5% (w/v) was added to the mixture and heated in a water bath at 40°C for 20 min. After being cool down for 15 min, the absorbance was measured at 750 nm by an Agilent Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, USA). The total phenolic content was expressed as gallic acid mg equivalents (mg kg^{-1}).

2.7. Statistical analysis

Unbalanced nested ANOVA was used to test the effects of the type of calcium carbonate and the percentages of addition on the fatty acids alkyl esters amount (Table 2). Two-way ANOVA was used to test the effects of the use of calcium carbonate and the olives days of storage on the fatty acids alkyl esters amount (Table 3) while three-way ANOVA was used for testing the influence of the experimental conditions on the enzymatic activities (Figure 1). In all cases, Tukey post-hoc test for multiple comparisons was carried out on the experimental data by means of Minitab 17 software (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1. Influence on the alkyl esters amount

Table 1 reports the characteristics of the oils extracted from the olives, without carbonate addition (C), immediately after harvesting. The samples fulfilled the European limits for the EVOO classification (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2013).

The fatty acids profiles matched those typical for olive oils and a variability among the cultivar studied was observed confirming, as it is well known, that fatty acids composition is strongly affected by the genotype (ROTONDI *et al*., 2010). Nociara cv. oil was richer in palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$) acids than Coratina cv. oil. The latter was richer in oleic ($C_{18:1}$) and eicosenoic ($C_{20:1}$) acids. Overall, Nociara cv. oil showed a higher extent of the primary oxidative degradation, likely due to the higher pigmentation index (almost two-fold than Coratina) of the drupes and to the higher amount of polyunsaturated fatty acids. Indeed, the oxidative susceptibility of the oils rises with the increase of the fatty acids unsaturation degree (CHOE and MIN, 2006).

The mean values, standard deviations, and statistical analysis of the oils alkyl esters content obtained from non-stored olives are reported in Table 2.

Table 1. Basic analytical characteristics of the oils obtained from olives processed immediately after harvesting (n=2).

Parameter	Coratina		Nociara	
	Mean value	**SD**	**Mean value**	**SD**
FFA	0.31	0.02	0.43	0.04
PV	6.5	0.1	9.8	0.1
K232	1.829	0.016	2.215	0.023
K270	0.115	0.005	0.128	0.003
Fatty acid composition (%)				
< C14:0	-	-	-	-
C16:0	10.09	0.01	14.08	0.12
C16:1	0.68	0.02	1.73	0.04
C17:0	0.06	0.01	0.05	0.00
C17:1	0.08	0.01	0.10	0.01
C18:0	2.12	0.04	1.91	0.08
C18:1	78.97	0.05	67.91	0.02
C18:2	6.77	0.07	12.95	0.04
C18:3	0.55	0.01	0.67	0.03
C20:0	0.34	0.01	0.37	0.03
C20:1	0.33	0.02	0.23	0.02

FFA, free fatty acids (g 100 g^{-1}); PV, peroxide value (meq O_2 kg^{-1}); K_{232}, specific absorption at 232 nm; K_{270}, specific absorption at 270 nm; $C_{16:0}$, palmitic acid; $C_{16:1}$, palmitoleic acid; $C_{17:0}$, heptadecanoic acid; $C_{17:1}$, heptadecenoic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{18:3}$, linolenic acid; $C_{20:0}$, arachidic acid; $C_{20:1}$, eicosenoic acid.

Table 2. Mean values, standard deviations and results of unbalanced nested ANOVA followed by Tukey's HSD test of fatty acids alkyl esters determined in oils obtained from olives processed immediately after harvesting. Oils were obtained by adding or not calcium carbonate (n=4).

Cultivar	Trial	FAME (mg kg^{-1})		FAEE (mg kg^{-1})		FAAE (mg kg^{-1})	
Coratina	C	4.53±0.22	a	3.75±0.22	a	8.29±0.41	a
	Ca2-2%	3.90±0.16	ab	3.01±0.27	b	6.91±0.34	b
	Ca2-4%	3.94±0.17	ab	3.58±0.16	a	7.52±0.19	ab
	Ca5-2%	3.83±0.30	b	3.95±0.25	a	7.77±0.25	ab
	Ca5-4%	3.66±0.48	b	3.62±0.36	a	7.27±0.83	b
Nociara	C	3.73±0.02	a	6.31±0.31	a	10.04±0.31	a
	Ca2-2%	3.34±0.44	a	5.65±0.25	a	8.99±0.41	a
	Ca2-4%	3.46±0.34	a	6.08±0.32	a	9.53±0.36	a
	Ca5-2%	3.49±0.07	a	6.07±0.59	a	9.56±0.64	a
	Ca5-4%	3.75±0.38	a	6.00±0.48	a	9.75±0.72	a

Different letters in the same column for the same cultivar indicate significant differences ($p \leq 0.05$).
C, control without calcium carbonate addition; Ca2, calcium carbonate 2.7 μm; Ca5, calcium carbonate 5.7 μm; FAME, fatty acids methyl esters; FAEE, fatty acids ethyl esters; FAAE, fatty acids alkyl esters.

The initial levels of FAAE in the control samples were different for Nociara or Coratina cvs.: Nociara oils were richer particularly in ethyl esters and, as a consequence, in the total amount of alkyl esters. The different starting conditions might be imputable to the different maturity degree of the drupes. By the progressive advancement of the maturity, fruits become softer and more susceptible to alterations, such as yeast growth, leading to a higher availability of ethanol by anaerobic fermentation (CONDE *et al*., 2008; DI SERIO *et al*., 2017). Coadjuvant use showed a different effect depending on the processed cultivar. Indeed, no statistical influence was found on the FAAE amount considering Nociara cv., while a significant effect was highlighted in the case of Coratina cv. In particular, respect to the control, FAME amount was significantly reduced by Ca5, regardless the percentage used, while FAEE were significantly lowered by Ca2-2%. As a result, the total FAAE amount was significantly lower in the case of Ca2-2% and Ca5-4%. In the light of this, it is possible to suppose a cultivar-dependent effect or, more probably, dependent on the different maturation degree of the cultivar studied. Indeed, the different amount of water or interfering compounds such as high mass polymeric substances (MAFRA *et al*., 2001) might be determinant in modulating the action of calcium carbonate (AGUILERA *et al*., 2010).

In order to have a deeper understanding about the coadjuvant effect on FAAE, the same olives have been processed after 5 and 12 days of storage. It is known that FAAE amount rises during olive storage as a consequence of degradation and fermentative processes (JABEUR *et al*., 2015). Table 3 reports the mean values, the standard deviations, and the statistical analysis of the alkyl esters of oils obtained from stored olives added or not with calcium carbonate during malaxation.

Table 3. Mean values, standard deviations and results of two-way ANOVA followed by Tukey's HSD test of the fatty acids alkyl esters of oils obtained from olives stored for 5 (T5) and 12 (T12) days after harvesting. Oils were obtained by adding or not calcium carbonate (n=4).

Cultivar	Trial	FAME (mg kg^{-1})		FAEE (mg kg^{-1})		FAAE (mg kg^{-1})	
Coratina	C_{T5}	6.93±0.24	c	17.70±1.23	c	24.63±1.45	c
	$Ca2_{T5}$-2%	6.89±0.18	c	10.99±0.31	d	17.88±0.22	d
	C_{T12}	17.36±0.79	a	48.74±1.64	a	66.11±1.49	a
	$Ca2_{T12}$-2%	12.75±0.21	b	27.60±0.25	b	40.35±0.44	b
Nociara	C_{T5}	8.14±0.21	c	38.61±0.51	b	46.75±0.69	b
	$Ca2_{T5}$-2%	7.73±0.26	c	31.00±0.47	c	38.73±0.36	c
	C_{T12}	23.55±0.96	a	174.96±2.01	a	198.51±2.82	a
	$Ca2_{T12}$-2%	21.99±0.34	b	174.42±1.88	a	196.41±1.96	a

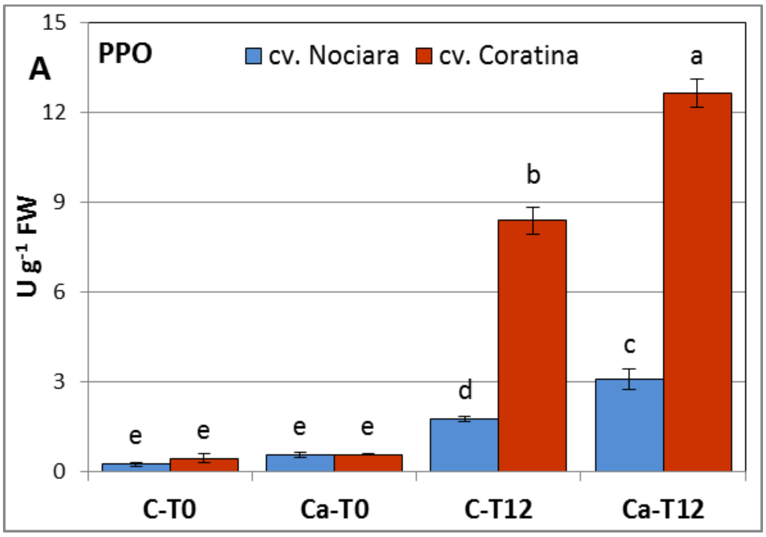
Different letters in the same column for the same cultivar indicate significant differences ($p \leq 0.05$).
C, control without calcium carbonate addition; Ca2, calcium carbonate 2.7 μm; Ca5, calcium carbonate 5.7 μm; FAME, fatty acids methyl esters; FAEE, fatty acids ethyl esters; FAAE, fatty acids alkyl esters.

As expected, the variable *days of storage* had a highly significant effect on the final alkyl esters amount in both the cultivars (in all cases $p < 0.001$, data not shown). In particular, the FAAE increment was mainly due to the increase of FAEE, in accordance with the findings of (JABEUR *et al*., 2015), confirming the role of ethyl esters of fatty acids as a marker of the raw material quality. However, the increment of these compounds was definitely more evident for Nociara cv. instead of Coratina one, probably due to the higher

maturity degree and/or greater substrate availability for the fermentative activities (GÓMEZ-COCA *et al*., 2016), showing the higher susceptibility of the Nociara cv. olives respect to the Coratina cv. fruits. In fact, it is noteworthy to highlight that in the period from 5 to 12 days of storage, the increase of the total alkyl esters and ethyl esters content was extraordinary in the case of Nociara cv. rising of about 325% and 353%, respectively.
Turning on the action of the coadjuvant, a highly significant effect of calcium carbonate addition was observed (in all cases $p < 0.01$) and, interestingly, the results were different for the cultivars studied. Similarly to what previously observed on fresh fruits, the strongest reduction caused by the coadjuvant in terms of FAAE was observed during Coratina olives processing, leading to a decrease of about 26% and 43% for FAME and FAEE, respectively, in the samples obtained after 12 days of storage. That is, the coadjuvant use at 12 days storage, in these experimental conditions, lowered the FAEE amount under the maximum EU limit for the extra virgin olive oil classification (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2016) moving from about 50 mg kg^{-1} to less than 30 mg kg^{-1}. After 5 days storage, it was already observed a significant reduction of FAEE and FAAE, even if sharper than what happened after 12 days. In the case of Nociara cv., a weaker effect of the calcium carbonate was observed, lowering the amount of FAEE and FAAE after 5 days of storage and the amount of FAME after 12 days. No significant effect was highlighted on the great content of FAEE and FAAE after 12 days of storage. Based on the behaviour observed in the case of Coratina cv. it seemed possible to suppose that the alkyl esters reduction by calcium carbonate was substrate dependent, i.e. the higher the content the higher the reduction. However, such hypothesis was not confirmed by the results obtained from the trials carried out on Nociara cv.

3.2. Enzymatic activity evaluation

Figure 1 depicted the enzymatic activities of polyphenol oxidase (PPO, Figure 1A) and peroxidase (POD, Figure 1B) measured on the Coratina and Nociara cvs. olive pastes added or not with calcium carbonate during malaxation.



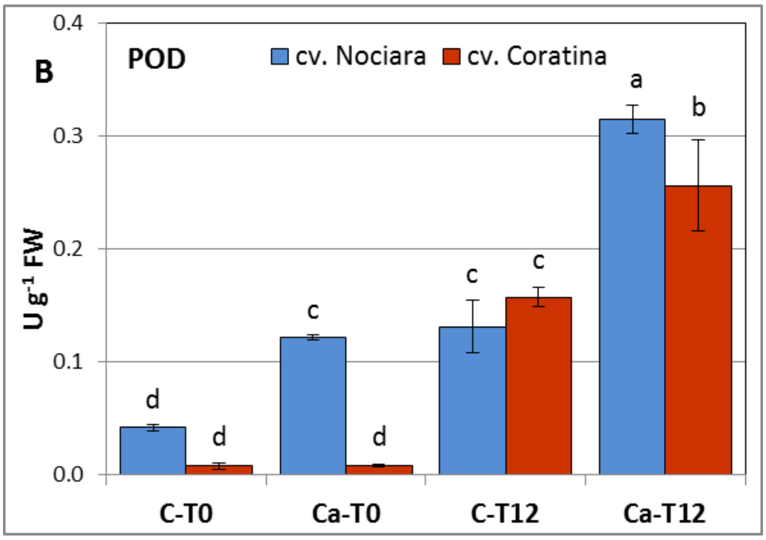


Figure 1. Polyphenol oxidase (PPO, A) and peroxidase (POD, B) activities measured on the olives pastes of Nociara and Coratina cvs. obtained from fresh olives (T0) and after 12 days of storage (T12) at ambient temperature added (Ca) or not (C) with calcium carbonate. Different letters indicate significant variations as determined by three-way ANOVA followed by Tukey's HSD test for multiple comparisons.

PPO activity (Fig. 1A) of fresh fruits was not affected by the coadjuvant use in both the cultivars. At T12, the enzyme activity was significantly higher than T0, in particular in the case of Coratina cv. The increased activity might be due to the progressive structure degradation of the fruits, due to the storage, and to the consequently increase in the substrate availability. This hypothesis seems to be confirmed by the higher enzymatic activity observed in the case of Coratina cv., which is known to be rich in phenolics (ROTONDI *et al*., 2010). Concerning the coadjuvant treatment, it is noteworthy that the use of calcium carbonate significantly increases the PPO activity in both the varieties at T12. The most plausible explanation might be the shift of the olives paste pH to optimal values for the enzyme activity due to the basic hydrolysis of the salt. POD catalyses the oxidation of phenolic compounds using hydrogen peroxide or others organic peroxides from the medium as oxidant agents (GAJHEDE, 2001; KADER *et al*., 2002). At T0, the enzyme activity was higher in the case of Nociara cv. respect to Coratina one, likely due to the higher value of peroxides (Table 1) necessary for the POD action, as previously stated. Calcium carbonate significantly affected the enzyme activity in the case of Nociara cv. while no statistical difference was observed for Coratina. At T12, the POD activity was significantly higher in both the cases respect to T0, and a further significant increase, due to the use of the processing aid, was highlighted. Overall, our findings might explain the reduction of the phenolic compounds of the investigated samples with the addition of calcium carbonate (Table 4), as also observed in previous studies (SQUEO *et al*., 2016; TAMBORRINO *et al*., 2017). Moreover, if these behaviours will be confirmed by further studies, even the action of the calcium carbonate, currently assumed as being merely physical, might be rethought.

Table 4. Mean values of phenolic compounds of the samples under investigation of oils obtained from fresh olives (T0) and after storage for 5 (T5) and 12 (T12) days after harvesting.

Trial	Coratina cv.	Nociara cv.
C_{T0}	470	188
$Ca2_{T0}$-2%	463	146
$Ca2_{T0}$-4%	406	127
$Ca5_{T0}$-2%	316	164
$Ca5_{T0}$-4%	324	121
C_{T5}	311	133
$Ca2_{T5}$-2%	262	113
C_{T12}	182	127
$Ca2_{T12}$-2%	139	105

C, control without calcium carbonate addition; Ca2, calcium carbonate 2.7 μm; Ca5, calcium carbonate 5.7 μm.

4. CONCLUSIONS

The results reported highlighted that the use of calcium carbonate modifies the amount of fatty acids alkyl esters as well as the activity of selected enzymes. Coadjuvant addition during malaxation led to a general reduction of FAAE and an increase in the PPO and POD activities, the former useful for the virgin olive oil classification, the latter involved in the phenolic content of VOO. The effect of the coadjuvant seems to be cultivar dependent or, more realistically, linked to the raw material ripening degree and oxidative degradation. In the light of this, further studies are needed to confirm such results considering more thoroughly the effect of the olives maturation degree and even concerning other coadjuvants commonly used during EVOO extraction.

ACKNOWLEDGEMENTS

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PAPER

ITALIAN WINES IN CHINA'S E-COMMERCE MARKET: FOCUS ON PIEDMONT REGION PRODUCTS

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ABSTRACT

In this study an analysis was conducted on the market performance of Italian wines, in particular from Piedmont region, and consumer preference in the Chinese e-commerce market. The Chinese e-commerce platform company, Taobao, and the professional online wine businesses, Yesmywine, Wine9 and Juxian, were investigated. Chinese consumers were willing to buy famous wines at a high price (i.e. Barolo). Furthermore, sparkling wines, from Piedmont and Emilia Romagna, and typical red wines, from Veneto and Tuscany, were frequently chosen. However, Italian wineries did not pay much attention to the Chinese e-commerce market, missing potential for increasing business.

Keywords: Chinese consumers, e-commerce, Italian wine

1. INTRODUCTION

China is currently one of the world's most dynamic markets for wine imports (MASSET *et al.,* 2016). The country ranks fourth in world-class destinations for wine, with 2.1 billion Euros and 6.3 million hectolitres of imported wine. For many decades, Chinese perceived grape wine was as a symbol of Western-sophistication and wealth, often consumed only by the elite. This trend has changed significantly only in the past decade (HUANG, 2000). The demand was mostly from high-level Chinese bureaucrats and wealthy businessmen for top ranked Bordeaux wines. These wines were purchased as gifts and were rarely consumed for pleasure (YANG and PALADINO, 2015; LOCKSHIN *et al.,* 2016; SEIDEMANN *et al.,* 2016). In the last ten years, economic factors such as the rural exodus to urban areas with its ensuing access to more expensive goods, together with an increase in wealth, has spurred a wider-range of fine wine consumption (MASSET *et al.,* 2016). The interest and enjoyment of foreign wines has become especially prominent in the largest cities (Shanghai, Beijing, Shenzhen, Guangzhou or Hong Kong) where a mix of expatriates, western-educated Chinese and an open-minded population have an appetite for imported goods (MU *et al.,* 2017; TROTTA *et al.,* 2015; MITRY *et al.,* 2009; LEE, 2009). This recent trend has also affected China's social structure, which has been influenced by younger consumers who tend to purchase wine by using new e-commerce platforms, and it's continuing to grow. For example, the demand for wine consumption in December 2012 for the younger target group was 31%, and reached all the way to 45% in March 2015 (LI *et al*., 2011). New data from 2015 shows China's per capita consumption of wine increased from 1.9 to 2 liters and the market potential is expanding (BACCA, 2015). Before the 2012 nation-wide crackdown on corruption in China, a large extent of wine consumption depended on group-purchases by public institutions, and the products tended to be high-end. However, after the Chinese Government ban on lavish banquets with great displays of wealth, this led to an almost immediate reduction in the purchase of very expensive wines from France. Italian wine imports also fell due to the Chinese anti-corruption laws, by almost 27%, but increased 7% in 2014, followed by an increase of 14% in 2015.
The outcome has been a growth of individual interest in wine and increasing efforts from almost every wine producing country to tap into the growing Chinese market for Western wines (DATAMONITOR INTERNATIONAL, 2015; LANNES *et al.,* 2012; LOCKSHIN, 2014; MARKETLINE, 2015; LOCKSHIN *et al.,* 2016). Additionally, the accession of China to the World Trade Organization in 2005 stimulated imports of wine due to a decrease from 43% to 14% in tariffs (Masset *et al.,* 2016; IANCHOVICHINA and MARTIN, 2006). Wine imports have risen by more than 400% to 289 million litres from 2008 to 2013 with France accounting for a market share of around 53% (GEFFROY, 2014). This data corresponding to a growing business volume in this period of 60%. From 2014 to 2015, China's imports of international wine had a further increase (from 1.17 to 1.84 billion Euros), with France still in the top spot. In 2016, imports from France grew by 11% or 900 million euros (http://www.inumeridelvino.it/). It is 42% of the market, which becomes 45% when considering bottled wine and sparkling wine. Starting in 2009, bottled wine began to play an important role in total imports, while imported bulk wine gradually decreased. This reflects the maturity of the consumer`s taste, and, on the other hand, reflects the upgrade of the Chinese wine industry chain (LOCKSHIN *et al.,* 2016).
Currently, Italy represents 12% of China's wine import market. Italian imports increased by 33%; in terms of value, this translates to a 24% increase or 20.3 billion euros.
Huge business opportunities for imported wine in the Chinese market stem mainly from the reduction in tariffs, an immature Chinese wine brand market (only major domestic brands, such as Changyu and a few others, cover China's low-end wine market) and an increasing demand by consumers for high-end products. Data show that, before 2012,

French wine always held the largest share of China's imported wine market, and maintained a sustained growth. But since the second half of 2012, this growth has slowed down and decreased. This shows that Chinese consumers are increasing the diversity of their taste preference and willing to try a variety of wines from sources around the world. Clearly this trend offers abounding business opportunities for wine suppliers and brand operators. This is especially evident in large and medium-sized markets.

Imported wine sale volume in retail channels have also increased in the past 30 years. In China there are differences among commercial supermarket retail formats; there are supermarkets targeted to high-class consumer groups, such as the Ole store of Vanguard group, and City supermarket; there are supermarkets targeted to mass consumption like Carrefour, Wal-Mart, RT-Mart, Lotus, and membership warehouse storage supermarkets such as Sam's Club and Metro; there are neighborhood community supermarkets such as Vingo shop and Seven-11. All supermarket levels cooperate with traditional importers and distributors. In order to optimize the costs, supermarkets choose the large importers who have more varieties. The supermarkets take the entrance fee and commissions from each wine. Overall the retail price of wine includes the supermarket storage cost and its profit. However in 2013, with the development of an e-commerce platform, the price is more competitive and transparent because there no entrance fees or other costs in these platforms. As a result, massive consumer supermarkets like Carrefour choose to compete on price, so now they prefer to display low-grade wine to get a competitive price (QIN, 2017).

The rapid development of Chinese e-commerce in recent years, in particular the development of comprehensive platforms, has led to a rapid development of imported wine e-commerce (ZENG and SZOLNOKI, 2016; FANG *et al.,* 2017). On the comprehensive platform, importers and distributors set up their own webpage directly for the consumers. The vertical e-business includes Business-to-Customer professional wine platforms such as Yesmywine, Wine9 and Jiuxian. The vertical wine platforms work just like importers and also cooperating with exclusive importers for some particular brands. Due to the different supply channels, wine from e-commerce has a lower cost.

n overview of the Chinese consumer of Italian wine was provided using the search indexes and sale volumes provided by the Taobao online platform. Market performance and the evolution of Italian and Piedmont wines in the Chinese e-market were also analyzed.

2. METHODOLOGY

Two kinds of wine e-commerce platforms in China were used in this study: the Chinese website for online shopping, Taobao, and professional online wine business websites (Yesmywine, Wine9 and Juxian). In China, one of the mainstream comprehensive websites is Taobao and Tmall from Alibaba group. Taobao was founded in 2003 by Alibaba's founder Jack Ma. Taobao supports Business-to-Customer (B2C) and Customer-to-Customer (C2C) retail by providing a platform for business and individual entrepreneurs to open online stores (GONG, 2016; REN *et al.,* 2012). Every individual or company can open their own online shop in Taobao: consumers can buy almost everything they need online, often at much lower prices than the ones of traditional retail channels. Sellers on Taobao list items for sale with a posted price and without a time limit excluding auctions with a fixed time-frame. This mechanism broadly allowed the use of posted price format both in online and offline retailing (CHEN *et al.,* 2015). Despite the posted price in Taobao, compared to e-Bay's online auctions, the price formation process includes several factors related to the communicated information by the seller, but above all the reputation deriving from the feedback received: reputable sellers are found to sell more products or services at higher prices (CHEN *et al.*, 2015; ZHANG *et al.*, 2016; QIU *et al.*, 2016; FAN *et al.*, 2014; MELNIK and ALM, 2002). This aspect influences also the recommender system which represents one important advantage of online shopping, which, contrary to offline market, suggests to the consumer products they might be interested in (MO and CHEN, 2015). In 2011, Taobao split into two websites: Taobao and Tmall. Only authenticated brands can join Tmall. This new B2C platform integrates thousands of brands, manufacturers, and provides the quality assurance of goods and a seven-day without reason return service (YU *et al.*, 2013). Today it has 500 million registered users and more than 60 million people visit the network daily. Over 48,000 items are sold every minute on this platform (GONG, 2016).

As currently happens for the most used online wine sales platforms (MCGECHAN, 2013), Taobao members can use computers or smart phones to visit its website or application to shop for goods. Sellers using Taobao can show their goods to customers who can select products and order from this "market". Customers can also correspond and bargain with the sellers, similar to traditional markets in China (LU *et al.*, 2009). Taobao is an incredibly user-friendly platform, providing a shopping guide for users to learn about selecting products and making online payments (Gong, 2016). In contrast, Tmall takes advantage of the "shopping mall" model by renting out online booths to brands for a fee (ZOU *et al.*, 2014). China is new to the concept of C2C. Taobao brings the small businesses to the Internet by adopting a "small businesses to consumers" model, whereas Tmall uses a "brand to consumers" model (HU & CHECCHINATO, 2015). In 2014, Alibaba launched Tmall-International, which directly supplies the imported goods from abroad for domestic consumers. Similar to the original Tmall, the Tmall-International platform only allows authenticated foreign suppliers to join. The suppliers can directly communicate with Chinese consumers. Tmall lends them warehouses located in bonded areas (the tax free zones), and the foreign suppliers send their goods to the warehouse. Due to the cooperation between Tmall-International and customs, each package passes the customs separately and quickly. However, due to strict policies and regulations, this system can't be used with wine and cigarettes for now.

Payments are done by means of Alipay for both platforms, which is not only an intermediary payment tool, but also protects the legitimate rights and interests for both the seller and the buyer. Alipay was created in 2003 by the Alibaba Group team as an offline payment method designed to increase the use of Taobao, improving the security of payments. Unlike PayPal, which takes high proportion commission when cooperates with some e-commerce site, Alipay is free for both sellers and buyers (LI and LIU, 2007). Additionally, Taobao provides the online chatting platform, Aliwangwang, to ensure a successful transaction and increase transparency (GAO *et al.*, 2016; HOLSAPPLE *et al.*, 2014).

Taobao's reputation system is the most popular and successful one in China (LIN and LI, 2005): among the reputation growth strategies, Taobao also provides a lot of information about its activities on its website by regularly posting information on the number of buyers and sellers transacting. In addition, Taobao provides details on the total transaction volumes, for each product category. Taobao also makes publicly available several indices: the Taobao Index provides information on searches, transactions and characteristics of buyers at product category level. The Taobao Interest Index tracks searches, bookmarks, and transactions by category by day and by week (CHU and MANCHANDA, 2015).

Data from Alibaba official software IndexTao were used for our analysis. IndexTao software can analyze users data related that had searched a specific keyword in Taobao. All the data with the distribution curve have to go through an indexation process, so here the "Search index" (daily keywords search data) is an indexation of search volume,

reflecting search trends, and it is not equivalent to the times this keyword had been searched on Taobao (LI *et al.*, 2014). Users are considered as potential consumers of the specific keyword. Italian wine performance was analyzed from January 2013 to December 2015 using "Italian wine" and "Italian red wine" keywords. Data obtained are reliable, because each Alipay is associated with users personal bank account and ID card. To protect privacy, Alibaba doesn't disclose any of the searches and the specific number of associated data. Chinese wine consumers characteristics based on the platform data were analyzed: consumers registration information were extracted using Taobao Search Index to analyze users characteristics (age, gender, wine consumption levels - based on the user's shopping record within six months- hobbies, and the geographical distribution).
For the analysis of age range, IndexTao introduced the concept of the Preference Index (P_I), the ratio between the proportion of all the potential consumers in a specific age range and the proportion of the same consumer group of Taobao users. For example, the Preference Index of age group 18-24 is equal to the proportion of 18-24 potential consumers in all potential consumers (Alibaba) divided to the proportion of 18-24 consumers in the Taobao users. Age data is divided from 18 to 24 years, 25-29 years, 30-34 years, 35-39 years, 40-59 years, 50-59 years and more than 60 years. The level of consumption is defined by the frequency of choosing a product, either expensive or not, on the website within six months. The range is defined by low, under average, medium, over average and high level of consumption.
Data from Yesmywine were employed to analyze Italian wine market performance, available wines on the platform and the sales volume of Italian and Piedmont wines. The same research was carried out using the Wine9 and Juxian platforms. Many Chinese industries began to create their own vertical Business-to-Customer (B2C) websites. China now has many professional online websites for the imported wines. The selected B2C online wine business websites presented different wine products and established a double service, both online and offline sales platforms.
Yesmywine, a website founded in 2008, has already become one of the biggest B2C wine specific platforms in China focusing on imported wine. Its success can be attributed to an all-round, three-dimensional, interactive customer information management system (GAO and LI, 2015). It keeps a close relationship with customers through wine tasting events and other programs. According to a recent study, consumers who buy wine on Yesmywine were 18-35 year-old, middle class, located in first or second-tier cities, and frequently used the Internet (LI, 2016). This consumer group has some common characteristics: they are located in large cities and a majority of them are sensitive to the price. They pursue high consumption efficiency and a high-quality lifestyle. In the Chinese wine market, the majority of the offline wine shops are supplied by distributors, from importers to secondary and tertiary distributors. The cost increases due to changing hands in the supply chain. Some of the wines on Yesmywine come from direct purchase abroad since Yesmywine works as an importer. The other parts come from primary suppliers, or directly from the importers. Currently Yesmywine directly purchases from 11 countries, including Grands Crus Classés wine from Bordeaux, Olivier Leflaive from Burgundy, Spier from South Africa, Craneford from Australia, Beronia from Spain, Henri Abele from France, Rio Bueno from Chile, Fabiano from Italy, Erswnwe Praelat from Germany, and also some unknown brands from various wineries. Wine can be searched according to price and production areas.
Wine9 was founded in 2009 and is under the jurisdiction of three divisions: the Wine9 official website, a comprehensive platform (online store on Tabobao and Tmall) and an offline department (for wholesale). In 2015, Wine9 began to import beer, chocolate, food, and health products. Today it has 4 million registered users online and 80 offline franchisees.

Another professional wine business is Jiuxian. On the webpage layout of Jiuxian, the wine is sorted according to sales volume. Jiuxian uses low prices for their products, which determines their selection of wines, mostly the easily accepted cheap sparkling wine, or OEM (Original Equipment Manufacturer) wines, such as *Rosso&Rosso* series. Jiuxian only has a small selection of Italian DOCG (Controlled and Guaranteed Designation of Origin) red wines.

3. RESULTS

From the Taobao Index data analysis, S_I of keyword "Italian Red Wine" (Fig. 1) reached 688 in 2014 and received the highest index of 1187 in 2015. From 2013 to 2014, the average search index was about 100, while in 2015 after the peak, S_I of "Italian Red Wine", reached about 200 on average.
Fig. 2 reported the search index of the keyword "Italian Wine". From 2013 to 2014 the S_I had been maintained at a low level with an average of about 50. In 2014 the index appeared in a peak reaching 571. From 2014 to 2015, the S_I had been maintained at a high level. There was three index peaks: 571, 588 and 704.

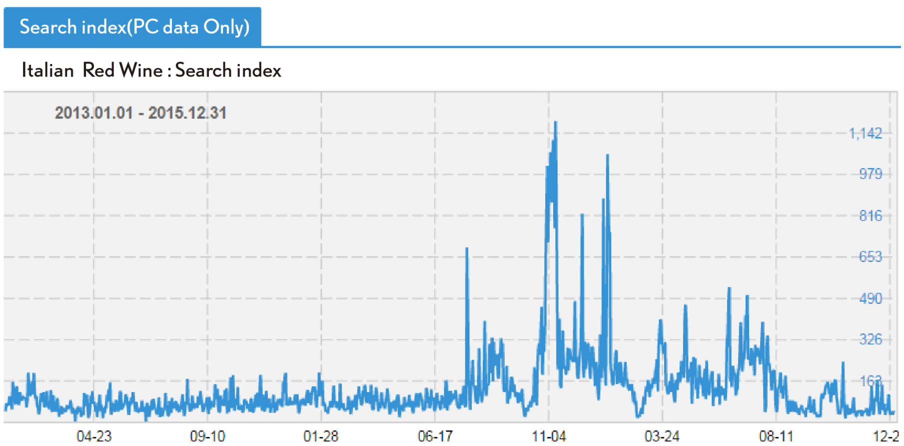

Figure 1. The S_I of keyword "Italian Red Wine".

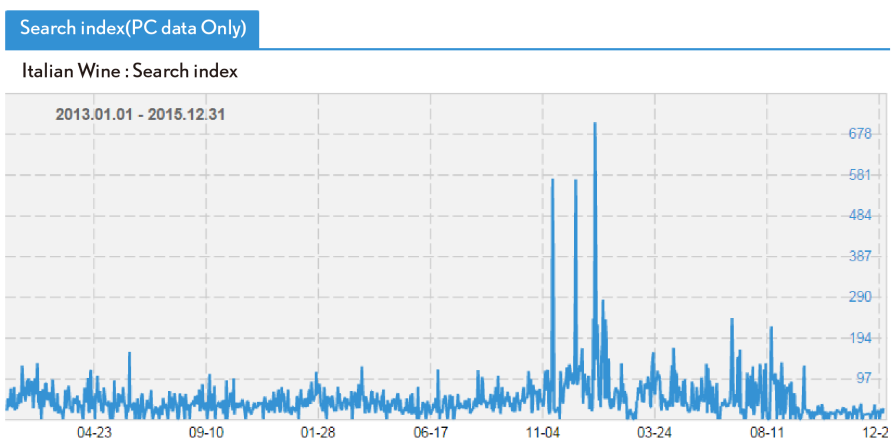

Figure 2. The S_I of keyword "Italian Wine".

Figs. 3 and 4 show the geographical distribution of consumers who searched the keywords “Italian Red Wine” and “Italian Wine”, respectively. Potential users were concentrated in southeastern coastal regions of China. In both cases, Guangdong province had the highest proportion, while the second was Zhejiang province. The proportions of the two key words were 18.73% and 15.92%, respectively.

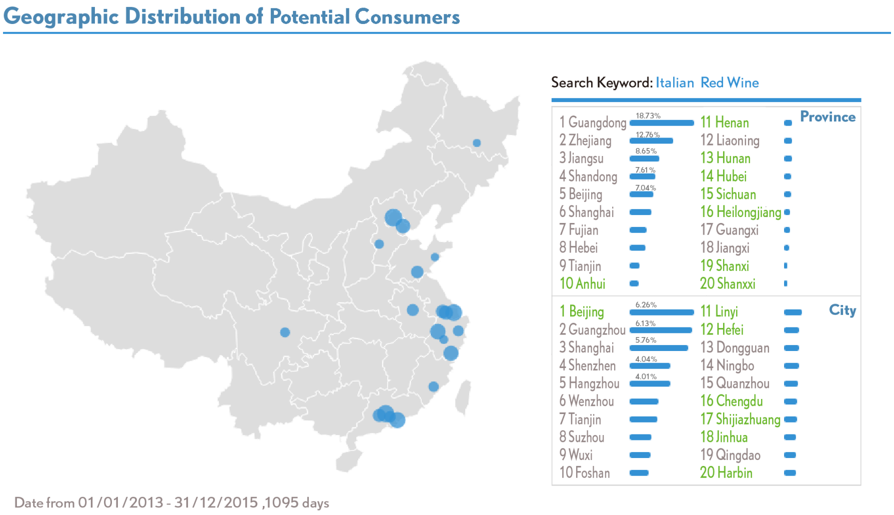

Figure 3. Geographic distribution of potential consumers under the keyword “Italian Red Wine”.

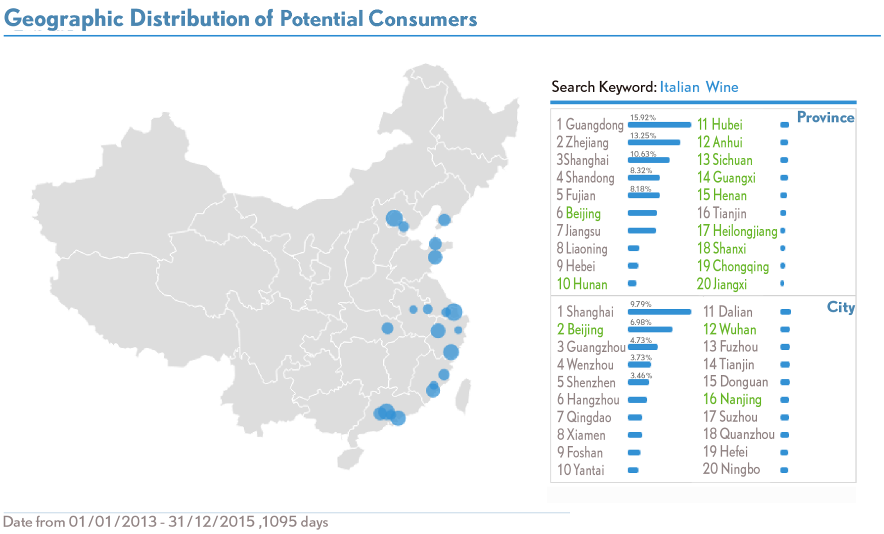

Figure 4. Geographic distribution of potential consumers under the keyword “Italian Wine”.

Preference Index (PI) was used for consumers’ age description: no users older than 60 years searched for “Italian Wine” online. Results of PI showed that consumers from the ages of 18-24 have no obvious or slightly low preference to Italian wines. The age groups 35-39 and 40-59 have a low preference for Italian wine, but the age group 50-59 has the highest preference. On the view of distribution, the 25-29 and 30-35 age groups accounted for 47% of the population in the "Italian Red Wine" keyword. Regarding the “Italian Wine” keyword, the latter two age groups accounted for 52.7%. However, a higher percentage does not mean a higher preference. This means the potential customers are concentrated mostly in the 25-34 age range. Due to people in this age range accounting for a large percentage of the e-commerce users, they have very large base. We can see the preference index of this age as average or slightly over average, and they have a preference use for the keyword "Italian Wine" instead "Italian Red Wine". In terms of gender, males are proportionately higher than that of females, but the difference is not significant.

Figs. 5 and 6 reveal the level of consumption (the frequency of choosing a product, either expensive or not, on the website within six months) Chinese wine consumers reported for both keywords. Potential consumers for Italian wine were concentrated in the average, over average and high-class levels, which mean they have a good acceptability of high price commodities.

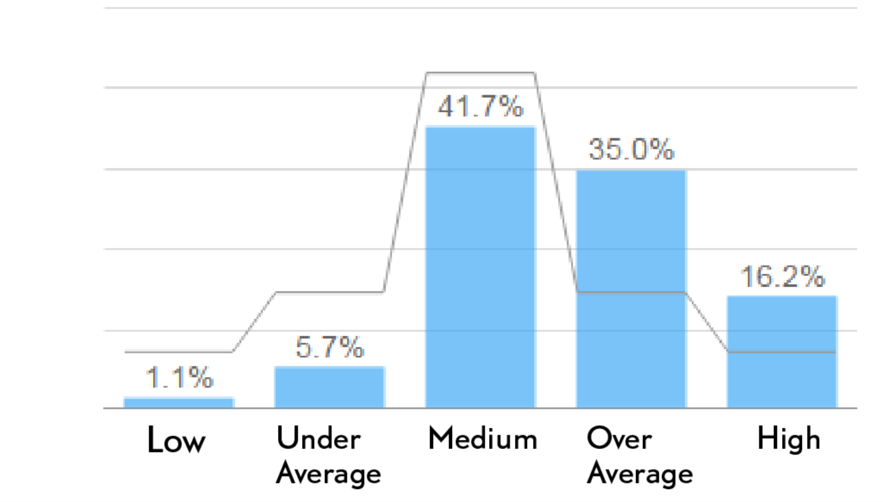

Figure 5. Consumption level of potential consumers under keyword "Italian Red Wine".

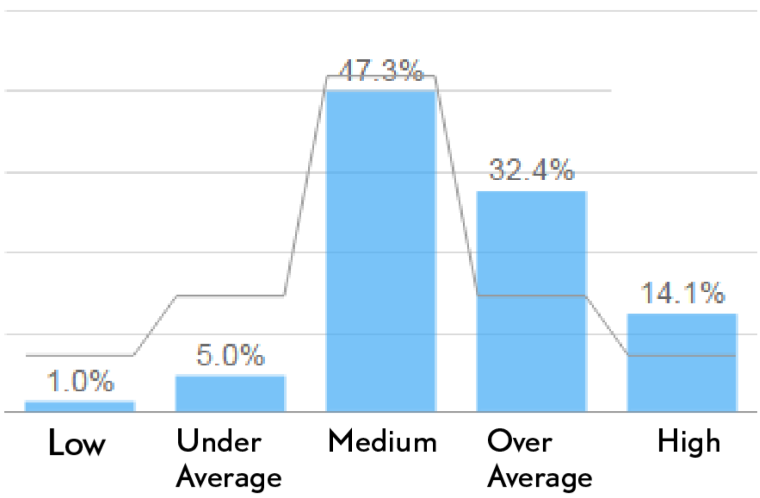

Figure 6. Consumption level of potential consumers under keyword "Italian Wine".

Figs 7. and 8 show the same trend of hobbies of Italian wine and Italian red wine. The Taobao users who search Italian wine also have an interest in the outdoors, sports, photography, home (quality life) and collecting.
Yesmywine presents a total of 6,824 wines from 13 different countries (Fig. 9) (http://www.yesmywine.com/). The top three countries were France with 3,111 wines, Australia with 684 wines, and Italy with 600 wines.

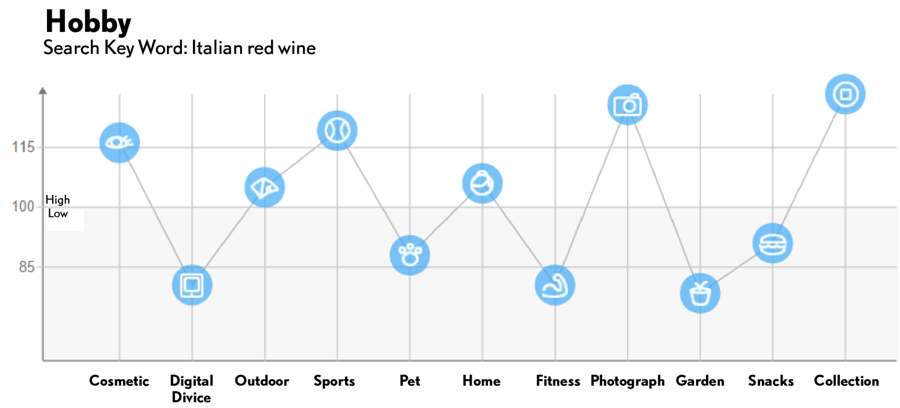

Figure 7. Hobbies of potential consumers of "Italian Red Wine".

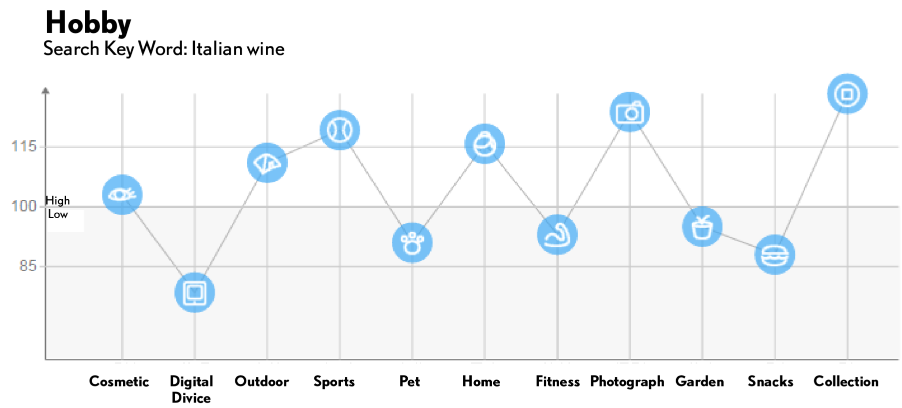

Figure 8. Hobbies of potential consumers of "Italian Wine".

Yesmywine had 600 Italian units of wine with a combination of sales (red, sparkling and white wines). This does not mean 600 varieties of wine. From the point view of price rank, Yesmywine has 8 price rankings (Table 1).
In the 1-49¥ (1-7€) range, there were 13 Italian wines. Out of this total, 6 were sparkling wines, 6 regional red wines, and 1 white wine. In the 50-99¥ (7-14€) range, there were 116 Italian wines, including 52 sparkling wines. Of the 41 red wines, there were regional wines such as Chianti, Barbera d'Asti, Montepulciano d'Abruzzo, Nero d'Avola, and Primitivo. 19 white wines, and 4 rose wines. In the 100-199¥ (14-27€) range, there were 130 varieties, including 72 red wines, 44 sparkling wines and 14 white wines. This section had 72 red wines, all which were based on the regional red wines. In this price range appeared Dolcetto d'Alba, Barbera d'Asti, Barbera d'Alba, Nero d'Avola, Primitivo, Nebbiolo, Chianti, Chianti Classico, and Chianti Riserva. In the 200-299¥ (27-42€) range, there were 108 wines, including 70 red wines, and 20 were combination sales. This price section was still strong in regional red wines, showcasing Amarone, Barbaresco, Dolcetto D'Alba,

Barbera D'Alba, Chianti, Chianti Classico, Langhe DOC, Corvina di Valpolicella, Montepulciano DOC, and Freisa D'Asti. The sparkling wines had 32 varieties, but most of them were combination sales.

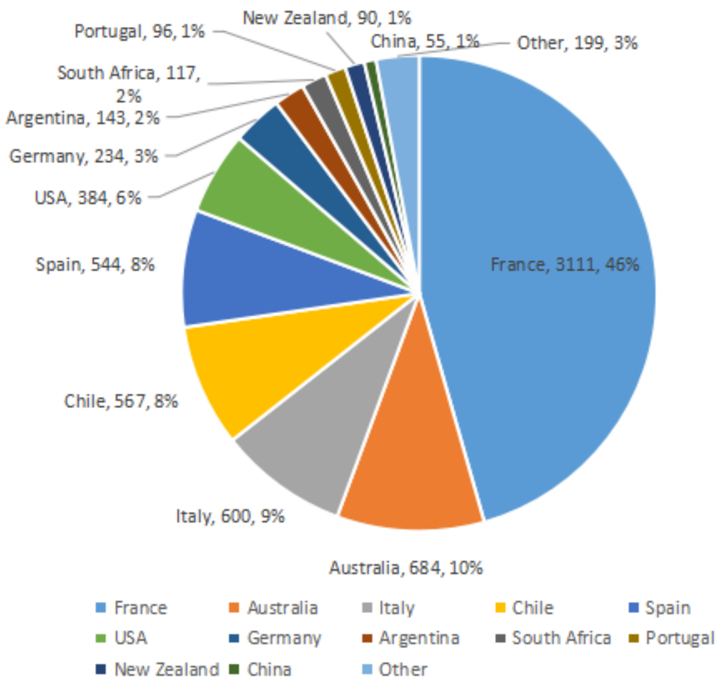

Figure 9. Origin of wine sales on Yesmywine.

Table 1. Italian wine numbers in 8 price ranges.

Price rank in Yuan	Price rank in Euro	Total number	Red wine	Sparkling wine	White wine
1-49¥	0-7€	13	6	6	1
50-99¥	7-14€	116	41	56	19
100-199¥	14-27€	130	72	44	14
200-299¥	27-42€	108	70	32	6
300-499¥	42-69€	80	55	23	2
500-799¥	69-111€	61	54	7	7
800-999¥	111-138€	22	21	0	1
over 1000¥	138€	70	59	4	7

In the 300-499¥ (42-69€) range, there were 80 commodities, including 55 red wines. Except for the combination of red wines, regional wines such as Brunello di Montalcino, Dolcetto D'Asti, and Roero rarely appeared. WGVT (Wine Grape Varietal Table) and GI (Geographical Indication), mainly were DOC (Controlled Designation of Origin) and DOCG (Controlled and Guaranteed Designation of Origin). This range has many brands of Barolo, Amarone, and Nebbiolo. There were also 23 kinds of sparkling wines. Combination sales accounted for 90% of the entire range. Other single commodities were the Proseccos. In the 500-799¥ (69-111€) range, there were a total of 61 commodities, including 48 red wines, of which 11 were Amarone, 8 Barolo, 7 Brunello di Montalcino, 5

Barbareco, 4 Barbera, and 3 Chianti. The remaining few wines were DOC regional red, as well as 7 white wines. Sparkling wine in this range only represented the Franciacorta by Berlicchi. In the 800-999¥ (111-138€) range, Italian wine had a total of 22 items in which 21 were red wines, 4 Brunello di Montalcino, 3 Barolo, 3 Amarone, 4 kinds of Tuscany IGT, 1 Barbaresco, 1 Langhe DOC, 1 Barbera D`Alba, and 4 group combination sales. Only 1 white wine combination was sold. In the over 1000¥ (138€) range, there were 70 kinds of wines. In addition to the combined products, 59 were red wine, except for the famous Brunello di Montalcino, Barolo, and Amarone. Other wines came from well-known wineries.

In Fig. 10, Italian wine has been classified by geographical division. Italy's Piedmont region has the largest number 151 kinds of wines, accounting for 25% of the total. Tuscany and Veneto both rank 2nd. Some regions have a few wines on Yesmywine, like Campagnia, which has only one wine, which is not labeled on the figure. In other northern Italian regions, Aosta Valley, Trentino Alto Adige, and Liguria do not have wines on Yesmywine. As for southern Italian regions, our research did not find wines from Molise and Basilicata on Yesmywine. However, wine from major regions can be found on Yesmywine. The most important region, Piedmont, presented a total of 151 commodities on Yesmywine. There are 109 red wines of which 32 brands are Barolo, 22 Barbera, 21 Barbaresco, 11 Dolcetto, 8 Lange DOC, 8 combination sales, 4 Nebbiolo, 1 Roero, 1 Freisa, and 1 Grignolino. Sparkling wines include 38 products, mainly the Moscato variety. White wine were 16, mainly chardonnay, Gavi and combination sales.

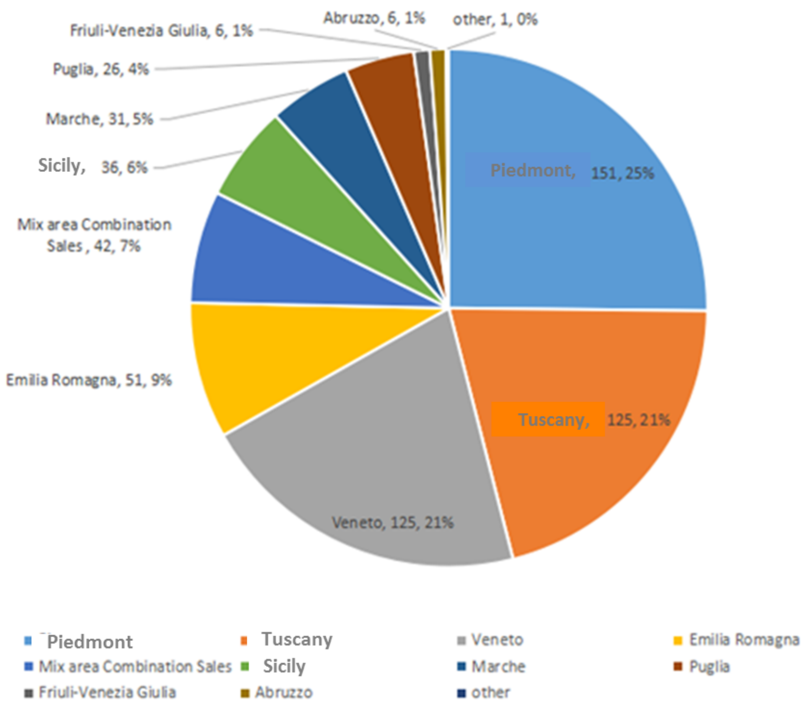

Figure 10. Italian wine geographical subdivision.

The sale volume data was collected from each sale page. In the Italian wine section, wines are ranked by sale volume. The wines sold from each Italian region are reported in Table 2 comparing the scenarios in the two considered years (2014 and 2016).

Table 2. Wines sold from each Italian region in 2014 and 2016.

Italian wines sold in 2014		Wines sold in 2016	
Region of origin	n. of labels	Region of origin	n. of labels
Veneto	9	Emilia Romagna	10
Emilia Romagna	6	Marche	5
Piedmont	2	Veneto	4
Marche	1	Piedmont	2

In 2014, in the Top 25 wines, 9 wines come from Veneto, 6 from Emilia Romagna, 2 from Piedmont. The average price was 74.16¥ (10€). The first wine sold for volume was an IGT white Malvasia sparkling sweet wine from Emilia Romagna (price at 78 ¥, 21,1582 bottes sold), followed by a Veneto IGT (price at 52 ¥, 11,2627 bottles sold), and by another Veneto IGT (price at 49 ¥, 106,920 bottles sold), which was the cheaper product in this classification. This ranking comprised mainly of wines from Emilia Romagna and Veneto. The most expensive wine was an Abruzzo DOC (price at 135 Yuan, 5,092 bottles sold), which ranked 14th. Data collected from Yesmywine in 2016 showed a different scenario compared to 2014. The best-selling wine in terms of volume remained the same as 2014, with an increase in price (88 ¥) and volume (237.839 bottles sold, +11% with respect to 2014). Moreover, new labels originating from the traditional brands were included in the rankings. Marche wine held the highest price (159 ¥). Of the Top 25 wines in 2016 were 4 wines coming from Veneto, 10 from Emilia Romagna, 2 from Piedmont, and 5 from Marche.

Number of Italian wines sold by Yesmywine platform classified in the price ranges is reported in Table 2, comparing the two considered years (2014 and 2016). None of the other wines were included in the higher price range in both the years (Table 3). The cheap wines still dominated the top sellers of Italian wines. In 2014 there were 12 sparkling wines, 12 red VDT or IGT wines, 1 white Garganega. In 2016, 18 sparkling wines in total, 5 red VDT or IGT wine, 1 white Garganega, and 1 rose Merlot were sold. The cheap sparkling wines from Emilia Romagna and Piedmont, as well as the VDT wine from Veneto, were very popular choices. Comparing 2016 with 2014, sparkling wine increased in 2016 and, on the contrary, red wines decreased from 12 to 5 products. The sparkling wine was very profitable and the red wine sold as usual. The average price of the top 25 Piedmont wines was 302¥ (42€). It was 4 times compared to the Italian wines, due to the high price of Nebbiolo.

Table 3. Classification of Italian wines in function of price range. The comparison between the scenarios in 2014 and 2016 is reported.

Price rank in Yuan	Price rank in Euro	Total number in 2014	%	Total number in 2014	%
1-49¥	0-7€	4	16%	4	16%
50-99¥	7-14€	16	64%	19	76%
100-199¥	14-27€	5	20%	2	8%

The top 25 Piedmont wines in 2014 were divided: 11 sparkling wines and 14 red wines. Moscato was the most popular variety (average price was 108¥, 14€). The most popular red variety was Nebbiolo with an average price of 728¥ (101€). In 2016, the top 25 Piedmont best sellers had changed. This may be due to some wines being sold out. The new data from February 2016 shows the same trend. There were 10 sparkling wines and 15 red wines. In comparison with the top 25 of Italian wines, the Piedmont ones had a higher price. Moscato was still the most popular white variety with an average price equal to 101¥ (14€), while Nebbiolo was the most popular red variety with an average price of 811¥ (112.5€). The best seller of Piedmont wine from both considered years was a sweet sparkling wine (80% Malvasia, 20% Moscato) with an increased sales volume, growing from 4,843 to 11,311 bottles (+57%). However, the increase of red wine was very slow, with sales volume only several hundred, even though some new red wines appeared in 2016. Overall, Yesmywine has sold a very important number of Italian wines. Most of them are cheap red wines and sparkling wines. Some of these wines are only produced for foreign market and can't be found in the Italian market. Some of the bulk wines are bottled in China. The real bottled and high quality Italian wines don't have a gratifying performance in the Chinese e-commerce market. Yesmywine does not assume the obligation to promote Italian wines. For each product, Yesmywine describes the wines in detail, including brand, winery information, origin, and also a brief introduction of the country. However, it is possible to find on the wine page some false information. This is especially true regarding information on the labeling of wine-producing areas, classification, the winery's name, and the verification method or the introduction of the wine. It is not comprehensive because oftentimes when the information is translated from Italian to Chinese it can lose the correct meaning.

In 2014, the best wine seller on Wine9 was Moscato from Piedmont. The first (price 168 ¥) has sold 13,372 bottles, while the second one (79 ¥) sold 13,456 bottles. Sale volumes were much higher than the third. In fact, in third place was a red wine from Veneto (389 ¥) with sales volume equal to 5,022. This is not only because of the lower price of Moscato, but also due to Moscato being an easy to drink wine with low alcohol percentage. In China, Moscato is popular for both male and female consumers. Moscato in 2014 had a beautiful blue bottle and label, according to the consumer`s comments. They consider Moscato to be the "best party wine". In 2016, the ranking of the top 25 Italian wines changed. This time the first place was a Moscato Spumante from Emilia Romagna (78 ¥, 7,097 bottles sold). Piedmont wines positioned in last place. Wine9 had a higher price in top 25 sellers wine compared to that of Yesmywine. Wine9 chose to cooperate with several famous wine brands like Zonin, Masi, Banfi, and Citra and has paid more attention to Italian red wines. The regional red wines from Veneto and Tuscany were also popular.

In 2014, 7 Piedmont products were sold on the Wine9 website (Table 4). Moscato, Gavi and Barbera varieties represented this rank with different prices (with a maximum of 1,299 ¥ of 2 Barbera, to 79 ¥ of Moscato2). However, in 2016, only Moscato and one brand of Malvasia were the only Piedmont wines that could be found on Wine9.

Table 4. Piedmont wines on Wine9.com.

Rank	Classification	Variety	Price/Yuan	Sales volumes (bottles)
1	/	Moscato 1	168	13772
2	/	Moscato 2	79	13456
3	DOCG	Gavi 1	398	4469
4	DOCG	Barbera 1	238	3702
5	DOCG	Moscato 3	99	3629
6	DOCG	Gavi 2	298	3304
7	DOC	Barbera 2	1299	3140

Ending in 2014, 7 from Piedmonts wines on Wine9.com.

Table 4. Continues.

Rank	Classification	Variety	Price/Yuan	Sales volumes (bottles)
1	/	Malvasia 1	118	1821
2	/	Moscato 4	89	1820
3	/	Moscato 5	78	1742
4	/	Moscato 6	88	698

Ending in 2016, 4 Piedmonts wines on Wine9.com

Analyzing the ranking of 25 Italian wines sold through Jiuxian, in 2014 the top 5 wines sold were Piedmont table wines sold at a maximum price of 59 ¥ and a minimum of 29 (Piedmont table wine is the lowest in the top 25 of Italian wines). In general Piedmont wine accounted for 52% of Italian wine sold. The most expensive wine sold in 2014, and ranked 17th, was a DOCG in Apulia at 269 ¥. In 2016, Piedmont wines were reduced by 36%, which fell to the last place in the ranking. However, regarding table wines, they were replaced in 2016 by important DOCGs. Nebbiolo, with high prices (1099 ¥) was in 22nd place. At the top of this ranking was a Lambrusco of Emilia Romagna (168 ¥), followed by a Merlot Veneto and, third, a Piedmont Muscat (79 ¥).
In 2016, Piedmont wines sold on Jiuxian decreased by -50% (from 18 to 9 products) (Table 5).

Table 5. Piedmont wine on Jiuxian.com.

Rank	Classification	Variety	Price /Yuan
1	WGVT	Grapes mixture 1	59
2	/	Grapes mixture 2	69
3	WGVT	Merlot, Sangiovese 1	49
4	WGVT	Merlot 1	29
5	WGVT	Moscato 7	39
6	DOCG	Moscato 8	128
7	/	Moscato 9	79
8	/	Moscato 10	69
9	/	Moscato 11	59
10	/	Moscato 12	69
11	WGVT	Dolcetto, Barbera, Pinot nero 1	99
12	WGVT	Chardonary, pinot grigio 1	59
13	WGVT	Barbera 3	79
14	/	Malvasia 2	96
15		Moscato 13	118
16	DOC	Barbera 4	88
17	DOC	Croatina1	398
18	DOC	Barbera 5	699

Piedmont wine present on Jiuxian.com in 2014.

Table 5. Continues.

Rank	Classification	Variety	Price /Yuan
1	DOCG	Moscato 8	79
2	/	Mix 1	118
3	/	Malvasia 1	79
4	/	Prosecco 1	158
5	/	Moscato 14	149
6	DOCG	Nebbiolo 1	1099
7	/	Moscato 13	118
8	/	Moscato 9	89
9	/	Moscato 4	78

Piedmont wine present on Jiuxian.com in 2016.

This development saw the elimination of table wines and the affirmation of sparkling wines. Furthermore, there was an introduction of quality wines, such as the Nebbiolo variety selling at the price of 1,099 ¥.

In Table 6 a comparison between the sales ranks of Piedmont wines on the various online sales platforms is reported.

Table 6. Comparison between ranks of Piedmonts wines sold by the different considered online platforms in the two years (2014 and 2016).

	Yesmywine				Jiuxian				Wine9			
	2014		2016		2014		2016		2014		2016	
Rank	Variety	Price/Yuan	Variety	Price/Yuan	Variety	Price/Yuan	Variety	Price /Yuan	Variety	Price/Yuan	Variety	Price/Yuan
1	Malvasia	68	Sparkling and sweet Malvasia	65	Grapes mixture 1	59	Moscato 8	79	Moscato 1	168	Malvasia 1	118
2	Martini	107	Fragolino	98	Grapes mixture 2	69	Mix 1	118	Moscato 2	79	Moscato 4	89
3	Moscato	89	Moscato 2	69	Merlot, Sangiovese 1	49	Malvasia 1	79	Gavi 1	398	Moscato 5	78
4	Moscato 2	79	Grapes mixture 1	68	Merlot 1	29	Prosecco 1	158	Barbera 1	238	Moscato 6	88
5	Barolo	259	Sweet white sparkling wine	59	Moscato 7	39	Moscato 14	149	Moscato 3	99	\	\
6	Moscato fruit	105	Moscato sparkling	110	Moscato 8	128	Nebbiolo 1	1099	Gavi 2	298	\	\
7	Moscato rose	148	Moscato 15	212	Moscato 9	79	Moscato 13	118	Barbera 2	1299	\	\

4. DISCUSSION AND CONCLUSIONS

Our research has confirmed the rapid evolution and development of the wine market, in particular Italian and Piedmont wines, in China. The use of online sales has made this market even more dynamic in the short-term and becoming increasingly accessible by more target consumers. The more important wine e-commerce platforms are Yesmywine, Wine9, Jiuxian, and the comprehensive platform, Taobao.

Italian wine is ranked fifth in the wine market in China, after France, Chile, Spain and Australia, but Italian sparkling wine ranks first. In fact, our research has found that buying sparkling wine is experiencing a period of strong expansion, especially for red sparkling wines. This was evident when comparing 2014 with 2016. Consumer profiles from Taobao data described young consumers being especially attracted to medium-high level products in terms of price. This result is confirmed also in FOUNTAIN and ZHU (2016). The evolution of red wine typology purchased from 2014 to 2015 also justifies the changes in the Italian wine rankings sold through the other online sales platforms considered in this work. The data confirmed that the Chinese red wine market has grown by 66% from 2009 to 2014 (EUROMONITOR, 2015), and 92% of wine consumption in 2013 was red (HERMOSO, 2014).

Potential consumers are those who are looking up wine on e-commerce platforms, but not necessarily purchasing the products. It's worth noting that consumers looking for "Italian wine" on Taobao do not mean that they are buying it (MITRY *et al.*, 2009; LEE, 2009). Most of the potential consumers are concentrated in the southeastern coast of China because are concentrated mainly in big cities. However, the society in rural China has changed under the network economy and the e-commerce developments (GENG *et al.*, 2016).

In recent years, the imported Italian bulk wine has had a slight increase in volume; however it still maintains a high average price among the important countries. This phenomenon ensures that the Chinese market does not have low-end Italian wines and protects the high-end Italian wine market.

Our analysis of the top 25 sellers from professional online platforms shows that Chinese consumers prefer sparkling wine from Piedmont and Emilia Romagna. The typical original red wines from Veneto and Tuscany are more saleable. The data shows Piedmont sparkling wines are much more popular in China compared to the Piedmont red wines. However, the tendency of Chinese consumers to increasingly buy expensive wines, compared to previous years (ZENG and SZOLNOKI, 2016), is confirmed by our results that highlight as consumers are willing to pay a high price for Piedmont red wines like Barolo. As reported in MASSET *et al.* (2016), the increased interest in fine wine consumption in China is linked to improved economic opportunities and political transparency has led the population to adopt traits from Western lifestyles. At the same time, Chinese government policies have altered consumer behavior by favoring healthier red wine consumption. Finally, a more educated and wealthy class has emerged a fondness for fine wine (SUN, 2009).

Compared to Wine9 and Jiuxian, Yesmywine has more Italian wine and better descriptions of its wine, but problems still exist. Italian wineries should enhance cooperation with the Chinese importers like Yesmywine. This includes supervising the marketing process and assisting the importers with the wine descriptions on their websites. This could be done by wine producers in Italy translating its brand and wine in Chinese for the convenience of local consumers, and try to associate the wine with its cultural and historical background, an element loved by Chinese consumers. Additionally, Italian wine producers should try to explain how the wine can be combined with various Chinese dishes on the description pages. If there is no appropriate Chinese dish to pair with the wine, try to introduce some Italian typical dishes, and combine with Italian food culture to attract consumers (LOCKSHIN *et al.,* 2016). Traditionally, wine is developed in each locality as an accompaniment to local foods. Wine is now traded globally, creating challenges for matching wine with cultural customs and cuisines typical of countries where wine is not a traditional beverage. Furthermore, imported wine is also considered a status symbol in some countries (LOCKSHIN, *et al.,* 2012; 2016). To buy wine in China is more than just the product, but it's also buying the foreign wine culture (CHINA MARKET, 2011). The translation of wine brands and labels plays a key role in conveying the wine's message to potential consumers and these translations must deal effectively with culture-specific items (CHAMPNEY, 2014). Parallel, China's e-business environment is ripe full of opportunities for wine sellers due to the following reasons: first, China does not have a mature wine retail terminal chain compared with Italy. Second, China has a mature Internet platform system and a huge user-base. Third, China is mature in third-party payment providers and has low logistics costs.

A statistically significant difference between online and offline border effects in China has been demonstrate by LI *et al.* (2016). In online trade, unlike offline trade, local governments face difficulties in controlling border effect and to restrict online trade: this effect due to the existence of government protectionism in the offline market should be used by Italian producer of wines.

Data from Taobao showed that the 25-35 age group has more preference for Italian wines than French ones. Social media has significantly influenced this age group, which is a big opportunity for wine producers to advertise their products. This is seen as an effective tool compared to conventional advertising since it provides quick and informative details and gives rise to an increased number of responses (EISEND & KUSTER, 2011; LOCKSHIN *et al.,* 2016).

In 2015, Wine9 gradually ended cooperation with the importers and began to purchase and import wines all on their own for the purpose of increased profits. This reform had a negative impact on the Piedmont wine industry. The change also created new problems of developing a professional online wine platform. For example, they didn`t have experience with wine selection or strong financial support that large importers have. When they skipped the importers, Wine9 had difficulty with wine selection and the financial support was not enough to support the diversity of wine. These reasons caused the varieties to decrease Piedmont wine on Wine9. However, this phenomenon just shows how important the process is for the development of professional online wine platforms and the cooperation with importers.

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PAPER

EFFECT OF DIFFERENT STORAGE CONDITIONS ON THE SHELF LIFE OF NATURAL GREEN TABLE OLIVES

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ABSTRACT

The aim of this research is to study the effects of different storage conditions on Spanish (alkaline debittering) and natural (directly brined) green olives. Laboratory-processed olives were stored in 6% brine or in a vacuum bag without brine, at 6 or 20°C. After 18 months, natural olives showed higher microbial and olive oil stability than NaOH-treated olives. The lower pH (<4.80) and higher total phenol content (0.2 g/100 g wet pulp) influenced positively the long shelf life of natural olives. The packaging in 6% NaCl brine and in a vacuum bag stored at 20°C gave better performance, while growth of psychrophilic spoilage bacteria occurred at cold temperature.

Keywords: microbial count, natural green olives, oil oxidation, phenols, sensory profile

1. INTRODUCTION

World consumption of table olives (TO) continues to increase, passing from 957 to 2,480 thousand tones in the period from 1990/91–2014/15 (IOOC, 2017). Major producer countries are located in the Mediterranean basin (Spain, Greece, Italy, Turkey, Tunisia, Morocco, Egypt, and Algeria). Green Spanish-style, natural black Greek-style and black ripe Californian are the most popular TO types; however, many other typologies are produced according to local traditions (ERCOLINI *et al*., 2006). In fact, TO types can vary depending on olive fruit kind (green or black olives), debittering method (chemical or biochemical), fermentation conditions (temperature, levels salt, and type of acid) and finally, canning (SANCHEZ *et al*., 2006).
Debittering and fermentation are the main steps of the table olive process. The first is fundamental to remove natural bitterness, while the fermentation is important to prevent microbial spoilage. The typical strong bitter and pungent taste of green olives is due to the high presence of oleuropein, a secoiridoid containing elenolic acid glucoside linked to hydroxytyrosol (AMBRA *et al.*, 2017; GHANBARI *et al*., 2012). In the chemical process, such as the Spanish-style method, olives are debittered in a few hours through a bath in a sodium hydroxide solution that hydrolyzes oleuropein quickly into less bitter compounds (CHAROENPRASERT and MITCHELL, 2012); subsequently, olives are water-washed repeatedly and then brined in 8-10% NaCl where a spontaneous lactic acid fermentation occurs in the following months (APONTE *et al*., 2010). Olive fermentation is caused by indigenous microbiota, including lactic acid bacteria (LAB) and/or yeasts, which transform the sugars in lactic acid with a consequential decrease in pH; in addition, substances inhibiting undesirable microorganisms are produced (BEVILACQUA *et al*., 2009). Moreover, several microbial strains are able to hydrolyse oleuropein contributing to olive debittering (IORIZZO *et al*., 2016; SERVILI *et al*., 2006). IOOC (2017) indicates the natural olives as "green olives, olives turning colour or black olives placed directly in brine in which they undergo complete or partial fermentation, preserved or not by the addition of acidifying agents". Therefore, raw olives are placed in water or brine as long as required for the spontaneous loss of bitterness. In this case, oleuropeinolytic bacterial/yeast strains degrade oleuropein in synergy with specialized enzymes naturally occurring in the olives (RAMIREZ *et al*., 2017a; RAMIREZ *et al*., 2016). However, olive debittering is slow and several months are needed to obtain a satisfactory good-tasting product (SANCHEZ *et al*., 2006).
In recent years, demand of organic and natural foods has become trendy (ROZIN *et al*., 2004). In this contest, the demand for natural TO risen since they have become popular and appreciated for the nutritional value and distinctive sensory characteristics. Several studies on natural TO have been carried out focusing on the content of bioactive components (CHAROENPRASERT and MITCHELL, 2012; SAKOUHI *et al*., 2008) and microbial characterization (BLEVE *et al*., 2015; PEREIRA *et al*., 2008). Moreover, also new systems to accelerate olive debittering with biotechnological approaches as an alternative to chemical treatment have been tried obtaining positive results by using enzymes (DE LEONARDIS *et al*., 2016), selected LAB (PERRICONE *et al*., 2013; HURTADO *et al*., 2010), batches in modified brine (RAMIREZ *et al*., 2017b) and vacuum impregnation (TAMER *et al*., 2013).
In this study, several chemical, microbial and sensory parameters have been tested in green olives laboratory-processed through both the Spanish-style and natural methods and stored for a period of time 18 months, under different conditions. Specifically, the effects of storing in brine were compared with those in vacuum bags, without brine, in order to evaluate the possibility to reduce the salt, potentially harmful to health. Finally, considering that in the supermarkets the packaged olives are frequently placed in

refrigerator, the effects of cold temperatures have been studied. The results could be useful to the improvement of natural olives, in olive packaging and conservation systems.

2. MATERIALS AND METHODS

2.1. Chemicals

All reagents were of analytical or HPLC grade. Gallic acid, hydroxytyrosol and oleuropein were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). All the media and the supplements for the microbiological analysis, unless otherwise stated, were from Oxoid, Basingstoke, Hampshire, UK.

2.2. Olive processing and storage conditions

About 10 kg of green or yellow-green olive fruits were handpicked from 'Cazzarella' trees, a dual-purpose autochthonous cultivar grown in the Molise region (Italy). At the laboratory, the olive batch was split and processed as described synthetically in Table 1.

Table 1. Olive sample typologies.

1. Raw olives:	*fresh untreated olives.*
2. B1-NaOH / B1-Nat:	*olives brined in 6% NaCl solution (first brining) and stored in the dark under 20 °C constant temperature for 9 months.*
3. B2-NaOH / B2-Nat:	*olives of point 2 brined in 6% NaCl fresh solution (second brining) and stored in the dark under 20 °C constant temperature for other 9 months.*
4. C-NaOH / C-Nat:	*olives of point 2 packaged without brine in vacuum bags and stored in a refrigerator under 6 °C temperature (C = cold) for other 9 months.*
5. T-NaOH / T-Nat:	*olives of point 2 packaged without brine in vacuum bags and stored in the dark under 20 °C constant temperature (T = tempered) for other 9 months.*

-NaOH: processed by Spanish method;
-Nat: natural processed.

In the preparation of the B1-NaOH sample, olives were placed into a 2% NaOH solution for 16 hours (ratio olive/lye 1:1.3, w/v); olives were washed repeatedly with water until neutral pH and brined into 6% NaCl solution (ratio olive/brine 1:1.3, w/v) in three different closed glass jars. In the case of the B1-Nat sample, the olives were immersed directly into 6% NaCl solution (ratio olive/brine 1:1.3, w/v); three weeks after (21 days), the brine was renewed with a fresh 6% NaCl solution. All jars were stored in the dark under 20°C constant temperature for 9 months. Thus, the olives were water-washed repeatedly, analyzed, repackaged and stored for another 9 months as described in Table 1 (B2-, C-, T-).

2.3. Analytical determinations

Moisture and total fat were determined on the pulp separated manually from the stone and homogenized. Moisture was determined at 105°C on about 5 g of pulp; successively, oil content was measured in dry pulp by using Soxhlet extraction with 40-60°C petroleum ether. Lactic acid, pH and total phenol were determined on both olive pulp and brine.

Brine was filtered through a 0.45 μm PVDF syringe-filter before assaying. As for the pulp, about 20 g of fresh pulp was dispersed in 60 mL of distilled water (1:3, w/v) and the flask was put into a sonicator bath for 20 minutes at room temperature by filtering the aqueous phase on paper. The pH measurement was performed through a pH-meter, while the lactic acid was measured by titration with 0.1 M NaOH up to pH 7.0. Free acidity and fatty acid composition were determined on the oil extracted at cold temperatures from 100 g of olive pulp by following the methods described in PASQUALONE *et al*. (2014). The free acidity (expressed as g oleic acid per 100 g pulp olive) was measured following the olive oil European Union Commission (1991), while the fatty acid composition was determined by gas-chromatographic analysis as described in DE LEONARDIS and MACCIOLA (2012). *p*-anisidine value was determined on the cold extracted oil according to AOCS (1998). Phenol extraction from the olive pulp, determination of total phenols and the HPLC analysis with UV detector were carried out in the same conditions described in DE LEONARDIS *et al*. (2016). Hydroxytyrosol (Hy), dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (HyEDA) and oleuropein (OLE) were monitored and each of them was quantified through a hydroxytyrosol calibration curve derived from a plot of area counts versus concentration. Hy-compounds were calculated as the sum of Hy, HyEDA and OLE expressed as mg/100 g wet pulp olives as Hy equivalent.

2.4. Microbial counts

Microbiological analysis was carried out on the olive pulp according to the method reported in IORIZZO *et al*. (2016) with modifications. For each sample, about 30 g of olives were homogenized in 0.9% NaCl (w/v) for 1 min in a Stomacher bag (Bag Mixer-400). Pulp homogenates were diluted serially and plated in triplicate by using the following growth media and incubation conditions: Plate Count Agar (PCA) at 30°C (72 h) and 15°C (5 days) for total mesophilic and psychrotrophic aerobic bacteria; Violet Red Bile Glucose Agar (VRBGA) at 37°C for 24 h for *Enterobacteriaceae*; MRS Agar, added with 0.17 g/L of cycloheximide (Sigma-Aldrich Co, St. Louis, MO, USA), at 30°C for 4 days in anaerobiosis (Anaerogen kit, Oxoid, Basingstoke, United Kingdom) for lactic acid bacteria (LAB); YPD agar (1% yeast extract, 2% peptone, 2% glucose and 2% agar) supplemented with 100 mg/L chloramphenicol at 28°C for 72 h for yeasts; *Pseudomonas* Agar Base (PAB), added with CFC selective supplement, at 30°C for 48 h for *Pseudomonas* spp.

2.5. Sensory analysis

Sensory profile was evaluated according to Galán-Soldevilla and Pérez-Cacho (2012), with modifications. The panel group was composed of 15 untrained volunteers selected and led by one olive oil expert taster. Training and alignment of panel and sensory profile sheet was developed by using similar commercial products. Nine attributes (defects, fruit odor, salty, bitter, acid, firmness, fibrous, crunchy and overall opinion) were selected and evaluated through a seven-point hedonic scale (7: like extremely; 6: like very much; 5: like slightly; 4: neither like nor dislike; 3: dislike slightly; 2: dislike very much; 1 = dislike extremely). Value zero was given for absent attribute. During the panel training, panel leader held a discussion on the descriptors and a consensus lexicon was developed about the following nine attributes (GALÁN-SOLDEVILLA and PÉREZ-CACHO, 2012): a) defects (presence of abnormal negative odor/aroma); b) gustatory sensations (salty: typical taste produced by sodium chloride aqueous solutions; bitter: basic taste produced by diluted aqueous solutions of caffeine: acid: basic taste produced by aqueous solutions of substances like citric acid); d) fruity odor (odor/aroma characteristic of fresh olives); e) kinesthetic sensations (firmness: mechanical property of texture related to the strength required to attain a certain penetration of the olive; fibrous: geometrical property of texture related to the perception of strands oriented in the same direction; crunchy: mechanical property of texture related to the cohesion and strength necessary to break an olive with teeth); f) overall judgment (general grade of appreciation).

2.6. Elaboration data

Each jar was analyzed independently in duplicate (six independent replicates for every sample), by calculating mean and standard deviation. Means obtained were compared through ANOVA (Duncan's multiple range post hoc tests at $p<0.05$) by using the SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Basic olive pulp characteristics

Processing method strongly affects the final characteristics of table olive (SANCHEZ *et al.*, 2006) and the data reported in Table 2 confirm this evidence.
Olive water content was initially 69.4%. Overall, water content in the processed olives was generally higher in the olives treated with NaOH than in the natural-processed (Nat). B1-NaOH and B2-NaOH samples, stored in the brine, kept the original moisture (69.6 and 72.6%, respectively), while the matching B1-Nat and B2-Nat samples showed a lower value (63.7 and 67.2%, respectively). However, the observed differences were not statistically significant. Conversely, significant moisture reduction was observed in the olives stored in the vacuum bags. Specifically, water content was 60.1, 59.0, 53.0 % in the C-Nat, T-NaOH and T-Nat samples, respectively.
An increase of lactic acid and a decrease of pH expected as result of the microbial activity. All the NaOH-treated olives had about one-higher-point of pH than the natural olives. More specifically, pH values were 5.17, 5.39, 5.56 and 5.73 in B1-NaOH, B2-NaOH, C-NaOH and T-NaOH, respectively. These high pH values are potentially harmful being not fit to inhibit growth of spoilage microorganisms. Actually, brine acid characteristics of the NaOH treated samples (Table 2) were below the requirements for trade according to the IOOC (2004) that has a set the minimum brine pH value at 4.1; however, addition of acidity regulators is permitted.
The pH values of Nat olive pulp were 4.62, 4.42, 4.80 and 4.75 in B1-Nat, B2-Nat, C-Nat and T-Nat, respectively. The pH value was obviously related to lactic acid, which was found in the Nat olives more-than-three-times higher than NaOH-treated olives. According to MARSILIO *et al*. (2005), lye treatment and subsequent water washing presumably caused sugar and nutrient loss from olive pulp giving rise to insufficient acidification in the NaOH-treated samples. In the set group of Nat olives, the maximum and minimum values of lactic acid were C-Nat (0.24%) and B2-Nat (0.11%), respectively. These values are in line with literature (DE LEONARDIS *et al*., 2016; APONTE *et al*., 2012).

3.2. Phenolic content

Many studies have evidenced a close relationship between processing method and content/composition of phenols in table olives (AMBRA *et al.,* 2017; CHAROENPRASERT and MITCHELL, 2012; BLEKAS *et al*., 2002).
In Table 3 phenolic compound evolution is given only for the Nat olives due to the phenol content in the NaOH-treated olives was negligible.

Table 2. Analytical characteristics of the pulp olive samples and relative canned brine.

	Pulp olive			Brine	
	Moisture %	pH	Lactic acid %	pH	Lactic acid g/l
Raw olives	69.4(4.3)^{a,b}	5.67(0.06)^{a}	nd	7.02(0.06)^{a}	n.d.
Stored in the first brine					
B1-NaOH	69.6(5.2)^{a,b}	5.17(0.38)^{b,c,d}	0.02(0.00)^{a}	5.05(0.27)^{b}	0.4(0.0)^{a}
B1-Nat	63.7(5.3)^{b}	4.62(0.34)^{b,c}	0.22(0.02)^{b}	4.26(0.16)^{c}	2.5(0.2)^{b}
Stored in the second brine					
B2-NaOH	72.6(4.2)^{a,b}	5.39(0.41)^{b,c,d}	0.03(0.00)^{a}	5.02(0.25)^{b}	0.5(0.0)^{a}
B2-Nat	67.2(4.1)^{a,b}	4.42(0.26)^{b}	0.11(0.01)^{c}	4.10(0.22)^{c}	2.5(0.2)^{b}
Stored in vacuum bags at 6°C					
C-NaOH	69.1(3.8)^{a,b}	5.56(0.29)^{c,d}	0.07(0.03)^{a}		
C-Nat	60.1(3.3)^{b}	4.80(0.31)^{b,c,d}	0.24(0.02)^{b}		
Stored in vacuum bags at 20°C					
T-NaOH	59.0(3.6)^{b}	5.73(0.29)^{d}	0.03(0.00)^{a}		
T-Nat	53.0(3.2)^{b}	4.75(0.26)^{b,c,d}	0.19(0.03)^{b}		

Values are means (standard deviation) of six independent replicates; letters on the column point out significant difference at $p<0.05$.
-NaOH: lye processed; -Nat: natural processed; n.d. = not detected.

Table 3. Total phenol and hydroxytyrosol (Hy) compounds obtained in pulp olives and related brine (natural olives).

	Pulp olives		Brine
	Total phenols % GAE	Hy-compounds mg/100g as HyE	Total phenols mg/L GAE
Raw olives	1.19(0.08)^{a}	199(13)^{a}	
B1-Nat	0.21(0.01)^{b}	53(4)^{b}	1,791(102)^{a}
B2-Nat	0.14(0.01)^{b}	25(1)^{c}	1,006(132)^{b}
C-Nat	0.20(0.01)^{b}	47(3)^{b}	
T-Nat	0.20(0.01)^{b}	62(4)^{b}	

Values are means (standard deviation) of six independent replicates and different letters on the column point out significant difference ($p<0.05$).

Specifically, Nat olive samples showed total phenol values dropped from 1.19 (raw olives) to about 0.20 g/100g (Nat processed olives) as GAE (gallic acid equivalent), respectively. Although the reduction of the starting phenol content is a coveted result from an organoleptic point of view, presence of residual phenols may be positive from a nutritional point of view, since several phenols are recognized as antioxidant and healthy substances (D'ANTUONO *et al*., 2016; DE LEONARDIS *et al*., 2013; DE LEONARDIS *et al*., 2008; SANCHEZ *et al*., 2006).
At the end of the first brining (B1-Nat), Hy-compound content was 53 mg per 100 g olives and insignificant changes were observed in C-Nat (47 mg/100 g) and T-Nat (62 mg/100g); conversely, Hy-compounds halved during the second brining (B2-Nat, 25 mg/100g).

The European Union Commission (2012) recently approved a health claim for extra virgin olive oil stating that 'daily intake of 5 mg of hydroxytyrosol and its derivatives is able to prevent low density lipoprotein (LDL) oxidation'. Therefore, Nat olives may be claimed as a source of phenols having health-promoting properties. However, the Hy-compound values reported in Table 3 were certainly underestimated because other hydroxytyrosol linked compounds, known to be present in olives (AMBRA *et al.*, 2017; CHAROENPRASERT and MITCHELL, 2012), have been omitted in this study for purposes of simplification of the model.
According some studies (RAMIREZ *et al.*, 2017a; Ramirez *et al.*, 2016; DE LEONARDIS *et al.*, 2015), biological and enzymatic oleuropein depletion may occur through a rapid transformation in Hy-EDA and subsequent hydrolysis with gradual Hy release. A similar pathway occurred also in the Nat olives, as noted in the graph of Fig. 1.

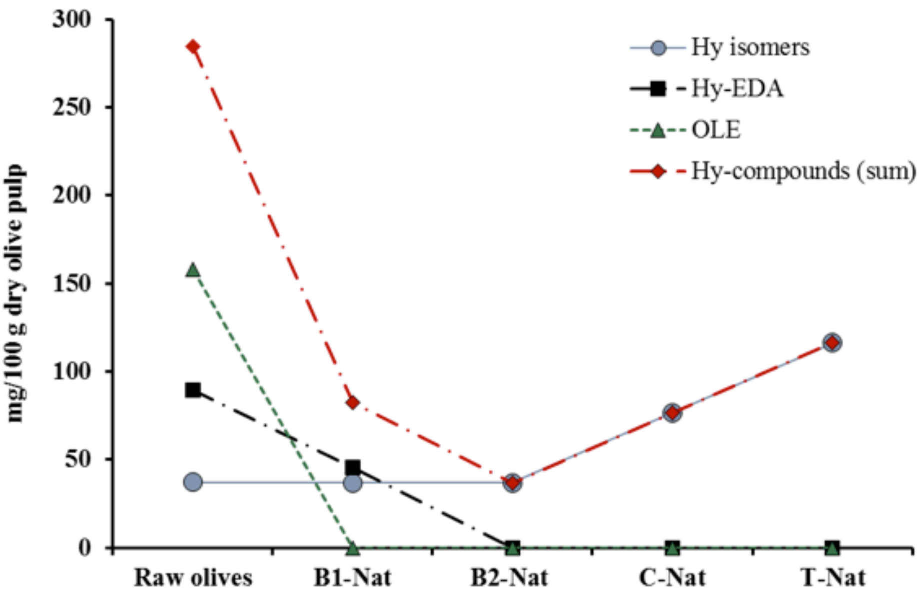

Figure 1. Changes in the table olives of the monitored phenolic compounds, expressed in mg (Hy equivalent) per 100 g dry olive pulp.

Raw olive pulp contained 37, 89 and 158 mg/100 g in dry matter (d.m.) of Hy, Hy-EDA and OLE, respectively. After 21 days of brining (when the first brine was replaced, see Material and Methods), the olive phenol profile was changed in 73, 167 and 22 mg/100 g d.m. of Hy, Hy-EDA and OLE, respectively (data not shown). Hy-EDA is a recognized antimicrobial compound inhibiting the growth of lactic bacteria (MEDINA *et al.*, 2007). After 9 of months brining (B1-Nat), OLE was completely depleted, while Hy-EDA was 45 mg/100 g d.m. Successively, Hy-EDA was undetectable in sample B2-Nat, C-Nat and T-Nat in which only Hy was found in the amounts of 37, 77 and 116 mg/100 g d.m., respectively.

3.3. Lipid fraction

The characteristics of lipid fraction of the raw olives and samples stored for 18 months are given in Table 4.

Table 4. Changes of lipid fraction of olives samples.

	Raw olives	NaOH-processed olives			Nat-processed olives		
		B2-NaOH	C-NaOH	T-NaOH	B2-Nat	C-Nat	T-NAT
Total oil % w.m.	18.4 (1.1)[a]	19.5 (1.2)[a]	19.3 (1.2)[a]	17.5 (1.1)[a]	22.1 (1.3)[b]	22.2 (1.3)[b]	21.6 (1.3)[a,b]
Total oil % d.m.	26.3 (1.6)[a]	26.8 (1.6)[a]	28.2 (1.7)[a]	29.3 (1.8)[a]	33.0 (2.2)[b]	36.5 (2.2)[c]	40.5 (2.5)[d]
Free acidity %	0.5 (0.0)[a]	11.2 (0.7)[b]	15.3 (0.9)[c]	13.7 (0.8)[d]	2.8 (0.2)[e]	5.5 (0.3)[f,a]	3.4 (0.2)[e]
p-Anisidine value	5.9(0.3)[a]	33.5(1.7)[e]	30.7(1.6)[d]	31.6(1.7)[d,e]	12.8(0.7)[b]	29.2(1.5)[d]	23.1(1.2)[c]
Fatty acids %							
C16:0	16.5 (1.0)[a]	23.7 (1.4)[b,c]	24.4 (1.5)[b]	21.6 (1.3)[c,d]	17.0 (1.0)[a]	19.9 (1.2)[d]	18.2 (1.1)[a,d]
C16:1	1.4 (0.1)	1.4 (0.1)	1.3 (0.1)	1.2 (0.0)	1.3 (0.1)	1.4 (0.2)	1.3 (0.1)
C18:0	2.9 (0.2)[a]	4.2 (0.3)[b]	4.6 (0.3)[b]	4.5 (0.3)[b]	3.4 (0.2)[c]	3.9 (0.3)[b,c]	3.8 (0.2)[c]
C18:1	65.5 (4.0)[a]	60.5 (3.7)[a,b]	57.1 (3.5)[b]	60.0 (3.6)[a,b]	64.5 (3.9)[a]	63.0 (3.8)[a,b]	64.3 (2.7)[a,b]
C18:2	11.6 (0.7)[a]	4.2 (0.3)[b]	3.2 (0.2)[c]	5.1 (0.2)[d]	11.3 (0.5)[a]	8.2 (0.3)[e]	9.3 (0.3)[f]
C18:3	0.6 (0.0)[a]	0.1 (0.0)[b]	0.1 (0.0)[b]	0.2 (0.0)[b]	0.6 (0.1)[a]	0.3 (0.1)[a]	0.4 (0.1)[a]
C20:0	0.5 (0.0)[a]	0.7 (0.0)[b]	0.8 (0.0)[b]	0.7 (0.0)[b]	0.5 (0.0)[a]	0.6 (0.0)[a]	0.6 (0.0)[a]
C20:1	0.2 (0.0)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.2 (0.0)	0.2 (0.0)
C22:0	0.1 (0.0)[a]	2.9 (0.7)[b]	4.8 (0.2)[b]	3.8 (0.6)[b]	0.3 (0.0)[c]	1.2 (0.5)[c]	0.7 (0.3)[c]
C22:1	0.0[a]	1.5 (0.6)[b]	2.7 (0.2)[b]	1.9 (0.8)[b]	0.1 (0.0)[a]	0.7 (0.2)[c]	0.3 (0.3)[c]

Values are means (standard deviation) of six independent replicates and different letters on the lines point out a significant difference ($p<0.05$).

A different oil content was observed between NaOH- and Nat-processed olives. Apparently, total oil content increased in the Nat-olives, especially considering total oil on dry matter. TAMER *et al*. (2013) have found that alkali treatment caused oil loss from the olives. Moreover, the advanced fermentation status (with large sugar consumption) and the reduced lipolytic activity (with low fat consumption) could explain the apparent increase of oil in the Nat-olives (Table 4). Unfortunately, we did not have enough data to establish a possible link between oil increase and microbial counts and types (Table 5). According to what was observed in previous studies (PASQUALONE *et al*., 2014; LOPEZ *et al*., 2011), a higher hydrolytic and oxidative oil degradation was observed in the NaOH- *vs* Nat-olives. Specifically, a considerable increase of free acidity was evident in B2-NaOH, C-NaOH and T-NaOH with values of 11.2, 15.3 and 13.7% on oil extracted, respectively; conversely, free acidity was less than 5.5% for the Nat-olives. Formation of free fatty acids could be catalysed by the lipases contained in the fruits or synthesized by the environmental microflora. A positive effect of low temperature on the triacylglycerol hydrolysis was evident due to the high free acidity values found in C-NaOH (15.3%) and C-Nat (5.5%). Similarly, oil oxidation was higher in the cold temperature stored olives, as the *p*-anisidine values show (Table 4). As known, *p*-anisidine assay measures secondary lipid oxidation compounds; the highest *p*-anisidine values were obtained in the oils extracted from the B2-NaOH, C-NaOH, T-NaOH and C-Nat olives.
A fatty acid composition of raw olives (Table 4) was in agreement with the literature (MALHEIRO *et al*., 2012). Oleic acid was the main fatty acid (65.5%), followed by palmitic acid (16.5%) and linoleic acid (11.6%). In order to make Table 4 more readable, the C17:0,

C17:1 and C24:0 fatty acids (found in equal or less amount 0.1%) were deliberately omitted; however, these fatty acids did not change during the storage of the olives.

Significant modifications of fatty acid composition occurred especially in the NaOH-olives, specifically, a reduction of linoleic and linolenic acid was registered in the B2-NaOH, C-NaOH, T-NaOH samples; in the same samples a decrease of oleic acid and an increase of palmitic and stearic acid were obtained. It is known that polyunsaturated fatty acids were more affected by oxidation and therefore decreased at a greater extent (CAPONIO *et al*., 2003; DE LEONARDIS and MACCIOLA, 2012). In the Nat-olives, especially in B2-Nat and T-Nat, the olive residual phenols have limited oil oxidation, as evidenced by the minor changes of *p*-anisidine and fatty acid profile. Unexpectedly, significant increase of the peaks corresponding to behenic (C22:0) and erucic (C22:1) fatty acids were found in the lipid fraction of stored olives. Generally, these long chain fatty acids are present in olive oil in amounts lower than 0.2%, as the data of raw olive oil shows (Table 4). In the NaOH treated olives, C22:0 and C22:1 were found in amounts higher than 2.9 and 1.5%, respectively. In particular, lipid fraction of the C-NaOH sample showed the highest content (C22:0 = 4.8%; C22:1 = 2.7%). Conversely, in the Nat-olives percentage of C22:0 and C22:1 was lower than NaOH olive oil counterpart, but higher than raw olive oil. We have no explanation for this and similar results are missing in literature. Perhaps, it is reasonable to hypothesise a microbial origin of C22:0 and C22:1 in the olives, by considering that few oleaginous microorganisms are able to synthesize long chain fatty acids (EL BIALY *et al*., 2011; RATLEDGE, 2004).

3.4. Sensory analysis

Graphical presentations of the obtained sensory evaluation (average values) are shown in Fig. 2.

The panel did not evaluate color; however, NaOH-olives were overall lightly yellow-green, while Nat-olives were dark gray-green. In general, sensory profile of NaOH-olives was very different from that of Nat-olives; these differences were amplified during the second storage. In general, 'overall opinion' was more positive for Nat- than NaOH- olive samples. The B1-NaOH obtained an 'overall opinion' of 4 points convincing us to continue the storage work for this sample despite its high pH (Table 2). However, at the end of study, B2-NaOH and T-NaOH showed perceptible sensory defects and loss of firmness, fibrous and crunchy, whereas C-NaOH was not evaluated due to its pronounced smell of rotten caused by an abnormal fermentation. In addition, salty was the prevailing, if not unique, taste in the tasted NaOH olives. Conversely, taste of Nat-olives was more complex and well-balanced between salty, bitter and acid tastes. Salty in the brined B1-Nat and B2-Nat was imperceptible, while bitter taste was perceived clearly in all Nat-olives. Effectively, bitter taste is a distinctive but pleasant flavor of the natural-style olives (LANZA and AMORUSO, 2016). The intensity of the bitter taste remained essentially unchanged between the B1-Nat, C-Nat and T-Nat samples, while it decreased in B2-Nat. Finally, a positive fruit odor was perceived clearly up to 18 storage months only in the Nat-olives.

3.5. Microbiological analysis

Fermentation of both NaOH- and Nat-olives occurred spontaneously without adding any starter culture. The microbial count of raw olives highlighted presence of yeasts (log 7.3 CFU/g), LAB (log 2.0 CFU/g) and *Enterobacteriaceae* (log 2.0 CFU/g).

Heperkan (2013) reports that microbiota of olives include principally yeasts and lactic acid bacteria (LAB), members of *Enterobacteriaceae*, *Clostridium*, *Pseudomonas*, *Staphylococcus*, and occasionally moulds. The processing method impacts microbial dynamics affecting greatly quality and shelf life of the TO (DE ANGELIS *et al*., 2015). Generally, indirectly brined green olives yeasts become the predominant population; however, a correct adding of LAB starter may improve lactic fermentation performance (CAMPUS *et al*., 2017; DE LEONARDIS *et al*., 2016; PERRICONE *et al*., 2013; CORSETTI *et al*., 2012).

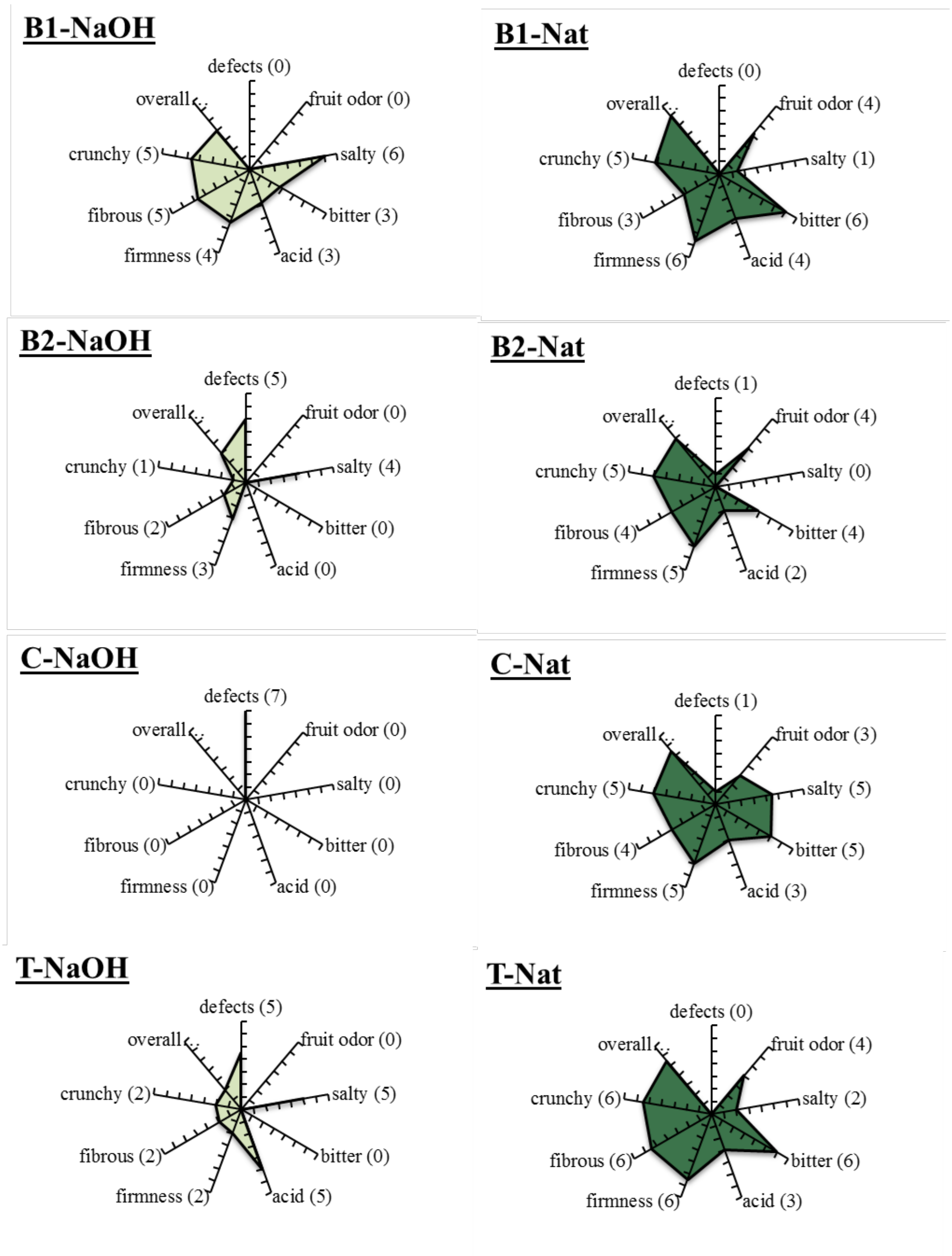

Figure 2. Sensory profile rings of the table olives.

The mean of microbial count and standard deviation of the microorganisms searched in the olive pulp are given in Table 5. The most relevant result was absence of detectable microorganisms in B1-Nat and B2-Nat, apart from yeast cells in B1-Nat (7.1 log CFU/g);

conversely, in all other samples LAB and yeasts coexisted until the end of study (Table 5). In addition, neither *Enterobacteriaceae* nor *Pseudomonas* spp. were found in B2-Nat, C-Nat e T-Nat. Therefore, low pH (Table 2) and high phenol level (Table 3) influenced the microbial profile of B1-Nat affecting positively the subsequent storage of the Nat-olives. Generally, the *Enterobacteriaceae* spp., eliminated during fermentation, are not detected at the end of the process (HEPERKAN, 2013). Nevertheless, *Enterobacteriaceae* cells were found in all NaOH-olives ranging from 3.3 to 4.8 log CFU/g (Table 5). Certainly, in these samples, high pH values and lack of phenols have favoured the development of *Enterobacteriaceae* and *Pseudomonas* spp., which have contributed to the spoilage of B2-NaOH, C-NaOH and T-NaOH samples. Moreover, in C-NaOH sample, *Enterobacteriaceae* and *Pseudomonas* spp. were not inhibited at cold temperature. Indeed, low temperature storage has penalized the populations of yeast and LAB favouring the growth of psychrophilic bacteria, which caused lipolysis, oil oxidation (Table 4), pectolytic action and, putrefaction (Fig. 2). Moreover, also for the Nat-olives, the conservation at cold temperature was proven to be less effective than that in brine and under temperate conditions.

Table 5. Cell count (log CFU/g) of microorganisms determined on pulp olives.

	Media					
	YPD	MRS	PCA (at 30°C)	PCA (at 6°C)	VRBGA	PCFC
NaOH processed olives						
B1-NaOH	6.20 (0.10)[a]	4.90 (0.10)[a]	6.10 (0.20)[a]	6.13 (0.12)[a]	3.50 (0.20)[a]	4.10 (0.20)[a]
B2-NaOH	5.47 (0.15)[c]	4.00 (0.20)[b]	5.00 (0.10)[b,c]	4.90 (0.20)[b]	4.80 (0.20)[b]	0.00 (0.00)
C-NaOH	6.13 (0.15)[a]	5.47 (0.15)[c]	6.10 (0.30)[a]	5.07 (0.15)[b]	3.30 (0.20)[a]	3.80 (0.20)[b]
T-NaOH	5.90 (0.20)[a]	4.67 (0.25)[a]	5.70 (0.20)[d]	5.60 (0.20)[d]	4.03 (0.15)[c]	4.60 (0.30)[c]
Nat processed olives						
B1-Nat	7.10 (0.20)[b]	n.d.	n.d.	n.d.	n.d.	n.d.
B2-Nat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C-Nat	4.60 (0.10)[d]	4.83 (0.59)[a]	4.70 (0.10)[c]	4.00 (0.10)[c]	n.d.	n.d.
T-Nat	5.17 (0.35)[c]	4.50 (0.40)[a]	5.20 (0.40)[b]	n.d.	n.d.	n.d.

Values are means (standard deviation) of three independent replicates; letters on the column point out significant difference at $p<0.05$.
n.d. = not detected.

4. CONCLUSIONS

After 18 months of storage, natural green olives showed good nutritional features (hydroxytyrosol, unchanged fatty acid profile), organoleptic identity, microbial safety, low oil hydrolysis and oxidation. Conversely, after 9 months of storage, pH values below the requirements for the trade were obtained in the NaOH-treated olives. Natural olives preserved high total phenols (0.2 g/100 g wet pulp) and a significant level of the Hy-compounds determined in this study. It is reasonable to suppose that residual phenols have influenced positively polyunsaturated fatty acid preservation and, together with the low pH level, have inhibited the growth of *Enterobacteriaceae* and *Pseudomonas* spp.
Therefore, the slow olive debittering of natural olives was counterbalanced by a prolonged shelf life. The packaging in 6% NaCl renewed brine or in vacuum bag, under a storing

temperature of 20°C, gave the best results, while conservation at cold temperature proved to favor the growth of psychrophilic spoilage bacteria.

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REFERENCE EXAMPLES

EXAMPLES of use in a Reference list are given below. The bold-faced parenthetical type of citation above the example is indicated ONLY for information and is NOT to be included in the reference list.

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