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Development of instrumental and sensory analytical methods of food obtained by traditional and emerging technologies

Presentata da: Patrizia Comandini

Coordinatore Dottorato

Prof. Claudio Cavani

Relatore

Dott.ssa Tullia Gallina Toschi

Correlatore

Dott. Giampaolo Blanda

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Preface

The consumer demand for natural, minimally processed, fresh like and functional food has lead to an increasing interest in emerging technologies. Novel processing techniques are attracting the attention of academic as well as industrial research, becoming commercially very important.

The aim of this PhD project was to study three innovative food processing technologies currently used in the food sector. Ultrasound-assisted freezing, *vacuum* impregnation and pulsed electric field have been investigated. Furthermore, analytical and sensory techniques have been developed in order to evaluate the quality of food and vegetable matrix obtained by traditional and emerging processes.

This thesis has a compilation structure, and the research articles produced during the PhD project have been enclosed and discussed. Owing to the extent of the topics investigated, technological and sensory aspects have been split in different chapters.

The first two chapters contains the list of publications produced and the abbreviations used in the text.

Chapter 3 describes the innovative technologies studied: ultrasound assisted freezing of potatoes, aroma enrichment of apple sticks through *vacuum*, ultrasound and atmospheric impregnation technologies and pulsed electric field treatment of melon juice. The papers produced are attached at the end of each paragraph.

Chapter 4 details the chromatographic analysis developed: phenolic composition of vegetable matrix as olive mill waste water and chestnut bark extracts have been described.

Finally, in chapter 5, sensory techniques are discussed. In particular the development of quantitative-descriptive methods for the evaluation of sensory profile of boiled potatoes and the investigation of the volatile fraction of raw and processed potatoes are reported.

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1. LIST OF PUBLICATIONS

Research article 1. Comandini P., Blanda G., Soto-Caballero M.C., Sala V., Tylewicz U., Mujica-Paz H., Gallina Toschi T. Ultrasound assisted freezing: investigation of the effects on supercooled potatoes. To be submitted to *Journal of Food Engineering.*

Research article 2. Comandini P., Blanda G., Mújica Paz H., Valdez Fragoso A., Gallina Toschi T. (2010). Impregnation techniques for aroma enrichment of apple sticks: a preliminary study. *Food and Bioprocess Technology* 3, 861-866.

Research article 3. Comandini P., Lerma-García M. L., Massanova P., Simó-Alfonso E. F., Gallina Toschi T. Phenolic profiles of olive mill wastewaters treated by membrane filtration systems. To be submitted to *Water Research.*

Research article 4. Comandini P., Lerma-García M. L., Simó-Alfonso E. F., Gallina Toschi T. Tannin analysis of chestnut bark extracts (*Castanea sativa* Mill.) by HPLC-DAD-MS. To be submitted to *Food Chemistry*.

Research article 5. P. Comandini, L. Cerretani, G. Blanda, A. Bendini, T. Gallina Toschi (2011). Characterization of potato flavours: an overview of volatile profiles and analytical procedures. *Food* 5 (SI1): 1-14.

Research article 6. Blanda G., Cerretani L., Comandini P., Gallina Toschi T., Lercker G. (2010). Investigation of off-odour and off-flavour development in boiled potatoes. *Food Chemistry* 118, 283-290.

Research article 7. Comandini P., Blanda G., Soto Caballero M.C., Mujica-Paz H., Valdez Fragoso A., Gallina Toschi T. Sensory profile of three Mexican potato cultivars and off-odors development after boiling. To be submitted to *Journal of Sensory Studies.*

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Torres S.S., Comandini P., Gallina Toschi T., Valdez Fragoso A., Welti Chanes J., Mújica Paz H. (2011). Impregnación de agentes antioscurecimiento en manzana entera para conservar el color del jugo. *Proceedings XXXII Encuentro Nacional y 1er. Congreso Internacional de la Amidiq*, Cancun (Mexico), 3rd-6th May 2011.

Comandini P., Lerma-García M.J., Massanova P., Herrero-Martínez J.M., Simó-Alfonso E.F., Gallina Toschi T. (2011). HPLC-DAD-MS evaluation of phenolic profiles of olive mill wastewaters treated by membrane filtration systems. *36th International Sysmposium on High-Performance Liquid Phase Separations and Related Techniques*, 19th-23rd June 2011, Budapest, Hungary.

Comandini P., Lerma-García M.J., Massanova P., Herrero-Martínez J.M., Simó-Alfonso E.F., Gallina Toschi T. (2011). Tannin analysis by HPLC-DAD-MS of chestnut bark extracts (*Castanea sativa* Mill.). *36th International Sysmposium on High-Performance Liquid Phase Separations and Related Techniques*, 19th-23rd June 2011, Budapest, Hungary.

Comandini P., Valdez Fragoso A., Soto Caballero M.C., Soria Hernández C.G., Mujica-Paz H., Gallina Toschi T., Welti Chanes J. (2011). Effects of pulsed electric fields on total phenol content of melon (*Cucumis melo* L.) juice. *Innovation Food Conference 2011*, 12th-14th October 2011, Osnabrück, Germany.

Comandini P., Valdez Fragoso A., Soto Caballero M.C., Soria Hernández C.G., Mujica-Paz H., Gallina Toschi T., Welti Chanes J. (2011). Polyphenoloxidase and pectin methylesterase activity in melon (*Cucumis melo* L.) juice treated by pulsed electric fields. *Innovation Food Conference 2011*, 12th-14th October 2011, Osnabrück, Germany.

Comandini P., Soto Caballero M. C., Blanda G., Gallina Toschi T. Freezing by means of sonocrystallization. A learning procedure on distilled and sparkling water. To be submitted to *Journal of Food Science Education.*

DISSEMINATION EVENTS

Gallina Toschi T., Comandini P. (2010). "È una montatura! Gli ultrasuoni e la maionese light" *La Notte dei ricercatori*, 24th September 2010, Cesena, Italy.

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2. ABBREVIATIONS USED

US	Ultrasound
P_B	Blake threshold
P_v	Vapour pressure
Σ	Surface tension
R_0	Initial nanobubble radius
P_0	System pressure
VI	Vacuum impregnation
AI	Atmospheric impregnation
HDM	Hydrodynamic mechanism
X	Volume fraction of the sample impregnated
r	Compression ratio
ϵ_e	Effective porosity
ρ_1	Initial gas pressure in the pores
ρ_2	External system and capillary pressure
DRP	Deformation-relaxation phenomenon
γ	Sample volume deformation at the end of the impregnation process
γ_1	Sample volume deformation at the end of the vacuum application
USI	Impregnation assisted by ultrasound
VUSI	Impregnation assisted by vacuum and ultrasound
PEF	Pulsed electric field
PME	Pectin methylesterase
PPO	Polyphenoloxidase
LOX	Lipoxygenase
PG	Polygalacturonase
POD	Peroxidase
TP	Total phenol
a_w	Water activity
E	Electric field
F	Pulse frequency
T	Treatment time

RA	Relative residual enzyme activity
A_t	Enzyme activity units after treatment
A_0	Enzyme activity units before treatment
PC	Phenolic compounds
OMWW	Olive mill waste water
MF	Microfiltration
UF	Ultrafiltration
NF	Nanofiltration
RO	Reverse osmosis
HHDP	Hexahydroxydiphenic acid
HPLC	High performance liquid chromatography
DAD	Diode array detector
ESI-MS	Electrospray ionization-mass spectrometry
SDE	Simultaneous distillation and extraction
SPME	Solid-phase microextraction
GC-MS	Gas chromatography-mass spectrometry
GC/O	Gas chromatography/olfactometry
GC-FID/O	Gas chromatography-flame ionization detector/olfactometry
GC-MS/O	Gas chromatography-mass spectrometry/olfactometry
QDA®	Quantitative descriptive analysis

3. EMERGING TECHNOLOGIES IN FOOD PROCESSING

3.1 ULTRASOUND-ASSISTED FREEZING

3.1.1 Overview of ultrasound applications in food industry

The use of ultrasound within the food industry has been a subject of research and development for many years and the sound ranges used can be basically divided into diagnostic (5 MHz – 10 MHz) and power ultrasound (20-100 kHz) (Mason, 1998).

In table 3.1.1 are listed some uses of power ultrasound in food technology which have been widely described in several reviews (McClements, 1995, 1997; Mason *et al.*, 1996; Povey and Mason, 1998; Li and Sun, 2002; Knorr *et al.*, 2004; Zheng and Sun, 2006; Dolatowski *et al.*, 2007; Ulusoy *et al.*, 2007; Feng *et al.*, 2008; Patist and Bates, 2008; Chemat *et al.*, 2011).

Table 3.1.1 Some uses of power ultrasound in food processing (Mason, 1998).

Mechanical effects
Crystallization of fats, sugars, etc.
Degassing
Destruction of foams
Extraction
Filtration and drying
Freezing
Mixing, homogenization and emulsification
Precipitation of airborne powders
Tenderization of meats
Brining, pickling and marinating
Chemical and biochemical effects
Bactericidal action
Effluent treatment
Modification of growth of living cells (stimulation/destruction)
Alteration of enzyme activity
Oxidation processes
Sterilization of equipment

The energy amount of the sound field is an important criterion for the classification of ultrasound applications. High-intensity ultrasound parameters include sound power (W), sound intensity (W/m^2), sound energy density (Ws/m^3), treatment temperature, and treatment time (Knorr *et al.*, 2004; Bermúdez-Aguirre *et al.*, 2011).

Low energy ultrasound applications frequently range at intensities lower than 1 W/cm^2 and at frequencies higher than 100 kHz. They are used for non-invasive detection (process control) and for characterizing physicochemical properties of food material (product assessment or control) (Mason and Luche, 1996; Villamiel and De Jong, 2000).

High energy ultrasound applications are usually found at intensities higher than 1 W/cm^2 and at frequencies between 18 and 100 kHz (McClements, 1995; Povey and Mason, 1998; Villamiel and De Jong, 2000) however, the majority are restricted to the range 20-40 kHz, i.e. the traditional ranges employed for cleaning, cell disruption and plastic welding (Mason, 1998).

3.1.2 Ultrasonic cavitation

The application of power ultrasound in food processing takes advantage of the chemical and the mechanical effects of cavitation.

As the ultrasonic pressure wave passes through the medium, regions of high and low pressure are created. The size of these pressure variations are referred to as the amplitude of the pressure wave or the acoustic pressure, and they are directly proportional to the amount of energy applied to the system. In compressible fluids (such as air) or at low intensity ultrasonic waves this pressure will induce motion and mixing within the fluid (acoustic streaming) and this movement is sufficient to accommodate these pressure variations (Kentish and Ashokkumar, 2011).

However, most liquids are inelastic and incompressible and thus cannot respond as easily in this manner. If the changes in pressure are great enough, the local pressure in the expansion phase of the cycle falls below the vapour pressure of the fluid, causing microbubbles of gas and vapour or cavities to grow; they relieve the tensile stresses created by the pressure wave. Scientific theory would suggest that the acoustic pressure variation required for this to occur is very large, up to 3,000 MPa. However, in practice, these microbubbles form at relatively mild acoustic pressures. It is generally believed that this is because any liquid already contains cavities of gas or nanobubbles, and that these nuclei assist in the formation of microbubbles (Suslick, 1991; Kentish and Ashokkumar, 2011). The bubble formation process is known as cavitation: in the rarefaction phase of the pressure wave, a bubble is created by the local fluid tension, or is expanded due to the decrease in

pressure. During compression, the increase in pressure either contracts the void or bubble to a smaller size, or eliminates it by implosion (figure 3.1.1). Generally, these processes are nonlinear because the changes in the radius of the void are not proportional to the variation in acoustic pressure (Young, 1989).

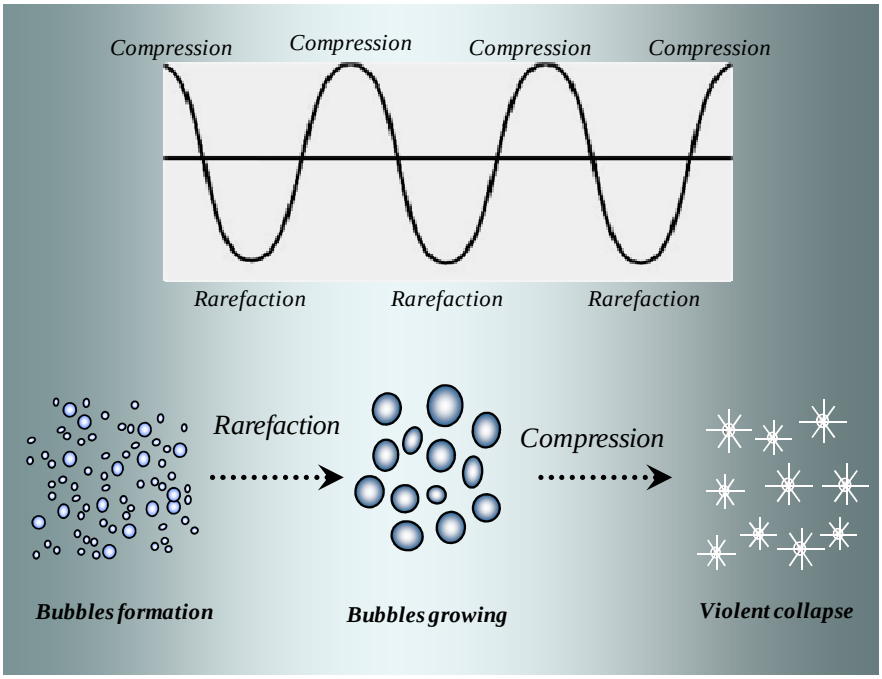

Figure 3.1.1 Sound transmission through a medium and generation of a cavitation bubble.

Cavitation is observed to occur above a definite threshold in ultrasonic intensity. Intensity, in this context, refers to the mechanical-power density supplied by the ultrasound device, and may vary spatially owing to the geometry of the container and the configuration of the point at which ultrasound is delivered. For example, if ultrasound is delivered by a probe the intensity is high near the tip, and falls off dramatically as the distance increases (McCausland *et al.*, 2001).

The lowest acoustic pressure at which cavitation is observed is called cavitation or Blake threshold (P_B), and is a function of the solution vapour pressure (Pv), the surface tension (σ), the initial nanobubble radius (R_0), and the system pressure (P_0) (Leighton, 1994; Kentish and Ashokkumar, 2011):

$$P_B = P_0 - P_v + \frac{4}{3}\sigma\sqrt{\frac{2\sigma}{3(P_0 + 2\frac{\sigma}{R_0} - P_v)R_0^3}}$$

The voids or cavities generated by ultrasound can be stable or transient. Stable cavities exist for a number o sonic pressure cycles and oscillate, usually nonlinearly, around some mean equilibrium size; usually their growth rate during rarefaction is equivalent to their rate of contraction during the compression phase (Thompson and Doraiswamy, 1999). Transient cavities exist for a single or a few acoustic cycles. They may enlarge several times, becoming larger than their original size during expansion, and collapsing violently during compression. This mechanism creates extreme temperatures and pressures (McCausland *et al.*, 2001). The order of temperature and pressure variations can be, respectively, of 5500°C and 50 MPa (Leighton, 1998). In the small spatial regions where cavitation occurs, also called “hot spots” (Henglein, 1987), the temperature can change at a rate of about 109°C/s (Suslick, 1991). In any cavitation field, the majority of visible bubbles are in stable oscillation, causing acoustic micro-streaming. However, the relationships between stable and unstable cavitation are quite complex and time-dependent, and stable cavities may evolve into transient ones via a number of mechanism (Young, 1989).

The cavitation has a variety of effects within the liquid medium depending upon the type of system in which it is generated. These systems can be broadly divided into homogeneous liquid, heterogeneous solid/liquid and heterogeneous liquid/liquid phase systems (Suslick, 1997).

When cavitation occurs in an homogeneous liquid-phase system, the rapid collapse of the bubble generates shear forces, which can produce mechanical effects in the bulk liquid immediately surrounding the cavity. Instead, the extreme conditions of temperature and pressure inside the collapsing bubble can produce chemical effects, reacting with any species introduced inside.

As regards heterogeneous solid-liquid systems, two main situations are considered: the presence of a solid surface in the liquid phase or the presence of dispersed powder.

The collapse of a cavitation bubble near a surface is non-symmetric (figure 3.1.2) because the surface provides resistance to liquid flow from its side. The powerful jet targeted at the surface can dislodge dirt and bacteria, determining the decontamination also of crevices that are not easily to clean by conventional methods (Mason *et al.*, 1996). This effect can also activate solid catalysts and increase mass and heat transfer to the surface by disruption of the interfacial boundary layers.

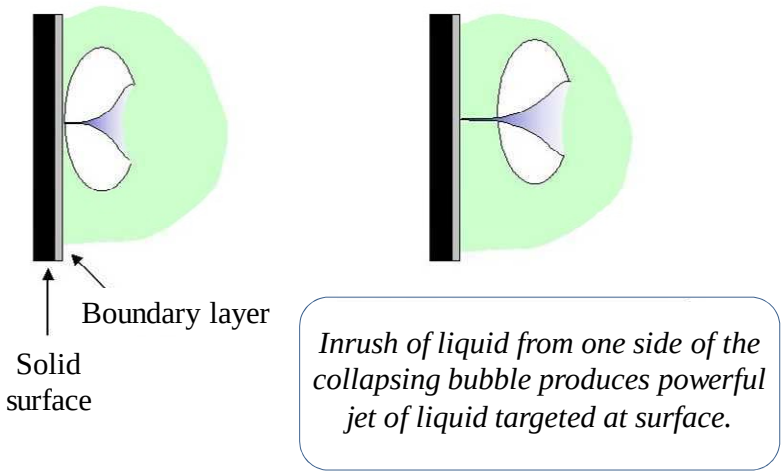

Figure 3.1.2 Cavitation bubble collapsing near a solid surface (www.sonochemistry.info).

When acoustic cavitation occurs in liquids containing suspended powders, dramatic effects may occur. In fact the powders (as well as the trapped gas) can act as nuclei for cavitation bubble formation and the subsequent collapse can lead to shock waves which break the particle. Cavitation bubble collapse in the liquid phase near to a particle can force it into rapid motion. Under these circumstances the general dispersive effect is accompanied by interparticle collisions which can lead to erosion, surface cleaning and wetting of the particles and particle size reduction.

In heterogeneous liquid-liquid systems, cavitational collapse at or near the interface cause its disruption and the liquid mixing, resulting in the formation of very fine emulsions.

3.1.3 The role of cavitation on ice nucleation

One of the first applications of ultrasound was sonocrystallization, that is the crystallization of ice due to ultrasonic irradiation. Sonocrystallization of supercooled water has been studied since 1965 and a significant body of literature is available (Hickling, 1965; Hunt and Jackson, 1966; Ohsaka and Trinh 1998; Inada *et al.* 2001; Chow *et al.* 2005), however, the exact action mechanism that explains the ultrasound effect is not yet well known. Different theories have been formulated and the most important mechanism by which ultrasonic irradiation can influence crystallization is ultrasonic cavitation (Hickling, 1965; Hunt and Jackson, 1966).

According to the model formulated by Hickling (1965), the positive pressures produced during the final stage of collapse of bubble cavitation (greater than 1 GPa) increase the equilibrium freezing temperature of water, and thus increase the supercooling degree, enhancing the rate of ice nucleation. Another model (Hunt and Jackson, 1966), instead, states

that nucleation is caused by the negative pressure that follows the collapse of the cavitation bubbles.

According to these conventional theories, the collapse of bubble transient cavities triggers off the nucleation of ice during ultrasonic-induced crystallization.

However, it has been demonstrated that ice nucleation can also be caused by an isolated, stable cavitation bubble in an ultrasonic field, without implosion phenomenon (Ohsaka and Trinh, 1998); in these cases nucleation could be triggered by micro-streaming, through an acceleration of heat and mass transfer processes.

The ultrasonic-induced nucleation of ice in water containing different size distributions of air bubbles was also investigated (Zhang *et al.*, 2003) and, owing to the different thermodynamic conditions involved, the effect of ultrasound could not be only explained by conventional models, but also secondary effects should be taken into account.

Primary and secondary nucleation of ice were both possible during the sonocrystallization of ice in sucrose solutions (Chow *et al.*, 2003). In particular a stimulation of the primary nucleation was caused by the increasing in the temperature at which the nucleation took place. Moreover the strong forces originated from the collapse of the cavitation bubbles also influenced the secondary nucleation mechanism, by fragmenting the pre-existing ice crystals, into smaller ones. The authors also noted that the secondary nucleation might be caused by the high shear flow in the vicinity of the cavitation (Chow *et al.*, 2005).

3.1.4 Application of power ultrasound during food freezing

Power ultrasound has been recently studied in assisting and accelerating freezing processes of vegetable products, but, at the best of our knowledge, a little research is reported on literature (Sun and Li, 2002, 2003; Zheng and Sun, 2006). The immersion freezing of potatoes associated with the application of power ultrasound was investigated by Sun and Li (2002) who compared different power levels, exposure time and freezing phases, during which ultrasound was applied. The authors showed that the higher output powers and the longer exposure times enhanced greatly the freezing rate. Also the structural changes of potatoes frozen by ultrasound-assisted immersion freezing were evaluated and an improving of the structure of frozen-then-thawed potato tissue was observed (Sun and Li, 2003).

The mentioned investigations on ultrasound-assisted immersion freezing (Li and Sun, 2002; Sun and Li, 2003; Zheng and Sun, 2006) were carried out at a fast freezing rate, by using an immersion freezing solution at a very low temperature (of about -18°C). In these operative conditions, the formation of a large number of small crystals occurred both within

and outside the cells, ensuring the maintenance of tissue integrity and minimizing drip loss during thawing. However, the supercooling process, which is the sample cooling below the initial freezing point without ice formation (Rahman *et al.*, 2002), was not observed.

The aim of the investigation carried out (research article 1) was to delve into the study of ultrasound-assisted immersion freezing of potato cubes, with particular attention to the effects of the application of ultrasound during the supercooling phase.

Moreover in several of the investigations published on ultrasound assisted freezing, the main ultrasound effect involved was the enhancement of heat transfer produced by sonication (Chemat *et al.*, 2011; Li and Sun, 2002). A second aim we followed was to verify if the application of ultrasound on supercooled potatoes could trigger off the freezing through cavitation on the surface of the sample, causing an anticipation of the beginning of the freezing process.

Ultrasound was applied in different times of the freezing process and the freezing parameters, as nucleation temperature and time, initial freezing temperature, transition phase and global freezing duration, were evaluated and discussed.

Ultrasound assisted freezing: investigation of the effects on supercooled potatoes

P. Comandini[a]*, G. Blanda[a], M. C. Soto-Caballero[a,1], V. Sala[b], U. Tylewicz[a], H. Mujica-Paz[c], T. Gallina Toschi[a]

[a] University of Bologna, Department of Food Science, Piazza Goidanich 60, Cesena, 47521, Italy

[b] Startec Ultrasuoni S.r.l., Viale Lombardia, 148, Brugherio, 20047, Italy

[c] Instituto Tecnológico y de Estudios Superiores de Monterrey, Departamento Biotecnología e Ingeniería de Alimentos, Eugenio Garza Sada 2501, Monterrey, Nuevo León 64849, México

*Corresponding author. Tel.: +39-0547-338121; fax: +39-0547-382348.

E-mail address: patrizia.comandini2@unibo.it (P.Comandini).

Key words: Freezing, Nucleation, Potato, Ultrasound, Supercooling

Running title: Ultrasound assisted freezing of potato

Abstract

Ultrasound has attracted great interest in recent years and its application in food freezing has shown promising advantages. In the present study the application of ultrasound during immersion freezing of potato cubes was studied and particular attention was given to the effects on supercooling process. Ultrasound, produced through a 35 kHz sonotrode, was applied when the temperature in the geometrical center of potato sample was in the range from -0.1 to -3.0°C. Several freezing parameters, as nucleation temperature and time, initial freezing temperature, transition phase and global freezing duration, were evaluated and discussed.

A significant anticipation of the nucleation process was detected when the ultrasound application temperature was lower than -0.1°C, moreover a reduction in freezing time was recorded when ultrasound was applied at -2.0°C.

1. Introduction

[1] Present address: Departamento Biotecnología e Ingeniería de Alimentos, Instituto Tecnológico y de Estudios Superiores de Monterrey. Eugenio Garza Sada 2501. Monterrey, Nuevo León 64849, México

Ultrasound is a pressure wave oscillating at a frequency above the threshold of human hearing, in the range from 20 kHz to about 10 MHz (Denbow, 2001). Besides medicine and chemistry, ultrasound is used in several industrial sectors including food science and technology (Chemat et al., 2011). Ultrasound employed in the food industry can be mainly classified into two different fields: high frequency - low energy (diagnostic ultrasound) in the MHz range and low frequency - high energy (power ultrasound) in the kHz range (Mason et al., 1996; Zheng and Sun, 2006). The application of the first one is focused on non-destructive food inspection, assurance of product quality and process control. Power ultrasound is used to promote chemical and physical modification of processes and products. It has been applied to improve cleaning, microbial destruction and enzymatic inactivation, and to enhance extraction, drying and filtration (Mulet et al., 2002; Albu et al., 2004; Bermúdez-Aguirre and Barbosa-Cánovas, 2008). Power ultrasound is a promising technique also in freezing process thanks to its direct effect on heat transfer and on crystallization (Mason, 1998; Li and Sun, 2002; Sigfusson et al., 2004).

Sonocrystallization of supercooled water has been studied since 1965 and a significant body of literature is available (Hickling, 1965; Hunt and Jackson, 1966; Ohsaka and Trinh 1998; Inada et al. 2001; Chow et al. 2005), however, the exact action mechanism that explains the ultrasound effect is not yet well known. Different theories have been formulated and acoustic cavitation, which consists of the formation, growth and violent collapse of low-pressure bubbles in liquids, seems to be the most important effect involved in water sonocrystallization (Hickling, 1965; Hunt and Jackson, 1966).

According to the model formulated by Hickling (1965), the positive pressures produced during the final stage of collapse of bubble cavitation increase the equilibrium freezing temperature of water, and thus increase the supercooling degree, enhancing the rate of ice nucleation. Another model (Hunt and Jackson, 1966), instead, states that nucleation is caused by the negative pressure that follows the collapse of the cavitation bubbles.

According to these conventional theories, the collapse of bubble transient cavities triggers off the nucleation of ice during ultrasonic-induced crystallization. However, it has been demonstrated that ice nucleation can also be caused by an isolated, stable cavitation bubble in an ultrasonic field, without implosion phenomenon (Ohsaka and Trinh, 1998); in these cases nucleation could be triggered by micro-streaming.

The ultrasonic-induced nucleation of ice in water containing different size distributions of air bubbles was also investigated (Zhang et al., 2003) and, owing to the different thermodynamic conditions involved, the effect of ultrasound could not be only explained by conventional models, but also secondary effects should be taken into account.

Primary and secondary nucleation of ice were both possible during the sonocrystallization of ice in sucrose solutions (Chow et al., 2003). In particular a stimulation of the primary nucleation was caused by the increasing in the temperature at which the nucleation took place. Moreover the strong forces originated from the collapse of the cavitation bubbles also influenced the secondary nucleation mechanism, by fragmenting the pre-existing ice crystals, into smaller ones. The authors also noted that the secondary nucleation might be caused by the high shear flow in the vicinity of the cavitation (Chow et al., 2005).

Freezing is an important way to preserve food during the time, maintaining the characteristics of fresh products, as in the case of vegetables. Nucleation and crystal growth are the main steps of ice crystallization, which affect the quality, the stability and the sensory properties of frozen foods.

It has been reported that a fast nucleation and a production of small and uniform crystals occur during sonocrystallization (Luque de Castro and Priego-Capote, 2007).

Power ultrasound has been recently studied in assisting and/or accelerating freezing processes of vegetable products, but a little research is reported on literature (Sun and Li, 2002, 2003; Zheng and Sun, 2006). The immersion freezing of potatoes associated with the application of power ultrasound was investigated by Sun and Li (2002), who compared different power levels, exposure time and freezing phases during which ultrasound was applied. The authors showed that the higher output powers and longer exposure times enhanced greatly the freezing rate. Also the structural changes of potatoes frozen by ultrasound-assisted immersion freezing were evaluated and an improving of the structure of frozen-then-thawed potato tissue was observed (Sun and Li, 2003).

The mentioned investigations on ultrasound-assisted immersion freezing (Li and Sun, 2002; Sun and Li, 2003; Zheng and Sun, 2006) were carried out at a fast freezing rate, by using an immersion freezing solution at a very low temperature (of about -18°C). In these operative conditions, the formation of a large number of small crystals occurred both within and outside the cells, ensuring the maintenance of tissue integrity and minimizing drip loss during thawing. However, the supercooling process, which is the sample cooling below the initial freezing point without ice formation (Rahman et al., 2002), was not observed.

Supercooling process has an important role in freezing food, because its extent influences both the rate of ice nuclei formation and their dimensions (Crivelli, 1992) and consequently affects the properties of frozen products.

The aim of this investigation was to delve into the study of ultrasound-assisted immersion freezing of potato cubes, with particular attention to the effects during the supercooling phase. In spite of the formation of a limited number of large ice crystals between cells, and the high

structural damage caused, a slow freezing rate was chosen, in order to obtain a clear visualization of the freezing curve and of the supercooling phenomenon in potato cubes (James et al., 2009).The capacity of ultrasound to promote the beginning of crystallization in supercooled potato cubes was investigated. Ultrasound was applied in different moments of the freezing process and the freezing parameters, as nucleation temperature and time, initial freezing temperature, transition phase and global freezing duration, were evaluated and discussed.

2. Materials and methods

2.1 Ultrasonic equipment

An ultrasound processor (300 W), working at a frequency of 35 kHz and equipped with a cone titanium sonotrode (Startec S.r.l., Milano, Italy) was used. The generator allowed to adjust the vibration amplitude from 0 % to 100 %. Treatment time was of 8 sec, with pulses duration of 1 sec.

Owing to the thermal effect, the absorption of ultrasonic power by the freezing solution caused a temperature increase, and a loss of nominal energy. Actual power transferred to freezing solution during sonication was measured calorimetrically by recording the temperature rise against the time of ultrasound application. Heat development was estimated from the slope of the straight portion of the line obtained. According to Raso et al. (1999) power output (P) was then calculated using the equation:

$$P = \frac{dT}{dt} c_p M$$

where *dT/dt* is the slope of the line representing the variation of temperature during the time (K s-1), *cp* is liquid medium heat capacity (kJ kg-1 K-1) and *M* is the amount of sample treated (kg). Power output was 21.1 W when the sonotrode operated at 100% of vibration amplitude.

2.2 Sample preparation

Potatoes (*Solanum tuberosum* L. cv. Safrane) were bought on local market in Cesena and stored at room temperature. Fresh potatoes were cut in cubes (1.5 cm^3 size) of about 8 g each one and immediately refrigerated until a temperature of 9 ± 1°C. After sample refrigeration, two thermocouples were positioned in each cube, one in the geometric centre and one close to the surface of the sample. T-type thermocouples (mod. GG-30-KK; Tersid, Milano, Italy) connected to a data acquisition system (mod. 2700; Keithley, Cleveland, USA) were used. About 1.5 g of dough (made with commercial wheat flour and water) was used to seal the

thermocouples incoming points and prevent the freezing solution to soak inside the potato cube during the immersion freezing.

2.3 Ultrasound assisted immersion freezing

Two potato cubes, cut from the same tuber, were frozen with and without ultrasound application, respectively. A solution of glycerol and water (50/50, w/w) was used for immersion freezing at a temperature of -6°C. Experiments were carried out in two vessels containing 1 kg of cooling solution each one (Fig. 1): in vessel A the immersion freezing was carried out without ultrasound application (control sample) and in vessel B the ultrasound-assisted immersion freezing was realized (US sample). In order to maintain the potato cubes in the same position inside the cooling solution, a retaining structure was used. Each sample was positioned in the centre of the vessel at a 2.5 cm depth in the freezing solution. The ultrasonic probe was lined up with the sample, and a distance of 1.5 cm, between the top of the ultrasonic probe and the sample, was maintained.

Control and US samples were immersed at the same time in the cooling solutions and when the geometric centre of potato cube reached the planned temperature (Table 1), 8 ultrasonic pulses (1 sec each) were applied to the ultrasound treated sample. As reported in Table 1, ultrasound effects were tested in 6 different moments of the freezing process: near 0°C (US-0.1), at temperatures higher (US-1.1, US-1.6, US-2.1) and lower (US-3.0) and near (US-2.6) to the initial freezing temperature of potato.

Immediately after the application of ultrasound, control and US samples, inside their own vessels, were put at - 45°C to complete the freezing process. Each experiment was repeated 5 times.

2.4 Statistical analysis

Data were analyzed using Statistica 8.0 (Statsoft Inc., Tulsa, OK); significant differences in the treatments were assessed by one-way analysis of variance (ANOVA, 95% significance level) and Fisher's least significant differences test was applied ($P < 0.05$).

3. Results and discussion

3.1. Preliminary experiments on water sonocrystallization and supercooling of potato cubes

Before ultrasound-assisted freezing of potato cubes, some preliminary studies were made in order to verify if the ultrasound equipment at our disposal was able to induce water sonocrystallization, and find the best operative conditions. Distilled and sparkling water were

undercooled until -1.5, -2.0 and -3.0°C and ultrasound was applied for 4 sec. The experiment was carried out for different volumes (50 and 500 mL) of water.

Ultrasound application promoted crystallization of distilled and sparkling water when the sample was undercooled until -2.0°C. The ice crystals formation was more evident in sparkling water, thanks to the high concentration of dissolved gases. An undercooling temperature of -1.5°C was not sufficient to promote ice crystallization by the application of ultrasound. On the other hand, when water was supercooled, until -3.0°C, the sample became very unstable, and crystallization was caused by little movements of the sample, before ultrasound application.

After observing the sonocrystallization of water, ultrasound was decided to be applied to supercooled potatoes, in order to evaluate the effects promoted on a solid matrix.

Previous studies on ultrasound-assisted immersion freezing of vegetable products (Li and Sun, 2002; Sun and Li, 2003) highlighted the capacity of power ultrasound to improve the freezing process by reducing the characteristic freezing time. These effects were due to an enhancement of heat transfer induced by acoustic streaming and cavitation of the coolant liquid at the interface with the product treated. However, the heat produced by ultrasound transmission through the medium limited the power applied and the exposure time. For these reasons, an efficient cooling system, able to remove the heat developed by ultrasound was used (Li and Sun, 2002; Sun and Li, 2003). In a following investigation, concerning the application of power ultrasound on immersion freezing of apples, the improvement of heat transfer was found to be an important mechanism of ultrasonic action, but also some evidence of power ultrasound to induce primary nucleation was observed.

Ultrasound has been used for a long time to initiate nucleation in supercooled aqueous solutions (Chalmers, 1964). For these reasons it was decided to operate in conditions that permitted to supercool potato cubes during the freezing process. In order to reach the highest supercool degree, a slow freezing rate was selected (Reid, 1998). Potato cubes were firstly refrigerated at about 8°C and then were frozen in a cooling solution having a temperature of -6.0°C. Different from previous experiments, where the coolant temperature was set at -18 or -20°C (Li and Su 2002; Sun and Li, 2003), the freezing rate was reduced by the higher temperature of the cooling solution used (-6°C), but a high supercooling of about 5.6°C could have been reached. Ultrasound application was tested before and during supercooling, as previously described.

Another important difference respect to previous investigations is related to the duration of ultrasound application, in fact owing to the absence of a cooling system able to remove the heat produced by ultrasound, it was decided to reduce as more as possible the duration of

ultrasonic exposure. Analyzing the results obtained during the preliminary experiments on water sonocrystallization, 8 seconds appeared to be the best choice.

3.2. Freezing parameters evaluated

The immersion freezing of control samples, without ultrasound application, was compared to ultrasound-assisted one. As summarized in Table 1, ultrasound was applied when the temperature of the geometrical centre of the potato cube was in the range from - 0.1°C to - 3.0°C. Both temperatures at the surface and at the geometrical centre were recorded. However, the temperature on the potato cube surface was very variable, because of handling the samples during the introduction and the positioning of the thermocouples; for these reasons it was not taken into account.

The freezing curve is one of the most accurate and widely adopted methods to determine the freezing point and other food freezing parameters (Rahman et al., 2002), thanks to its easy and cheap use. Freezing curves recorded in the geometrical centre of ultrasound immersion freezing and control samples were analyzed and the following characteristic factors of the freezing process were evaluated: nucleation temperature, initial freezing temperature (NTem and IFT respectively), nucleation time (Ntime), transition phase time (TPtime) and global freezing process time (Ftime).

A typical freezing curve obtained by immersion freezing of a potato cube and illustrating the parameters evaluated, is reported in Fig. 2. The effects of ultrasound application on these factors, summarized in Table 2, are individually discussed.

3.2.1 Nucleation temperature (NTem) and time (Ntime)

The nucleation temperature (NTem) is the lowest temperature, reached by the sample during the cooling phase, without ice formation. When crystal nuclei dimensions exceed the critical radius for nucleation, the cooling process stops and nucleation begins by releasing latent heat in the system. This phenomenon is associated with a rapid increase of temperature from the NT to the initial freezing temperature (IFT) (Rahman et al., 2002), generating the nucleation peak in the freezing curve. The time of appearance of the nucleation peak is defined nucleation time (Ntime). The term supercooling is referred to the number of degrees below the freezing point reached during the cooling phase without freezing, thus a higher value of supercooling corresponds to a lower nucleation temperature.

Although there is a significant body of literature concerning the sonocrystallization of ice, the mechanisms involved are still not completely clarified. In general sonocrystallization induces a faster primary nucleation (Sun and Li, 2003), accelerating the crystallization of a solution

free of nucleation seeds; it has been demonstrated that primary nucleation of ice in sucrose solution can be achieved at higher nucleation temperatures in the presence of ultrasound (Chow et al., 2003). Ultrasound application can also initiate the secondary nucleation, determining the production of smaller crystals with better size uniformity (Chow et al., 2005).

In this investigation an early primary nucleation of potato cubes was noted when ultrasound was applied. The main effect of ultrasound application was the interruption of the supercooling process and the beginning of nucleation. When ultrasound was applied in the range -1.1 - -3.0°C, higher nucleation temperatures (and a lower supercooling) were recorded (Table 2).

The consequence of the abort of supercooling process was the anticipation of the beginning of nucleation process, as revealed Ntime results. The nucleation peak in ultrasound samples appeared before than in the control and it was anticipated from 26.12 min (control sample) to about 9.23 min (US-3 samples). Also in this case, the US-0.1 sample was not significantly different from the control.

In the experimental conditions used, ultrasound could cause a rapid nucleation of ice in the outer layers of the sample; this phenomenon was evident also thanks to the formation of a thin stratification of ice on the surface of the sample, during and immediately after the acoustic exposure. Next, ice propagation carried on toward the centre of the product by determining an early nucleation peak formation. If ultrasound application had had an immediate effect on the whole mass of the product, the comparison of the nucleation peak in the geometrical centre of potato cubes would have been recorded immediately after ultrasound exposure, but this event did not occurred. Maybe the transmission of ultrasound inside potato tissues was limited by the low intensity of the acoustic waves.

When ultrasound was applied at -0.1°C, no significant differences were observed, in NTem and Ntime, respect to the control sample (Table 2), probably owing to the too high temperature of ultrasound application.

3.2.2 Initial freezing temperature (IFT)

As foodstuffs are a mixture of different constituents, they do not have a single freezing point, but a freezing range. The ‘freezing point’ of potato was considered as the initial freezing temperature (IFT), the temperature at which ice growing started following supercooling (Comini et al., 1974). In particular, as shown in Fig. 2, the IFT was considered as the initial temperature of the plateau formed after the end of supercooling process. Owing to the growth of ice crystals and the release of latent heat, the slowest rate of change of temperature is observed in this area of the freezing curve (James et al., 2009).

According to theoretical models concerning the cavitation-induced nucleation of ice, the high positive pressure at the end of the collapse or the negative pressure that follows the collapse of a cavitation bubble, increases the equilibrium freezing temperature of water, causing the nucleation of ice (Hickling, 1965; Hunt and Jackson, 1966). At the best of our knowledge no scientific information is reported on the variation of the freezing point of ultrasound-assisted frozen food.

Some publications concerning the evaluation of physico-chemical properties of food components, as *a*-lactalbumin (Jambrak et al., 2010) and whey proteins (Krešić et al., 2008), have shown that acoustic waves caused a freezing point depression.

The IFTs of ultrasound treated samples, in particular US-1.1, US-1.5, US-2.0, were significantly higher than the control (IFT -2.7°C), as reported in Table 2. A slight IFT variation was also recorded for US-2.6 , US-3.0 and US-0.1, in the last case ultrasound had not have a significant effect on NT and Ntime. Ultrasound was supposed to have caused a slight increase of cooling solution temperature (about 0.5°C), reducing the freezing rate of the potato cubes. This could have facilitated the freezing of extracellular water, which has a lower solute concentration, determining a higher initial freezing temperature of potato cubes.

According to a different hypothesis the rise of IFT could be a consequence of physical, chemical or structural modifications caused by ultrasound on potato cubes, but a direct effect of ultrasound on freezing transition phase was excluded.

3.2.3 Transition phase and global freezing duration

The freezing time (Ftime) was expressed as the duration of the whole freezing process, starting from the beginning of the cooling phase until the end of freezing (Fig. 2). The end point of freezing was evaluated as the moment when the freezing curve reached the maximum slope after crystallization (Rahman et al., 2002).

The duration of the temperature plateau after sample supercooling, on the plot time-temperature and having a slope near zero, was identified as transition phase time (TPtime); it was calculated as the distance between Ntime and Ftime.

Ftime and TPtime values were significantly affected by the heat produced by ultrasound, which could not be efficiently removed from the system.

Although such preface, the total Ftime of control sample was statistically identical to the ultrasound-treated ones, unless US-2 sample that froze more quickly. The lowest freezing time of US-2 (53.39 min) was due to the fact that it had started before the nucleation process, as occurred for the samples US-1.1, -1.5, -2.6, -3.0, but unlike the other US treated samples, its transition phase time was slightly lower, determining the lowest FTime value.

The application of ultrasound caused an increase in TPtime, respect to control samples. This result could be explained by the lower supercooling reached by these samples.

In this regard, further investigations are required to verify the effect of ultrasound on the quality parameters of frozen and thawed potatoes. In particular, it is necessary to evaluate if the anticipation of the nucleation is accompanied by the formation of smaller ice crystals, or if the effect of ultrasound is only a mechanical interruption of the supercooling process, without affecting the ice crystal structure and distribution inside vegetable tissue. Texture and sensory studies of the products obtained by ultrasound-assisted freezing should be evaluated.

4. Conclusions

The application of ultrasound during immersion freezing of potatoes modified important freezing parameters, as revealed by the analysis of the freezing curves.

The acoustic waves exposure caused an anticipation of the nucleation beginning, when the temperature of ultrasound application in the centre of the sample was lower than -0.1°C.

Despite an efficient removing of the heat developed by ultrasound was not possible, owing to instrumental limitations, when ultrasound was applied at -2.0°C the freezing time resulted significantly lowered respect with the control samples.

The anticipation of nucleation beginning and the reduction of freezing time could have a great impact for food industry. However further investigations are necessary in order to better explain the effect of acoustic waves on food immersion freezing, and evaluate quality properties of the product treated.

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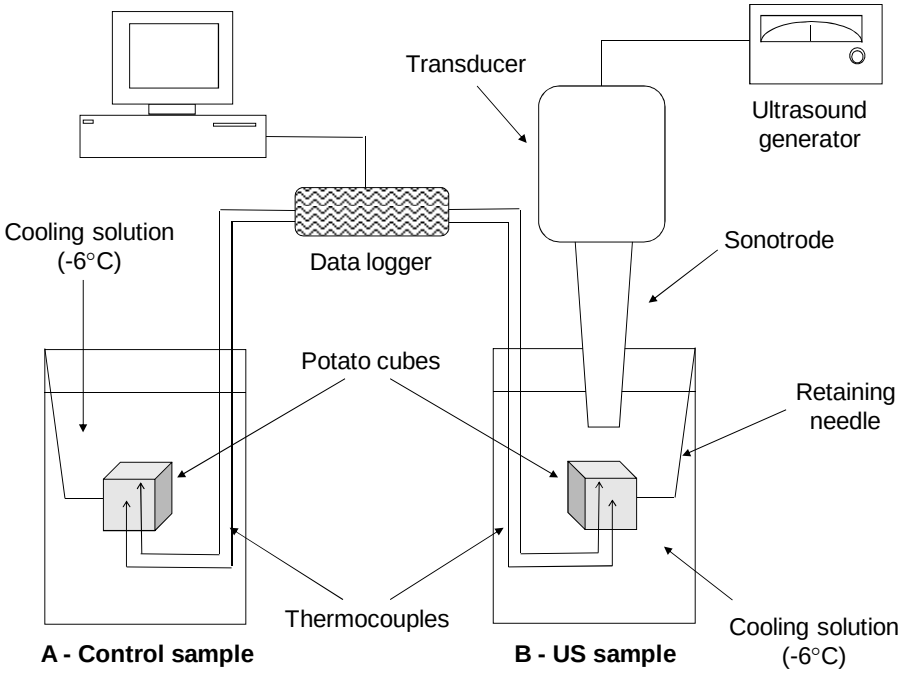
436 **Figure 1** Schematic representation of the experimental equipment for control (A) and
437 ultrasound-assisted immersion freezing (B).



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440 **Figure 2** Freezing curve of a potato sample and freezing parameters evaluated.

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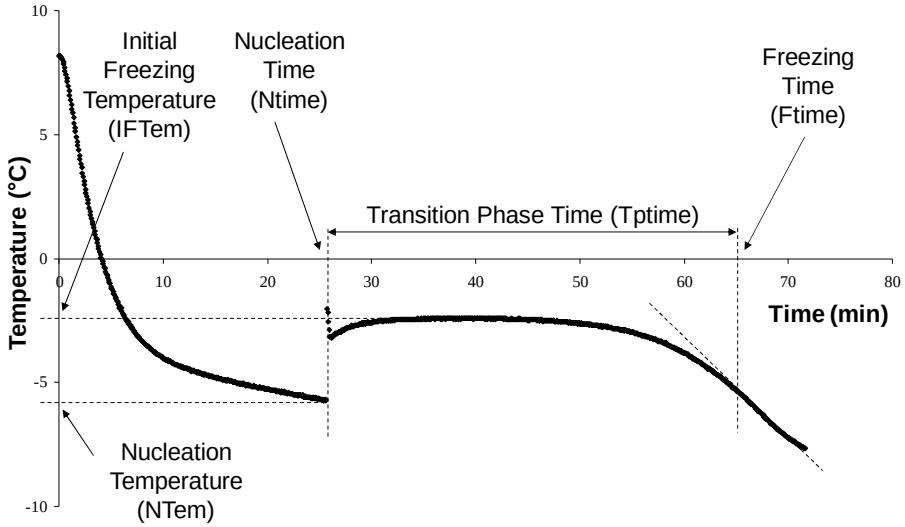

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Table 1

Immersion freezing treatments.

Sample ID	US[A] temperature (°C)
Control	US not applied
US-0.1	-0.13 ± 0.11
US-1.1	-1.12 ± 0.13
US-1.6	-1.54 ± 0.09
US-2.1	-2.08 ± 0.08
US-2.6	-2.58 ± 0.12
US-3.0	-3.00 ± 0.03

All values are means ± standard deviation.

[A] US: ultrasound.

Table 2

Freezing parameters of control and ultrasound-assisted samples.

Treatments	Freezing parameters				
	NT[A] (°C)	Ntime[B] (min)	IFT[C] (°C)	TPtime[D] (min)	Ftime[E] (min)
Control	-5.67 ± 1.03^{c}	26.12 ± 6.08^{a}	-2.70 ± 0.37^{d}	39.21 ± 3.33^{c}	62.44 ± 10.15^{a}
US-0.1	-5.49 ± 0.42^{bc}	24.95 ± 3.16^{a}	-2.33 ± 0.28^{abcd}	41.55 ± 5.29^{bc}	66.50 ± 4.58^{a}
US-1.1	-4.01 ± 0.87^{ac}	17.37± 3.52^{b}	-2.26 ± 0.25^{abc}	47.83 ± 6.67^{a}	60.51 ± 5.83^{ab}
US-1.5	-3.74 ± 0.95^{a}	9.63 ± 3.79^{c}	-2.17 ± 0.47^{a}	46.60 ± 6.73^{ab}	58.99 ± 7.66^{ab}
US-2.0	-3.62 ± 1.22^{a}	8.64 ± 3.90^{c}	-2.11 ± 0.48^{ab}	41.50 ± 6.59^{c}	53.39 ± 5.19^{b}
US-2.6	-3.85 ± 0.98^{a}	11.44 ± 3.82^{bc}	-2.53 ± 0.21^{bcd}	51.27 ± 5.83^{a}	64.32 ± 7.16^{a}
US-3.0	-3.73 ± 1.08^{a}	9.23 ± 2.96^{c}	-2.47 ± 0.48^{cd}	50.01 ± 4.18^{a}	62.23 ± 8.82^{a}

All values are means ± standard deviation. Means within columns with different letters are significantly different ($P < 0.05$).

[A] NT: nucleation temperature (°C).

[B] Ntime: nucleation time (min).

[C] IFT: initial freezing temperature (°C).

[D] TPtime: transition phase time (min).

[E] Ftime: freezing time (min).

3.2. IMPREGNATION TECHNOLOGIES

3.2.1 Impregnation process: atmospheric pressure vs. *vacuum* application

Impregnation technologies can be used to incorporate physiologically active compounds into fresh food, thus obtaining functional foods. In this way, the product composition, as well as its physical and chemical properties may be changed in order to improve its characteristics (Fito and Chiralt, 2000; Fito *et al.*, 1996, 2000, 2001). Impregnation processes can be carried out at atmospheric pressure (atmospheric impregnation, AI), under vacuum conditions (vacuum impregnation, VI) or by a combination of vacuum impregnation followed by large periods at atmospheric pressure (Anino *et al.*, 2005).

The operating pressure is one of the factors affecting the composition and the structural characteristics of the final product. During AI, plant cellular structure acts as a semi-permeable membrane and water and solutes fluxes are usually considered as diffusion driven. When AI is applied, longer treatment times are required but a great solute final concentration can be achieved (Anino *et al.*, 2005).

In VI process, a porous product is immersed in an adequate liquid phase and is submitted to a two step pressure change. First, a vacuum pressure promotes the gas flow throughout the porous product until mechanical equilibrium is achieved. In this moment, capillary penetration will be higher than at atmospheric pressure. When atmospheric pressure is restored in a second step, residual gas compression leads to the external solution inflow while pressure gradients persist. This phenomenon, explained for the first time by Fito (1994), is called (hydrodynamic mechanism, HDM).

From the HDM model it is possible to predict the amount of liquid that can be introduced into a porous food (Fito and Chiralt, 1995). The volume fraction of the initial sample (χ) impregnated by the external liquid, when mechanical equilibrium is achieved in the sample, has been modeled in a simplified way for stiff products, as a function of the compression ratio (r), sample effective porosity (ϵ_e), initial gas pressure in the pores (ρ_1) and external system and capillary pressure (ρ_2), as described in the following equations (Fito, 1994):

$$\chi = \varepsilon_e \left[1 - \frac{1}{r} \right]$$

Where

$$r = \frac{\rho_2}{\rho_1}$$

These equations permit to evaluate the amount of sample volume that will be occupied by an external liquid of a determined composition at the mechanical equilibrium status, in terms of the product porosity and the and the compression ratio, when the porosity remains constant during compression, that is the relative deformation is unimportant (Fito and Chiralt, 2000).

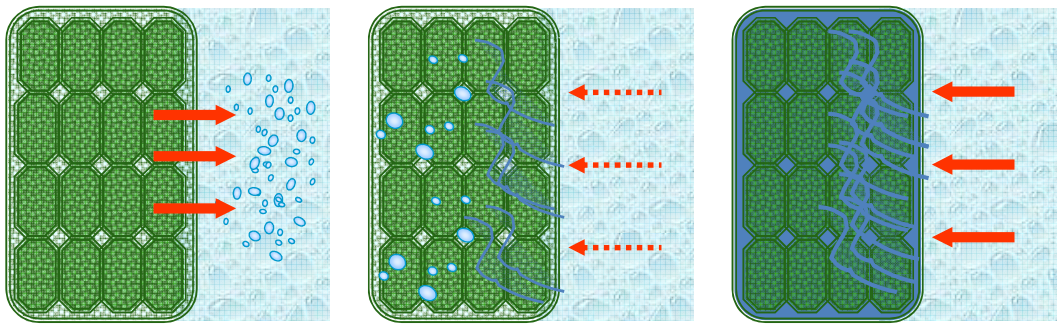

Figure 3.2.1 HDM mechanism in a vegetable food immersed in a liquid system.

The HDM was extended for viscoelastic porous products, where pressure changes cause not only gas or liquid flow but also solid matrix deformation-relaxation phenomena (DRP). In viscoelastic materials expansion-compression processes lead to changes in pore volume, which will be time-dependent. During the first VI step, product volume usually swells, associated with gas expansion, and, afterward, the solid matrix relaxes; capillary penetration or expelling of internal liquid also occurs in this period. In the second step, compression causes volume deformation and subsequent relaxation, coupled with the external liquid penetration in the pores. Mechanical properties of the solid matrix and flow properties of the penetrating liquid in the pores will define characteristic penetration and deformation-relaxation times responsible for the final impregnation and deformation status of the sample at equilibrium. The following equation describes the relation between the compression ratio (r), the initial sample porosity (ϵ_e), the final sample volume fraction impregnated by the external solution (χ) and the sample volume deformations at the end of both the process (γ) and the vacuum step (γ_1) (all of these are referred to the sample initial volume):

$$\varepsilon_e = \frac{(\chi - \gamma)r + \gamma_1}{(r - 1)}$$

The substitution of internal gases by a liquid phase allows direct formulation of a food, without exposing the food structure to the stress due to the long exposure to gradient solute concentration as in atmospheric process (Chiralt *et al.*, 1999).

HDM can allow in some cases a great mass transfer velocity and a better quality product, although the effectiveness of VI for the incorporation of a specific component is limited by its solubility and/or by the vegetable matrix porosity (Anino *et al.*, 2005).

VI efficiency has been reported to depend on:

-process parameters, including vacuum level and time, holding time in the impregnation solution after pressure release (Hoover and Miller, 1975);

-food matrix, including effective porosity (Mujica-Paz *et al.*, 2003a) and tortuosity;

-impregnation solution properties, such as osmolarity (Mujica- Paz *et al.*, 2003b) and viscosity (Barat *et al.*, 2001; Guillemin *et al.*, 2008).

Impregnation technologies have been used to fortify fruit and vegetable matrices with probiotics and minerals (Alzamora *et al.*, 2005), such as calcium (Mujica-Paz *et al.*, 2002; Gras *et al.*, 2003) and zincum (Tapia *et al.*, 2003; Zhao and Xie 2004). Some of these applications aimed to improve also the texture characteristics, through the incorporation of calcium salts and chitosan-based edible coatings (Anino *et al.*, 2005; Vargas *et al.*, 2009).

3.2.2 Aroma enrichment of food through impregnation technology

Vacuum impregnation technology can also be used to change the food matrix composition with the final aim of improving its sensory characteristics (Fito *et al.*, 2001). Modifying the flavour of foods is a longstanding practice that has been developed in response to various factors, starting with an initial attempt to ensure survival of the human race and now to deliver the desired properties to the foods (Reineccius, 2006).

Fruit preparations, which are important ingredients of several milk products, are often added of natural or artificial flavourings, but to the best of our knowledge, there are no methods for the production of food ingredients enriched with aromas; for these reasons it was decided to investigate the enrichment of apple sticks with green apple aroma, by comparing traditional techniques such as AI and VI, with more innovative technologies such as impregnation assisted by ultrasound (USI) and the combination of vacuum plus ultrasound technologies (VUSI).

Ultrasound is extremely effective in accelerating heat and mass transfer kinetics (Kentish and Ashokkumar, 2011); recently high power ultrasound has been used to improve osmotic dehydration (Fernandes *et al.* 2008), prickling and marinating processes (Kingsley and Farkas, 1990; Carcel *et al.*, 2007; Hatloe, 1995; Sanchez *et al.*, 1999).

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COMMUNICATION

Impregnation Techniques for Aroma Enrichment of Apple Sticks: A Preliminary Study

Patrizia Comandini · Giampaolo Blanda · Hugo Mújica Paz · Aurora Valdez Fragoso · Tullia Gallina Toschi

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Abstract The food industry often utilizes flavor-enriched, semifinished food products as ingredients in more complex preparations. To the best of our knowledge, there are no methods to produce minimally processed food items to which flavorings have been added. In this investigation, apple sticks were enriched with a green apple aroma using different techniques. In particular, vacuum impregnation (VI), ultrasound technology (USI), and the combination of these two techniques (VUSI) were compared with atmospheric pressure impregnation (AI). An isotonic solution of fructose containing ascorbic acid and green apple flavoring was used for impregnation of apple sticks. Different treatment times (2.5, 5.0, and 12.5 min) were investigated, and the relative amount of the major compounds of impregnation flavoring was recorded. Significant differences between treatments were detected: VI and VUSI gave the highest aroma enrichment at 5.0 min of treatment. Different impregnation behaviors were recorded for alcohols and esters: the former increased even after 5.0 min of treatment, and the other components increased until 5.0 min and then decreased, mainly when ultrasound was applied (USI and VUSI). Some possible explanations of these results are proposed, although additional studies are needed to explain the mechanisms involved.

P. Comandini (✉) · G. Blanda · T. Gallina Toschi
Dipartimento di Scienze degli Alimenti, Facoltà di Agraria,
Università di Bologna,
P.zza Goidanich 60,
47023 Cesena (FC), Italy
e-mail: patrizia.comandini2@unibo.it

H. Mújica Paz · A. Valdez Fragoso
Departamento de Biotecnología e Ingeniería de Alimentos,
Instituto Tecnológico y de Estudios Superiores de Monterrey,
Col. Tecnológico,
Monterrey, NL, Mexico

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Introduction

Modifying the flavor of foods is a longstanding practice that has developed in response to various factors. Dairy products, such as flavored milks, yogurts, and frozen desserts, represent a major market for flavoring materials (Reineccius 2006). Fruit preparations, which are important ingredients of several milk products, are often supplemented with natural or artificial flavorings, but to the best of our knowledge, there are no methods for the production of food ingredients enriched with aromas.

The production of convenient, fresh, and healthy foods has been widely investigated in recent years and has included fortified foods with physiologically active compounds like probiotics (Alzamora et al. 2005), calcium (Mújica-Paz et al. 2002; Torres et al. 2006; Saxena et al. 2009) and zinc salts (Zhao and Xie 2004), osmodehydrofreezed fruits enriched with cryoprotectants (Talens et al. 2002a, b), as well as minimally processed foods incorporated with edible coatings to improve texture (Vargas et al. 2009). Such impregnation processes are usually performed under vacuum conditions or at atmospheric pressure.

Another technology that has gained a considerable interest in minimally processed food manufacturing is high-intensity ultrasound (Patist and Bates 2008), which has been applied in several food processes (McClements 1995). Recently, high-power ultrasound has been used to accelerate the mass transfer kinetics involved in osmotic dehydration (Fernandes et al. 2008). The high intensity of acoustic waves can generate the growth and collapse of bubbles inside liquids, a phenomenon known as cavitation.

The asymmetric implosion of such cavitation bubbles close to a solid surface generates microjets in the direction of the product that can affect mass transfer (Mason et al. 1996).

In this study, the methodologies used for manufacturing of functional food enriched with physiologically active compounds, such as vacuum and atmospheric impregnation, and innovative ultrasound technology were employed to promote flavor enrichment of apple sticks. In particular, this investigation compared different impregnation techniques, including impregnation at atmospheric pressure (AI), vacuum impregnation (VI), impregnation assisted by ultrasound (USI), and the combination of vacuum plus ultrasound technologies (VUSI), with the aim of selecting the best procedure.

Material and Methods

Raw Material

Apples (*Malus domestica*, var. Golden Delicious) were bought from a local market in Cesena and stored at 4 °C until analysis. Fruits were cut in regular sticks (45×9×9 mm^3) with a handle cutter; the core, peel, and seeds were removed. Each sample, obtained from six apples, was divided into four aliquots of 50 g each and submitted to the impregnation treatments described below.

Impregnation Treatments

All experiments were carried out at room temperature. A fructose isotonic solution (14.0–15.0°Bx) containing ascorbic acid (0.5% wt/wt) and dry, food-grade green apple flavoring (0.5% wt/wt) was used. The mass ratio of fruit to syrup was 1:17. Apple sticks were dipped in the impregnation isotonic solution and maintained immersed with a stainless steel net. Impregnation treatments, summarized in Table 1, were carried out in three steps. Steps 1 and 2 lasted 5.0 and 1.5 min, respectively, while during step 3, different times (2.5, 5.0, and 12.5 min) were tested.

- AI: Apple sticks were maintained at atmospheric pressure.
- VI: Samples were placed in a chamber connected to a vacuum pump (Incofar s.r.l., Modena, Italy), and a pressure of 280 mbar was applied to the system for 5.0 min (step 1). Next, atmospheric pressure was restored over 1.5 min (step 2), and apple sticks were maintained immersed for 2.5, 5.0, or 12.5 min (step 3).
- USI: Samples were maintained at atmospheric pressure during the first two steps and then were put in a 35-kHz ultrasonic bath (Liarre s.r.l., Bologna, Italy) during step 3.
- VUSI: Vacuum pressure (280 mbar) was applied during step 1, after which atmospheric conditions were restored (step 2). Samples were then placed inside the ultrasonic bath (step 3) to complete the treatment.

After impregnation, apple sticks were removed from the solution, drained on a wire net for 5 min and gently blotted on tissue paper. Samples were weighed before and after impregnation treatments. Each experiment was performed in triplicate.

Volatile Analysis

Each impregnated sample was ground in a blender with 100 mL of saline solution (NaCl 0.34 M) and homogenized for 1 min at 14,000 rpm (Ultra Turrax, IKA-Werke T25 basic; Staufen, Germany). Next, the mixture was centrifuged at 3,500 rpm (1,880×*g*), for 10 min, and 1 g of supernatant was recovered and diluted 50-fold with distilled water. Fifty microliters of butan-1-ol (0.04 μL * 100 mL^{-1}) was added as internal standard.

The headspace-solid phase microextraction (HS-SPME) devices and the fused silica fiber coated with divinylbenzene/carboxen/polidymetylosiloxane (DVB/CAR/PDMS, 50/30 μm, 2 cm) were purchased from Supelco (Bellefonte, PA). Five grams of the diluted sample was placed in a 10-mL vial hermetically sealed with a polytetrafluoroethylene/silicone septum. The SPME device was inserted through the septum and the system was equilibrated for 5 min at 50 °C with constant agitation, without exposing the fiber. Extraction at 50 °C was then performed for 30 min. After sampling, the fiber was desorbed for 5 min at 250 °C.

Volatile compounds were analyzed by a gas chromatograph QP2010 Plus (Shimadzu, Kyoto, Japan) equipped

Table 1 Impregnation conditions of apples with green apple flavoring

Treatment	Impregnation steps		
	Step 1 (5min)	Step 2 (1.5min)	Step 3 (2.5/5.0/12.5min)
AI	Atmospheric pressure	Atmospheric pressure	Atmospheric pressure
VI	Vacuum application	Restoring atmospheric pressure	Atmospheric pressure
USI	Atmospheric pressure	Atmospheric pressure	Ultrasound treatment
VUSI	Vacuum application	Restoring atmospheric pressure	Ultrasound treatment

with gas chromatography-mass spectrometry solution software (Shimadzu, version 2.5). Analytes were separated on a ZB-WAX column 30 m×0.25 mm ID, 1.00-μm film thickness (Phenomenex, Torrance, CA). The column temperature was held at 40 °C for 5 min and increased to 130 °C at 3 °C min^{-1}; the temperature was held at 130 °C for 1 min and was then increased to 240 °C at 10 °C min^{-1}. Finally, the column was kept at 240 °C for 3 min. The ion source and the transfer line were set to 175 and 280 °C, respectively. Electron impact mass spectra were recorded at 70-eV ionization energy in the 20- to 250-amu mass range (2 scan s^{-1}). Volatile compounds present in flavor-enriched apple sticks were tentatively identified basing on computer matching against commercial libraries, as well as an in-house laboratory spectral library of pure substances, Kovats retention indices, and literature data.

After identification of the major components of the green apple flavoring, analyses were carried out using a gas chromatograph Carlo Erba AUTO/HR/GC (Carlo Erba Instruments, Milan, Italy) with a flame ionization detector (FID), equipped with a ZB-WAX column 30 m×0.25 mm ID, 1.00-μm film thickness (Phenomenex). Helium was the carrier gas (1.5 mL min^{-1}). The chromatographic parameters had the same values as GC/MS analysis; the detector temperature was 250 °C.

Semiquantitative Analysis

The relative amount (RA) of the analytes was calculated. Peak areas of volatile compounds were normalized by using the peak area of the internal standard; RA [g^{-1}] was obtained as the ratio of normalized areas to the initial weight of samples, according to the following equation:

$$\mathrm{RA} = \frac{A_{\mathrm{vol}}}{A_{\mathrm{i.s.}}} / W_{\mathrm{i}}$$

where A_{vol} is peak area of volatiles (μV s), $A_{\mathrm{i.s.}}$ is peak area of the internal standard (μV s), and W_{i} is the initial weight of the sample (g) before the impregnation treatment.

RA was determined for the individual compounds of green apple flavoring and for the overall aroma, obtained by the sum of the RAs of ethyl 2-methylbutanoate, 3-methylbutylacetate, hexyl acetate, and hexan-1-ol.

Statistical Analysis

Data were analyzed using Statistica 8.0 (Statsoft Inc., Tulsa, OK); two-way analysis of variance was carried out and Fisher's least significant differences test was applied ($p<0.05$).

Results and Discussion

Preliminary Experiments

Initial experiments developed and optimized flavoring impregnation techniques, volatile extraction, and GC analytical conditions. Magnetic agitation was chosen during SPME extraction since an improvement of the signal to noise ratio of 1.35 was noted. The range of linearity of SPME/GC response was evaluated by analyzing different aqueous dilutions of the flavor-enriched apple samples; four dilutions were tested, 1:10, 1:25, 1:50, and 1:100. The correlation coefficients between RAs and dilution employed were calculated for the major compounds of green apple flavoring, which were 0.9997 (ethyl 2-methylbutanoate), 0.9981 (3-methylbutyl acetate), 0.9999 (hexyl acetate), and 0.9855 (hexan-1-ol). The aqueous dilution 1:50 was selected.

Identification of the Components of Artificial Flavoring

A typical gas chromatographic trace obtained by analyzing flavor-enriched apple sticks is shown in Fig. 1. Nine compounds were identified: seven esters (ethyl butanoate, ethyl 2-methylbutanoate, butyl acetate, 3-methylbutyl acetate, 3-methylbutyl butanoate, hexyl acetate and (*E*)-hex-3-enyl acetate) and two alcohols (3-methylbutan-1-ol and hexan-1-ol); peak 5 was butan-1-ol, which was added to the samples before SPME/GC analysis as an internal standard.

All these compounds were components of the external flavoring since, due to the dilution used, the native volatiles of apples were not detectable, as observed in preliminary experiments.

Overall Green Apple Flavoring Impregnation

Significant differences ($p<0.05$) were detected between treatments and times for green apple flavoring impregnation. As detailed in Table 2, VI and VUSI gave the highest impregnation, compared with AI and USI, mainly at 5.0 min. USI treatments, however, were not different from AI, and for this reason, it was assumed that the higher RAs obtained for VUSI were closely related to the vacuum effect. The higher volatile impregnation obtained in VI and VUSI was due to the fraction of isotonic solution that penetrated inside the apple sticks by a hydrodynamic mechanism, i.e., the pressure gradient that develops when atmospheric pressure is restored. In fact, VI and VUSI samples had a variation in weight of about 14%; no significant differences in weight were detected for AI and USI samples.

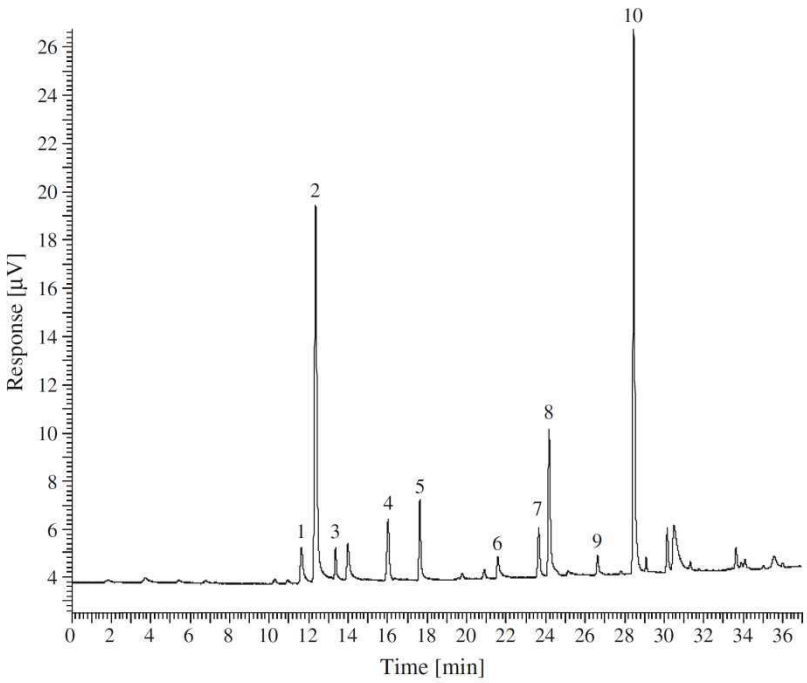

Fig. 1 SPME-GC/MS chromatogram of flavor-enriched apple sticks with the VI technique. Peaks: 1 = ethyl butanoate, 2 = ethyl-2-methylbutanoate, 3 = butyl acetate, 4 = 3-methylbutyl acetate, 5 = butan-1-ol (IS), 6 = 3-methylbutan-1-ol, 7 = 3-methylbutyl butanoate, 8 = hexyl acetate, 9 = (*E*)-hex-3-enyl acetate, and 10 = hexan-1-ol

Evolution of Esters and Alcohols Impregnation

The RAs of the major components of green apple flavoring (ethyl 2-methylbutanoate, 3-methylbutylacetate, hexyl acetate, and hexan-1-ol) were studied at different relaxation times (2.5, 5.0, and 12.5 min), as shown in Fig. 2. The quantities of ethyl 2-methylbutanoate, 3-methylbutyl acetate, and hexyl acetate increased until 5.0 min of impregnation with all treatments.

In AI samples, the impregnation of esters increased until 5.0 min of treatment and then reached a maximum level. One exception was ethyl 2-methylbutanoate, for which the amount gradually rose until 12.5 min. It is possible that apple sticks reached an equilibrium state with the impregnation medium after a few minutes of treatment. No differences in weight (before and after AI treatment) were detected, and thus, volatile transfer was mainly due to selective diffusion mechanism of aromatic compounds.

Moreover, in VI samples, the RA of esters increased until 5.0 min before reaching a plateau. This highlights that, under the experimental conditions used, a 5.0-min relaxation time was associated with maximum impregnation; the increase of the relaxation phase up to 12.5 min did not lead to improvement of the volatile amount. The same weight variations (15%, $p<0.05$) were recorded for the three different relaxation steps of 2.5, 5.0, and 12.5 min. Based on these results, we hypothesized that 2.5 min of relaxation time was sufficient to determine the uptake of liquid inside apple tissues, but volatile transfers continued up to 5.0 min due to diffusion. For this reason, the 5.0-min relaxation time caused a higher RA for VI samples, although the volume of isotonic solution penetrated in apple sticks did not change.

The impregnation obtained with USI was mainly due to diffusion since no significant weight variations were detected for USI samples after treatment. The RA of esters increased until 5.0 min of impregnation, after which the quantity of 3-methylbutyl acetate and hexyl acetate decreased; the RA of ethyl 2-methylbutanoate, however, remained constant.

The impregnation of hexyl acetate was always higher in USI with respect to AI. The maximum USI amounts of ethyl 2-methylbutanoate and 3-methylbutyl acetate were obtained at 5.0 min; ultrasound application for 12.5 min, in contrast, caused a reduction in these compounds.

Table 2 RAs [g^{-1}] of the overall flavoring obtained with different impregnation techniques and treatment times

Treatment	Time		
	2.5 min[b]	5.0 min[a]	12.5 min[a]
AI[b]	0.15±0.04	0.23±0.04	0.24±0.03
USI[b]	0.19±0.04	0.24±0.04	0.23±0.03
VUSI[ab]	0.20±0.04	0.36±0.03	0.27±0.03
VI[a]	0.21±0.06	0.35±0.06	0.36±0.03

Values are means ± standard deviations (n=3). [a,ab,b] Different letters in rows show statistically significant differences between treatments ($p<0.05$). [a,b] Different letters in columns show statistically significant differences between times ($p < 0.05$).

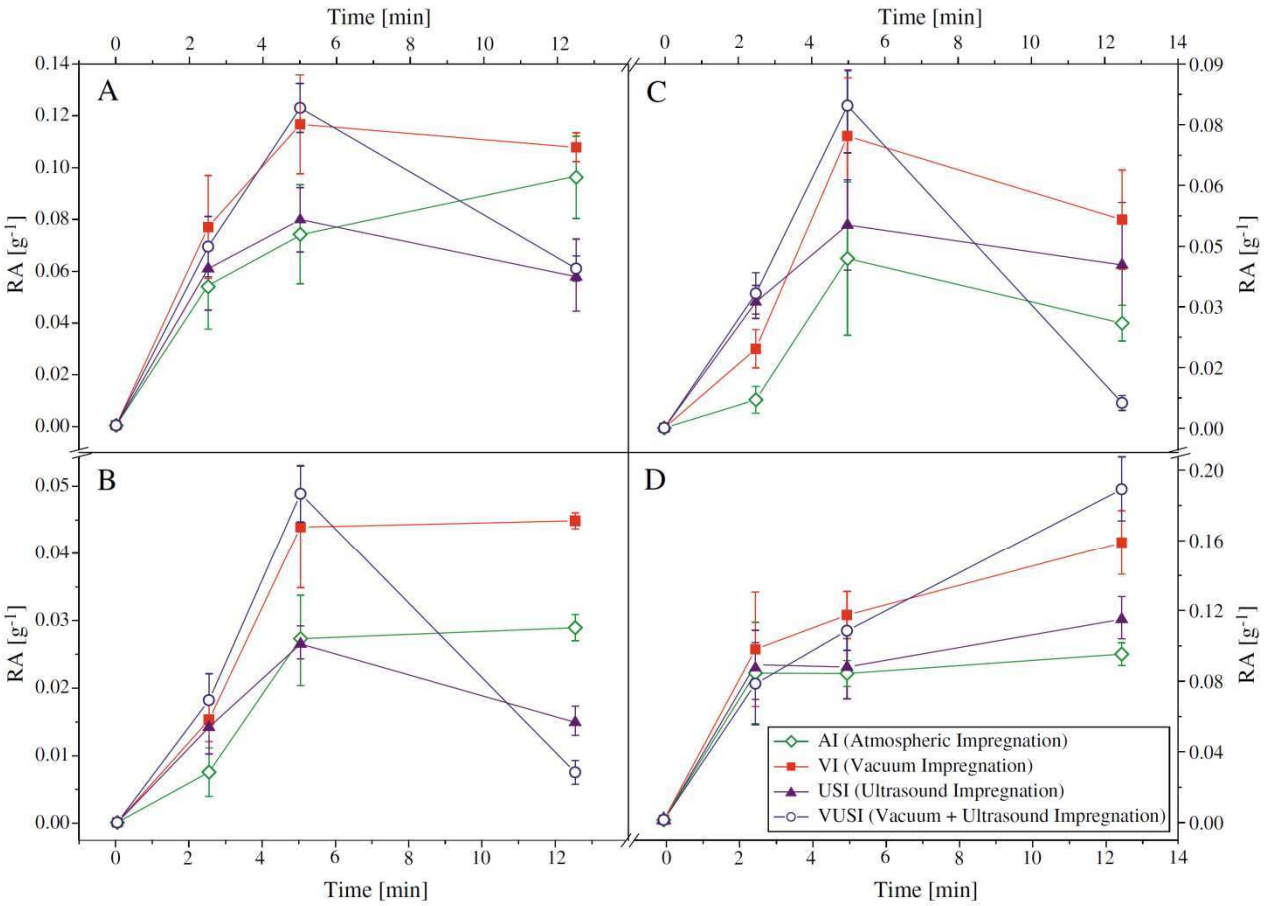

Fig. 2 Impregnation of ethyl 2-methylbutanoate (**a**), 3-methylbutyl acetate (**b**), hexyl acetate (**c**), and hexan-1-ol (**d**) at different relaxation times (2.5, 5.0, and 12.5 min)

The application of VUSI caused an elevated impregnation of esters during the first 5.0 min of treatment, with a trend similar to VI; after 5.0 min, a significant decrease in the amount of esters was noted.

At times up to 5.0 min, the main ultrasound effect was mixing the solution and maintaining a high concentration gradient at the surface of the product. After 5.0 min, however, the reduction in RAs obtained was related to acceleration of the evaporation rate. In fact, at 5.0 min of treatment, the increase in temperature was about 0.8 °C; at 12.5 min, the temperature of the isotonic solution increased by 2.1 °C, promoting evaporation of the most volatile components.

In general, the highest ester impregnation was obtained at 5.0 min with VI and VUSI. The decreasing RA of some volatiles after 5.0 min of impregnation with VUSI and USI might be explained by a progressive reduction of flavor in isotonic solutions. Possible causes are the breakdown by metabolism, preferential evaporation of some components, or hydrolytic breakage of volatile molecules (Tau et al. 1994).

Assuming that the reduction in esters content in apple sticks was related to a variation of flavor concentration in isotonic solution, several assumptions were made. As all experiments were carried out in open systems, the main mechanism responsible for flavor release from the isotonic solution was mass flux (Fm) from the bulk to the surface of the solution, calculated using the following equation:

$$\mathrm{Fm} = \mathrm{ks}(\mathrm{Css} - \mathrm{Cs})$$

where ks is the mass transport coefficient, Css is the aroma compound concentration at the surface of the solution, and Cs is the aroma compound concentration in the bulk of the solution (De Roos 2000, 2006).

Air flowing over the surface of the solution diluted the headspace flavor concentration, and mass transport took place from the solution to air to restore the phase equilibrium. Under the analytical conditions used, the depletion of flavor at the surface of the solution was nearing completion (Css→0), and the release of the volatile compounds was determined by the rate at which the molecules diffused from the bulk to the surface solution (ks).

The value of the mass transport coefficient (ks) is a function of the diffusion mechanism. In stagnant systems,

mass transport can take place only by molecular (static) diffusion, caused by the random movement of molecules. Dynamic systems are characterized by eddy or convective diffusion, i.e., transport of the fluid elements and the dissolved solutes, from one location to another (De Roos 2006).

In AI treatment, the main mass transport mechanism was static diffusion, which determined a relatively slow adsorption of esters by apple sticks and their saturation at 12.5 min. The dynamic mechanism, which was involved in USI, caused an increase of the impregnation of esters during the first 5.0 min; at 12.5 min, a reduction occurred, owing to an acceleration of the depletion rate.

In VI and VUSI treatments, the main mechanism responsible for flavoring enrichment was hydrodynamic mechanism, but in VI, the relaxation was realized under static conditions, at atmospheric pressure, and the ester impregnation did not vary significantly; in VUSI, the application of ultrasound during relaxation caused a notable depletion of flavorflavor for all esters at 12.5 min.

The RA of hexan-1-ol showed a different evolution during the relaxation time: it increased until 5.0 min in AI and USI samples, after which the RA was constant, and no significant differences were detected between 5.0 and 12.5 min. The impregnation of hexan-1-ol in VI and VUSI samples increased up to 12.5 min; in the first 5.0 min, the RA of hexan-1-ol was higher in VI, while at 12.5 min, the alcohol amount was higher in VUSI samples.

The low relative volatility of hexan-1-ol, which is about 100 times less than those of several esters (Ali et al. 2003), together with other physicochemical properties, such as polarity and functional groups, could have led to slower depletion of the alcohol from the isotonic solution and a longer impregnation time, with respect to esters, to reach saturation of the product.

Conclusions

The findings of the present investigation are consistent with those reported in the literature regarding the efficacy of vacuum application in flavor impregnation: VI and VUSI treatments gave the highest aroma enrichment at 5.0 min; USI, in contrast, was not significantly different from AI. Esters and alcohols also had different impregnation behaviors. Further studies are necessary to explain these results, as well as the reduction of ethyl 2-methylbutanoate, 3-methylbutyl acetate, and hexyl acetate after a few minutes of treatment.

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3.3 PULSED ELECTRIC FIELDS

3.3.1 Investigation of the effects of pulsed electric fields on total phenol content and on polyphenoloxidase and pectin methylesterase activity of melon (*Cucumis melo* L.) juice

Introduction

Thermal processing is the most common method to extend the shelf-life of fruit and vegetable juices, however, these treatments reduce the sensory and nutritional qualities of food products. Melon (*Cucumis melo* L.) is a commercially important crop in many countries. Owing to its high pH and low acidity, freshly cut melon or minimally-processed melon-based products have a short shelf-life. Moreover, the heat treatment of melon juice is a difficult issue because this fruit is thermosensitive, and a cooked off-odour is produced during thermal process.

Other undesirable changes of fruit juices are related to color, viscosity and flavour alterations. Many of these reactions are catalyzed by enzymes such as polyphenoloxidase (PPO), peroxidase (POD), lipoxygenase (LOX), pectin methylesterase (PME) and polygalacturonase (PG).

Non-thermal technologies, such as pulsed electric field (PEF) might be a valid alternative to heat treatment to obtain a cold pasteurization of melon juice. PEF treatment might reduce the quality losses of melon juice thanks to a small heat production. To date, only a little number of investigations have been carried out on the application of PEF on melon juice, and much of these researches have focused on microbial inactivation on inoculated products.

The objectives of our study were to investigate the effects of PEF on the total phenol (TP) content of melon juice treated by PEF and to compare it with non-treated juice. Moreover PME and PPO relative activities of fresh melon juice were evaluated through potentiometric and spectrophotometric techniques and were compared to those of melon juice treated by PEF.

Material and methods

Sample preparation for PEF treatment

Batches of melon (*Cucumis melo* L. - var. Chino) were bought on the local market in Monterrey. Fruits were washed with tap water, and with a disinfection solution of sodium hypochlorite, then they were rinsed with water and dried. In order to obtain melon juice they were peeled, cut and homogenized with a mixer. The obtained puree was filtered before PEF treatment.

Physico-chemical properties of fresh fruits

Three fruits were randomly selected from the batch used to produce melon juice. They were treated in the same way described in the previous paragraph for melon juice production. Titrable acidity was determined according to AOAC (1984) and expressed as g citric acid on 100 g of melon juice. Soluble solids (°Brix) were measured with an Atago Hand refractometer (ATAGO, Co. Osaka, Japan). Water activity was determined with a Decagon CX-1 hygrometer (Decagon Devices Inc., Pullman, Washington); the pH was evaluated with a Beckman pH-meter.

PEF equipment

An ELCRACK HVP5 apparatus was used to process melon juice. Pulsed electric field strength, pulse frequency and treatment time were ranged by applying a central composite design (three numeric factors, n = 3). A total of 20 experiments, including 14 non-center and 6 center points, were carried out in duplicate. Appropriate values of pulsed electric field strength (18.75-23.75 kV/cm), pulse frequency (100-900 Hz) and treatment time (4-8 µs) were selected in order to obtain the lowest heating effects; the highest temperature increase obtained in the product after treatment was 17°C.

Experimental design and statistical analysis

A central composite design was used to determine the effect of the voltage, frequency and treatment time on total phenol content, PME and PPO residual activity of melon juice treated by PEF. Experimental conditions are presented in table 3.3.1.

Table 3.3.1 Coded and real values of the independent variables in a central composite design.

Coded value	-1	0	1
E (kV/cm)	21.3	19.8	22.8
Frequency (Hz)	262	500	738
Time (µsec)	5	6	7

A total of 20 experiments, including 14 non-center and 6 center points, were carried out in duplicate, as reported in table 3.3.2. The experimental design allowed to establish a second order polynomial by:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_1x_1^2 + b_2x_2^2 + b_3x_3^2$$

The statistical analysis was performed using Design Expert V.5.0.3 (1996) to obtain the coefficients of the polynomial, the error probabilities (*p*), and the explained variability

percentage (R^2), which allows the evaluation of the global fitting of the model to the experimental values of independent variables.

Table 3.3.2 Central composite design followed to process *Cucumis melo* juice with different PEF treatments.

Experiment	*E* (kV/cm)	*F* (Hz)	*t* (µs)
1	22.8	262	5
2	19.8	262	7
3	22.8	738	7
4	21.3	500	6
5	18.8	500	6
6	21.3	900	6
7	21.3	500	8
8	19.8	738	5
9	21.3	100	6
10	19.8	262	5
11	21.3	500	6
12	19.8	738	7
13	22.8	262	7
14	22.8	738	5
15	21.3	500	6
16	21.3	500	6
17	21.3	500	6
18	23.8	500	6
19	21.3	500	6
20	21.3	500	4

Colorimetric determination of total phenol (TP) content

The total phenol (TP) content of melon juice was determined by the Folin–Ciocalteau method at 750 nm (Singleton & Rossi, 1965), using a multimode microplate reader (Biotek Sinergy HT, Biotek Instruments, Vermont, USA). TPs were calculated as gallic acid equivalent (GAE) from the calibration curve of gallic acid standard solutions (r^2 = 0.9996) and expressed as mg GAE/L of melon juice. The analyses were done in triplicate; and the mean values and the standard deviations were calculated.

PPO and PME measurements

For the assay of PPO, 0.5 mL of the sample, previously centrifuged and filtered through 0.45 µm nylon filters, was mixed with 2.0 mL 0.05 mol/L sodium phosphate buffer (pH=6.8). The sample was kept for 15 min at 30°C and then 1 mL of 0.2 mol/L catechol was added. PPO activity was determined by measuring absorbance of the mixture at 420 nm, using a

multimode microplate reader (Biotek Sinergy HT, Biotek Instruments, Vermont, USA) at 32°C.

The absorbance was acquired every 20 s for 30 min. The data obtained was plotted against time and the PPO activity was calculated from the slope of the initial linear part of the curves.

One unit of PPO activity was defined as the change in absorbance at 420 nm/min and per millilitre of melon juice.

PME activity was measured by adapting the method described by Elez-Martínez *et al.* (2007). A 10 mL aliquot of melon juice was added with 10 mL of NaCl 2 N, then the mixture was tempered at 30°C for 10 min. 10 mL of the diluted sample was mixed 40 mL of 1% pectin–salt substrate (also at 30°C) and incubated at 30°C. The solution was adjusted to pH 7.0 with 2.0 N NaOH, and then the pH of the solution was readjusted to pH 7.7 with 0.05 N NaOH. After the pH reached 7.7, 0.05 mL of 0.125 N NaOH was added. The time required for the solution pH to return to 7.7 was measured.

Both enzyme (PPO and PME) were expressed as activity units and the relative residual activity (RA%) of PPO and PME was calculated as the activity after treatment divided by the activity before the treatment. The residual activity was obtained with the following equation:

$$RA(\%) = \frac{A_t}{A_0} \cdot 100$$

where:

A_t: enzyme activity units of melon juice after PEF

A_0: enzyme activity units of untreated melon juice

Results and discussion

The physic-chemical properties of melon juice analyzed are presented in table 3.3.3. Each value is the mean of three measurements (n = 3).

Table 3.3.3 Physico-chemical properties of melon juice treated (mean value ± standard deviation).

Property	Value
a_w	0.97 ± 0.02
pH	7.20 ± 0.01
Acidity[a]	0.98 ± 0.01
Soluble solids[b]	9.90 ± 0.02

[a] g citric acid/100 g melon juice. [b] °Brix.

The analysis of variance of the results obtained showed that the second order models were well adjusted to the experimental data for both total phenols and enzyme activity ($p \leq 0.05$), as reported on table 3.3.4. The results obtained indicated that more than 95% of behavior variation of TPs could be explained by the quadratic fitted model; although lower R^2 were obtained for the residual enzymatic activities, the explained variation was respectively 74.8 and 88.0 for PPO and PME. The bold characters indicate that the corresponding parameters have a significant effect on y ($p < 0.05$).

Table 3.3.4 Analysis of variance for TPs, PME and PPO (y), using coded variables (x_1: voltage; x_2: frequency; x_3: time).

Source	SS	df	MS	*F*	*p*(F)
Model for TPs: $y = 301.44 - 5.63x_1 + 1.61x_2 - 1.63x_3 - 2.54x_1x_2 + \mathbf{7.20x_1x_3} - 3.05x_2x_3 - \mathbf{11.52x_1^2} - \mathbf{15.86x_2^2} - \mathbf{13.19x_3^2}$ ($R^2 = 0.9791$)					
Model	7791.7	9	865.7	52.02	< 0.0001
Residual	166.4	10	16.6		
Model for PME: $y = 94.28 + \mathbf{1.08x_1} - 0.08x_2 + 0.48x_3 + 0.24x_1x_2 - 1.27x_1x_3 + 0.76x_2x_3 + \mathbf{1.45x_1^2} + \mathbf{3.11x_2^2} + \mathbf{1.82x_3^2}$ ($R^2 = 0.8804$)					
Model	224.38	9	24.9	8.18	0.0015
Residual	30.49	10	3.05		
Model for PPO: $y = 92.99 + 4.31x_1 + \mathbf{7.24x_2} + 0.74x_3 + \mathbf{7.44x_1x_2} - 3.36x_1x_3 - 6.51x_2x_3 + 1.39x_1^2 - 2.81x_2^2 + 2.37x_3^2$ ($R^2 = 0.7483$)					
Model	2095.82	9	232.87	3.30	0.0382
Residual	705.10	10	70.51		

The TPs content of melon juice treated with PEF was well fitted with a quadratic model, as illustrated in figure 3.3.3.

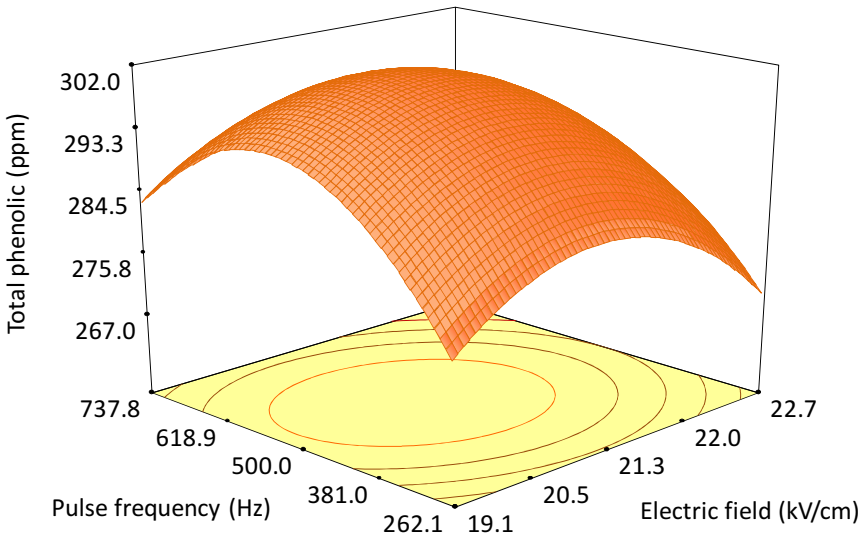

Figure 3.3.3 Surface response of the effect of pulse frequency and electric field strength on TP content of melon juice treated by PEF. Duration of electric pulse: 6 µs (R^2 = 0.9791).

PEF conditions corresponding to the central point of the experimental design (21.3 kV/cm, 500 Hz, 6 µs) determined the highest TP concentration, respect to non-treated melon juice.

As illustrated in figure 3.3.4, the TP content in fresh non-treated melon juice was initially 266.5 ppm, and it reduced to 231.4 and 222.5 ppm, after 3 and 6 days of storage, respectively. TP content of PEF treated juice resulted 13.2 %, 25.9% and 29.9% higher than non-treated sample, at 0, 3 and 6 days of storage, respectively.

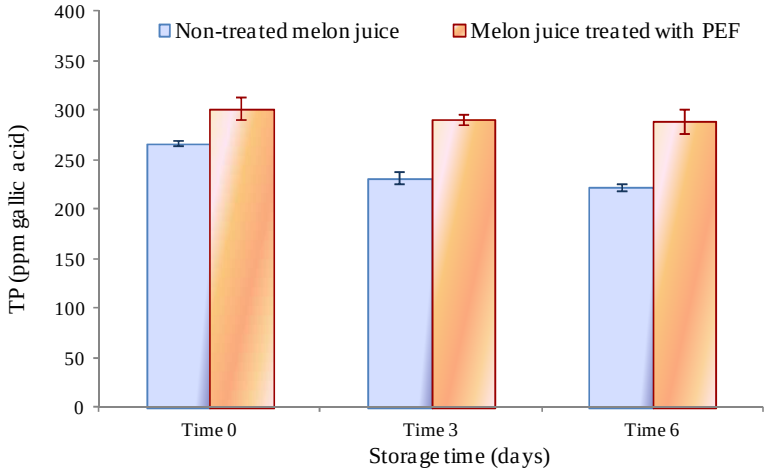

Figure 3.3.4 Comparison of TP content of melon juice non-treated and treated by PEF (21.3 kV/cm, 500 Hz, 6 µs), at different storage times.

PME results fitted well with a surface quadratic model design (R^2: 0.8804, $p<0.05$); a maximum reduction of the enzymatic activity was obtained in correspondence of the central point (21.3 kV, 500 Hz, 6 µs), as illustrated in figure 3.3.5.

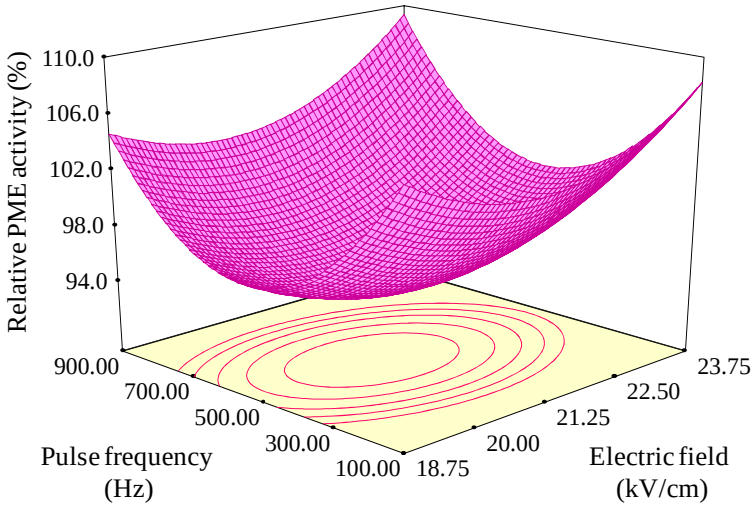

Figure 3.3.5 Surface response of the effect of pulse frequency and electric field strength on relative PME activity; pulse duration: 6 µs.

Also PPO activity was fitted well by a quadratic model (R^2: 0.7483, $p<0.05$). In several experimental conditions tested, the PPO relative activity was increased by PEF process, with respect to the correspondent non-treated melon juice. The highest PPO activity reduction was obtained with electric pulses of low frequency and high duration (5-6 µs), as reported in figure 3.3.6.

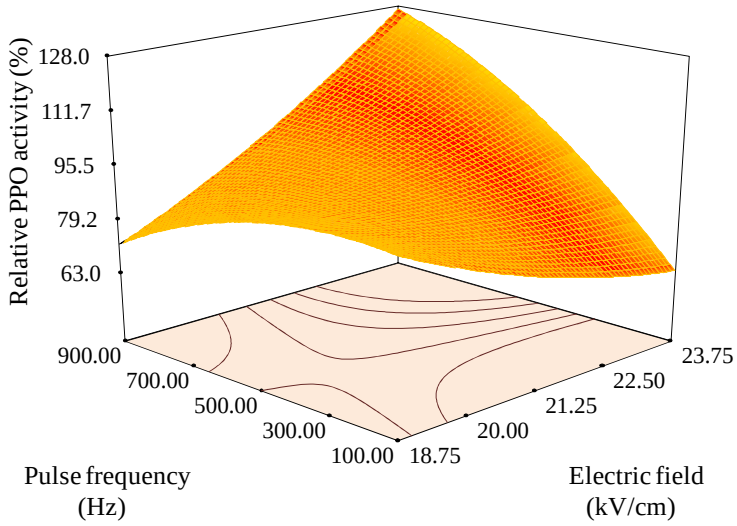

Figure 3.3.6 Surface response of the effect of pulse frequency and electric field strength on relative PPO activity; pulse duration: 6 µs.

Conclusions

A central composite design was used to compare the TP content, PME and PPO of melon juice treated by PEF to a non–treated product.

TPs of melon juice treated with PEF were well fitted with quadratic surfaces; PEF condition corresponding to the central point of the experimental design determined the highest TP content in PEF melon juice at each storage time (0, 3 and 6 days).

PME and PPO relative activities of melon juice treated by PEF were well fitted by quadratic surfaces. The maximum reduction of the PME activity was obtained in correspondence of the central point of the experimental design. The relative activity of PPO was increased or reduced respect to the un-treated melon juice, depending on the PEF conditions applied. Further studies are necessary in order to explain the variations detected.

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4.INSTRUMENTAL ANALYSIS: POLYPHENOLS IN VEGETABLE MATRIX

4.1 DEFINITION AND CLASSIFICATION

Phenolic compounds (PCs) are chemically defined by the presence of at least one aromatic ring bearing one (phenols) or more (polyphenols) hydroxyl substituents, including their functional derivatives (e.g. esters and glycosides) (Hättenschwiler and Vitousek, 2000).

PCs are the most widely distribute secondary metabolites of plants, in which they can act as natural antimicrobial agents, defensive compounds against herbivores, inhibitors of pre-harvest seed germination, or also as attracting agent for pollulants or UV protective agents (Haslam and Lilley, 1988; Haslam, 1998; Bravo, 1998).

Over 8000 phenolic compounds are currently known (Bravo, 1998) and they can be classified in a number of ways. Harbourne (1989) suggested a classification based on the number of carbons in the molecule (table 4.1.1).

Table 4.1.1 Classification of phenolic compounds (Harbourne, 1989).

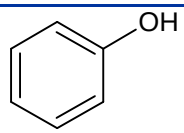
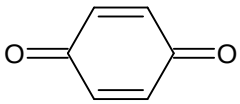
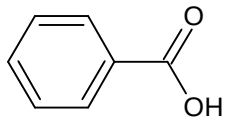
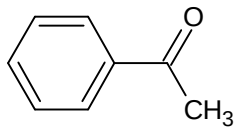
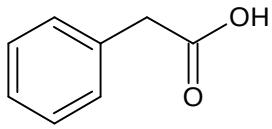
Structure	Class	Molecule
C_6	Simple phenolics	
	Benzoquinones	
C_6-C_1	Phenolic acids and related compounds	
C_6-C_2	Acetophenones	
	Phenylacetic acids	

Table 4.1.1 *Continued*

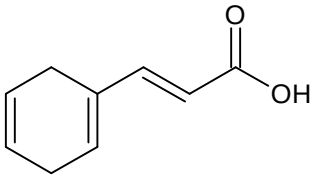
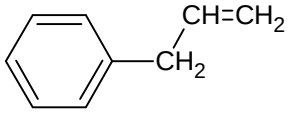
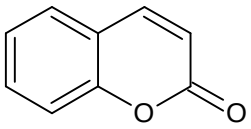
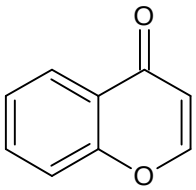
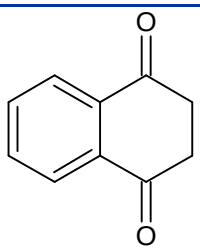
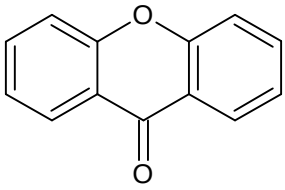
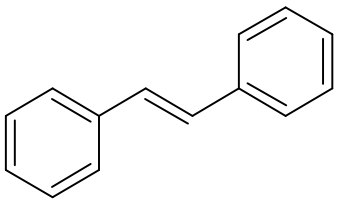
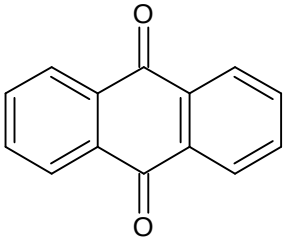
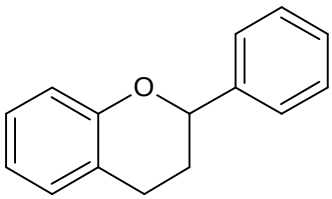
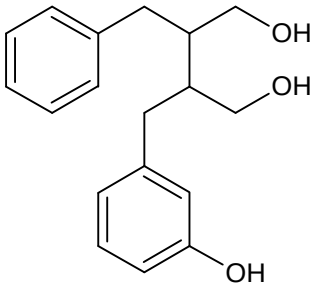
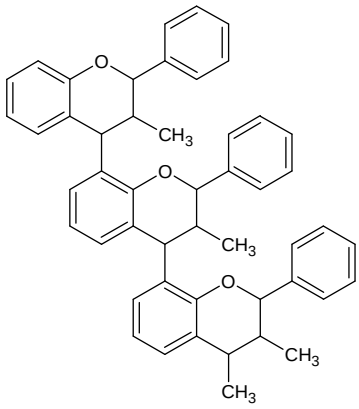
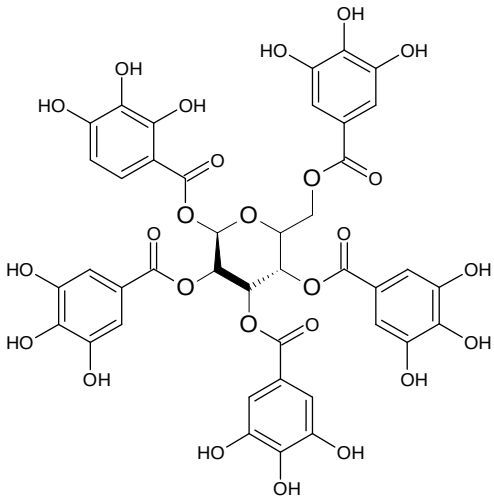
Structure	Class	Molecule
C_6-C_3	Cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols	
	Phenylpropenes	
	Coumarins, isocoumarins	
	Chromones	
C_6-C_4	Napthoquinones	
C_6-C_1-C_6	Xanthones	
C_6-C_2-C_6	Stilbenes	
	Anthraquinones	

Table 4.1.1 *Continued.*

Structure	Class		Molecule
C_6-C_3-C_6	Flavonoids		
$(C_6$-$C_3)_n$	$(C_6$-$C_3)_2$	Lignan	
	$(C_6$-$C_3)_{2+n}$	Lignin	
n	Condensed tannins		
	Hydrolysable tannins		

High molecular weight PCs include lignin (polymers of C6–C3 hydroxycinnamate-type compounds), condensed tannins (polymers of flavanols formed by oxidative condensation between the C-4 of the heterocyclic ring and the C-6 and C-8 carbons of the adjacent rings) and hydrolysable tannins (gallic acid, 3-digallic acid or hexahydrohydiphenic acid esterified to a polyol such as glucose or quinic acid(O'Connell and Fox, 2001).

An alternative classification has been used by Swain and Bate-Smith (1962), who grouped the phenols in "common" and "less common" categories. Ribéreau-Gayon (1972) grouped phenolic compounds into three families as follows:

1. Widely distributed phenols – ubiquitous to all plants, or of importance for a specific plant.
2. Phenols that are less widely distributed – limited number of compounds known.
3. Phenolic constituents present as polymers.

Polyphenols are important components of common foods, including tea, red wine, fruits, vegetables, beverages and various medicinal plants. The importance of polyphenols arises from their effects on sensory properties, including astringency and colour, and on the possible health effects that they may have (Vekiari *et al.*, 2008). In fact these molecules posses anti-tumoral, anti-allergic, anti-platelet, anti-ischemic, and anti-inflammatory properties, and most of these effects are believed to be due to their antioxidant capacity (Moure *et al.*, 2001). The antioxidant compounds from natural sources could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. Natural antioxidants have been also proposed for use in topical pharmaceutical and cosmetic compositions (Moure *et al.*, 2001).

Increasing interest in the replacement of synthetic antioxidants has led to research into natural sources of antioxidants, especially in plant materials (Vázquez *et al.*, 2008).

Because purified phenolic compounds are difficult to obtain and because extracts sometimes have antioxidant activities higher than those of pure molecules, there is a growing interest for the use of plant extracts (Calliste *et al.*, 2005).

The vegetable matrix that have been analysed in the following investigations (research articles 3 and 4) are olive mill waste water (OMWW) and chestnut bark extracts. The first is rich in simple phenols, while in the latter the main components are represented by complex molecules belonging to the class of hydrolysable tannins.

4.2 MAIN PHENOL COMPOUNDS IN OLIVE MILL WASTE WATER (OMWW)

OMWW is an important by-product of olive oil industry obtained in three-phase systems; it is the combination of the aqueous phase released by olives, and of the water used to process the fruits during extraction of oil (Bazoti *et al.*, 2006). Their phenolic composition has attracted great attention recently, in fact the isolation of phenolic bioactive compounds and the employment in pharmaceutical, cosmetic, food and other industrial sectors, has been proposed as a viable alternative for valorising this by-product (Obied *et al.*, 2005c; Russo, 2007). Moreover, the development of a low-cost processing method for these residues could lead to the generation of valuable co-products, reducing the overall extra virgin olive oil processing costs, and thus increasing the competitiveness and economic profits for mill companies.

In addition to solvent extraction techniques used to fractionate and recover polyphenols in small scale experiments (Lesage-Meessen *et al.*, 2001; De Marco *et al.*, 2007; Galanakis *et al.*, 2010), the development of membrane technology adopting different filtration techniques, such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), has been proposed, as an alternative to traditional physical-chemical, biological and thermal treatments (Paraskeva and Diamadopoulos, 2006), with the objective of reducing environmental pollution while simultaneously recovering and concentrating OMWW useful by-products (Paraskeva *et al.*, 2007; Russo, 2007; Bódalo *et al.*, 2008; Akdemir and Ozer, 2009; Coskun *et al.*, 2010; Garcia-Castello *et al.*, 2010).

Other recent studies concerned the treatment of olive mill waste water in vertical subsurface flow constructed wetlands (Yalcuk *et al.*, 2010) and the investigation of the addition of OMWW concentrates to extra virgin olive oils (Zunin *et al.*, 2011).

The phenolic composition of OMWW has been widely studied in the last few years. Several aspects, ranging from extraction, analysis, identification and quantification procedures (Bazoti *et al.*, 2006; Bianco *et al.*, 2003; Della Greca *et al.*, 2004; Mulinacci *et al.*, 2001; Obied *et al.* 2005a; Obied *et al.*, 2005c; Zafra *et al.*, 2006) to the evaluation of phenol antioxidant (Lesage-Meessen *et al.*, 2001; Visioli et al, 1999), antimicrobial (Ramos-Cormenzana *et al.*, 1996) and molluscicidal activities (Obied *et al.*, 2005b) have been taken into account. A list of the bioactivity of the major biophenols in OMWW could be found also in Obied *et al.* (2005c). Gómez-Caravaca *et al.* (2011) studied different hydrolysis processes of OMWW and found that hydrolysis with hydrochloric and citric acids had a good efficiency and proposed them as a pretreatment to recover antioxidant compounds from OMWW.

Recently, the phenolic composition of solid and liquid wastes generated during the storage of extra virgin olive oil was studied (Lozano-Sanchez *et al.*, 2011) and, as previously

reported for OMWW (Servili *et al.*, 1999; De Marco *et al.*, 2007; Obied *et al.*, 2007) an high amount of hydroxytyrosol was found, together with other phenolic alcohols and acids, secoiridoids, lignans and flavones, whose origin was tentatively established based on proposed degradation pathways.

In research article 3, the composition of OMWW treated with a semi-industrial membrane filtration system, including UF and RO modules, was investigated. The determination of phenol and antioxidant content of OMWW treated with different filtration systems was studied, with particular attention to the qualitative and quantitative phenol composition of UF and RO permeates and retentates.

Phenolic Profiles of Olive Mill Wastewaters Treated by Membrane Filtration Systems

PATRIZIA COMANDINI,[*,†] MARÍA J. LERMA-GARCÍA,[§] PIETRO MASSANOVA,[†] ERNESTO F. SIMÓ-ALFONSO,[#] AND TULLIA GALLINA TOSCHI[†]

[†]Department of Food Science, University of Bologna, Piazza Goidanich 60, I-47521 Cesena, FC, Italy

[§]Department of Analytical Chemistry and Food Technology, University of Castilla – La Mancha, Avda. Camilo José Cela 10, E-13004, Ciudad Real, Spain

[#]Department of Analytical Chemistry, University of Valencia, Doctor Moliner 50, E-46100, Burjassot, Valencia, Spain

*Corresponding author (telephone +390547338121, fax +390547382348, e-mail patrizia.comandini2@unibo.it).

ABSTRACT

Olive mill wastewater (OMWW) is an important by-product obtained during the extraction of olive oil. In this investigation, the phenolic composition of OMWW treated with a semi-industrial membrane filtration system, including ultrafiltration (UF) and reverse osmosis (RO) modules, was studied. In particular, the composition of untreated OMWW was compared to the permeate and to concentrate fractions obtained at each filtration step. 3,4-(dihydroxyphenyl) ethanol and *p*-(hydroxyphenyl) ethanol were found as the main compounds of all OMWW analyzed. A total of 32 compounds detected at 240 or 280 nm by HPLC-DAD were considered for quantification of phenols. UF reduced phenol concentration by about 40% with respect to the initial level; in the permeate of RO, the phenol concentration ranged from 0 to 1% of the initial content. In contrast, the content of phenolic compounds was increased of about 2.6 fold in RO concentrate. The same phenolic profile, more or less intense, was obtained at all stages of filtration.

Keywords: Olive mill waste water (OMWW); phenolic compounds; reverse osmosis; ultrafiltration

INTRODUCTION

Olive mill waste water (OMWW) is an important by-product of olive oil industry obtained in three-phase systems; it is the combination of the aqueous phase released by olives, and of the water used to process the fruits during extraction of oil (*1*). In addition to OMWW, other olive mill wastes (OMWs) include pomace (solid waste produced in the three-phase systems) and alperujo (semi-solid waste generated by two-phase systems).

The composition of OMW has been widely investigated (*2*, *3*) and the main components of the organic fraction comprise sugars, tannins, phenolic compounds, polyalcohols, pectins and lipids (*4*, *5*).

Several aspects of OMW composition have been investigated, ranging from extraction, analysis, identification and quantification of phenols (*1*, *6–10*), and evaluation of their antioxidant (*11–13*), antimicrobial (*14*) and molluscicidal activities (*15*). Several analytical methods used to characterize the major biophenols in OMW, and their bioactivity has been reviewed by Obied et al. (*16*).

Secoiridoid derivatives, namely, 3,4-(dihydroxyphenyl) ethanol (hydroxytyrosol, HYTY) and *p*-(hydroxyphenyl) ethanol (tyrosol, TY) are the main compounds of OMWW, whereas secoiridoid glycosides are present at high concentrations in pomace and olive fruit (*17*). However, the phenolic composition of OMW is characterized by a large complexity; in fact, Obied et al. (*18*), using reversed phase HPLC coupled with photodiode array detection (DAD), electrospray ionisation mass spectrometry (ESI-MS) and fluorimetric detection (FLD), confirmed the presence of 52 phenolic compounds in olive extracts and 44 in OMW, which belonged to the classes of simple phenols, benzoic and cinnamic acids, flavonoids and secoiridoids.

Owing to the toxicity of OMWW (*19*) and its antimicrobial (*20–21*) and degradation properties (*22*), initial studies on OMWW phenol fraction were aimed to remove these compounds through physico-chemical and biological treatments. More recently, the isolation of phenolic bioactive compounds and their employment in pharmaceutical, cosmetic, food and other industrial sectors, has been proposed as a viable alternative for valorizing this by-product (*16*, *23*). In addition to solvent extraction techniques used to fractionate and recover polyphenols in small scale experiments (*11*, *24–25*), the development of membrane technology adopting different filtration techniques, such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), has been proposed for both

pre-treatment of OMWW, reducing their polluting load (*26–30*), and for the recovery and fractionation of polyphenols (*23*, *31*).

In particular, De Marco et al. (*24*) were able to purify HYTY from all other biophenols of OMWW, yielding 1 g of pure HYTY from 1 liter of OMWW. Several methods for obtaining HYTY from OMWW and other by-products derived from olive trees have also been patented (*32–34*).

In this investigation, the composition of OMWW treated with a semi-industrial membrane filtration system, including UF and RO modules, was studied. The aim of this work was the determination of phenol and antioxidant content of OMWW treated with different filtration systems. In particular, the concentration of phenols in the retentate and permeate of UF and RO were evaluated and compared with the untreated sample. Qualitative and quantitative phenol composition of UF and RO permeates were compared to the initial sample (OMWW before filtration) to determine the selectivity of these membranes towards one or more phenolic classes.

MATERIALS AND METHODS

Reagents and standards. The standards and reagents used for the quantification of phenols by spectrophotometry (gallic acid (GA), Folin-Ciocalteau reagent, sodium molybdate dihydrate), HPLC (3-hydroxyphenylacetic acid (3-HPA), vanillin (VAN), vanillic acid (VA), hydroxytyrosol (HYTY), oleuropein (OLE), ferulic acid (FA), cinnamic acid (CIN), (-)-epicatechin (EPI), (+)-catechin (CAT), caffeic acid (CAF), *p*-coumaric (*p*-COUM) and syringic acid (SYR)) and for the evaluation of the antioxidant capacity (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid diammonium salt ($ABTS^{\bullet+}$) and potassium persulfate) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other solvents used were: *n*-hexane, methanol, ethyl acetate (Sigma-Aldrich, St. Louis, MO, USA); acetonitrile, formic acid and ethanol (Merck, Darmstadt, Germany). Deionized water was obtained with a Barnstead deionizer (Sybron, Boston, MA).

Samples. OMWW samples were collected during the 2009-2010 production years from a three-phase olive oil mill in Emilia-Romagna (Italy). After collection, OMWW samples were pretreated with a semi-industrial membrane filtration system, including UF and RO. The following abbreviations have been used: FEED, initial OMWW before filtration; UF Perm,

OMWW permeate from ultrafiltration module; RO Perm, OMWW permeate from reverse osmosis module; RO RET, OMWW retentate from reverse osmosis.

Dry matter pH measurements. Dry matter (DM) was determined by drying about 50 g of OMWW samples at 105°C until a constant weight was reached. pH was measured on OMWW samples previously centrifuged (3000 rpm, 5 min) and filtered (cellulose acetate filter 0.45 µm). The pH-meter was a Basic 20 model (Crison Instrument, Barcelona, Spain).

Spectrophotometric determination of total phenol (TP) content. Before each photometric determination (TP, *o*-DPH content, $ABTS^{\bullet+}$ and COD evaluation), OMWW samples were centrifuged (3000 rpm, 5 min), filtered through cellulose filters (0.45 µm) and the oil residues were removed by washing the samples three times with *n*-hexane. The TP content of the extracts was determined by adapting a previously published method (*35*). After suitable dilution of the samples, TP content was determined using the Folin-Ciocalteau reagent and measuring the absorbance at 750 nm (Shimadzu Spectrophotometer UV-VIS 1204, Kyoto, Japan). Total phenols were expressed as mg GA mL^{-1} sample (calibration curve with r^2 = 0.9932). Spectrophotometric analyses were repeated three times for each OMWW sample.

Spectrophotometric determination of *o*-diphenols (*o*-DPH). 4 mL of a solution prepared by mixing 0.5 mL of pretreated OMWW sample and 5 mL of methanol/water (1:1 v/v) were added to 1 mL of a 5% solution of sodium molybdate dihydrate in ethanol/water (1:1 v/v) and vigorously shaken. After 10 min at room temperature, the mixture was centrifuged for 5 min at 3000 rpm and the absorbance of the supernatant was measured at 370 nm. The calibration curve (r^2 = 0.9954) was constructed with GA solutions. The results were expressed in mg GA mL^{-1} sample. The spectrophotometric analysis was repeated three times for each sample.

$ABTS^{\bullet+}$ scavenging activity of OMWW. An aqueous solution of $ABTS^{\bullet+}$ (concentration of 7 mM) was prepared. The radical cation of ABTS was obtained by reaction with potassium persulphate until it reached a final concentration of 2.45 mM, while maintaining the stock solution in the dark at room temperature for at least 12 h. Before use, the $ABTS^{\bullet+}$ solution was diluted with ethanol to reach an absorbance of 0.70 ± 0.02 at 734 nm at 30°C. Next, 1 mL of the diluted $ABTS^{\bullet+}$ solution was added to 0.01 mL of pretreated OMWW sample and the decrease in absorbance was recorded for 10 min (*36*). Absorbance values were corrected for radical decay using a blank solution (0.01 mL of 50% aqueous methanol). Measurements were made in triplicate and the antioxidant activity was calculated as Trolox Equivalent

Antioxidant Capacity (TEAC, mmol Trolox L^{-1} sample), using a calibration curve with r^2 = 0.9811.

Chemical oxygen demand (COD). COD of OMWW was determined using a multiparameter bench photometer dedicated for COD analysis (HI 839800, Hanna Instruments United States Inc., Woonsocket, RI, USA). OMWW samples, previously centrifuged and filtered, were appropriately diluted with distilled water and analyzed using reagents for measurement of high range COD (0 - 15 g O_2 L^{-1}). Samples were added to the reagent vials, mixed and heated for 2 h at 150°C. At the end of the digestion, when room temperature was reached, the photometric measurement was made. Distilled water was used as blank.

Extraction of phenolic compounds. According to Gómez-Caravaca et al. (*37*), about 10 g of OMWW were centrifuged (3000 rpm, 10 min), the supernatant was filtered through nylon filters (0.45 µm) and washed three times with *n*-hexane. 5 mL of OMWW were extracted three times with 7.5 mL of ethyl acetate. Next, the three extractions were combined and evaporated under nitrogen to complete dryness. The concentrated extract was dissolved in 1 mL of aqueous methanol (50%), filtered through 0.20 µm nylon filters and injected in HPLC.

Instrumentation and working conditions. A 1100 series liquid chromatograph provided with a quaternary pump and UV–Vis diode array detection (Agilent Technologies, Waldbronn, Germany), was used. Separation was carried out with a reverse phase C18 (2) 100A Luna column (5 µm, 150 x 4.60 mm I.D., Phenomenex, Torrance, CA, USA). The mobile phase was composed of solvent A (0.5% v/v formic acid in HPLC-grade water) and solvent B (acetonitrile). Solutions were filtered through 0.20 µm cellulose acetate filter discs (Albet, Barcelona, Spain) and sonicated for 10 min before use. The following linear gradient elution was employed: from 0 to 27 min solvent B increased from 5 to 18%, at 50 min solvent B reached 25%, and finally at 72 min solvent B was 95%; at 77 min 5% solvent B was restored. A 5 min post-run equilibration was performed. An injection volume of 10 µL and a flow rate of 0.5 mL min^{-1} were used. Absorption spectra were recorded in the range of 200–600 nm, while the detector wavelength was set at 240, 280 and 320 nm. Peak quantification was carried out at 240 and 280 nm. The main phenolic compounds were identified by comparison with the relative retention times of reference standards, when available, or by comparing the relative elution order and UV spectra with those reported in literature. The identity of each peak was also confirmed by HPLC-MS. In fact, the liquid chromatograph was also coupled (in series with the UV–vis detector) to the ESI source of an HP 1100 series ion trap mass spectrometer (ITMS) (Agilent). The ITMS working conditions were: nebulizer gas

pressure, 0.24 MPa (35 psi); drying gas flow, 7 L min^{-1} at 300°C; capillary voltage, 2.5 kV; voltages of skimmers 1 and 2, −41.0 and −6.0 V, respectively. Nitrogen was used as nebulizer and drying gas (Gaslab NG LCMS 20 generator, Equcien, Madrid, Spain). The mass spectrometer was scanned within the *m/z* 100–900 range in the negative and positive ion mode. Maximum loading of the ion trap was 3×10^4 counts, and maximum collection time was 300 ms. To enhance the sensitivity of detection, a flow divisor of 1:10 ratio located after the UV–Vis detector and before the ESI source was used. Total ion chromatograms (TIC) and extracted ion chromatograms (EIC) were smoothed using a Gaussian filter set at 9 points. Quantification of phenolic compounds in HPLC was achieved by comparing the peak areas with those of HYTY as external standard, according to the procedure described by Tsimidou et al. (*38*). Data were expressed as mg HYTY kg^{-1} OMWW for both simple and hydrolysable phenolic compounds.

Moreover, a cumulative quantification of phenols detected at 240 and 280 nm was made. The compounds detected at 240 nm were kept separate from those detected at 280 nm to monitor the behavior of the different classes of phenols to filtration. These results were expressed in percentages with respect to the feed sample, which was considered 100%.

Statistical analysis. All experiments were performed in triplicate. Data were expressed as means ± standard deviation (SD) and analysed using Statistica 8.0 (Statsoft Inc.,Tulsa, OK). Analysis of variance (ANOVA) was used to determine if significant differences existed at a level of confidence of $p < 0.05$ (Honestly Significant Differences or HSD by Tukey).

RESULTS AND DISCUSSION

The composition of OMWW during the different stages of filtration is reported in **Table 1**. Filtration caused a reduction of the DM of samples from 3.02 % (FEED) to 0.08 % (RO PERM); DM of RO RET was almost doubled with respect to the initial sample (FEED), indicating the level of concentration reached in the retentate of RO during filtration. The treatments reduced the COD values of OMWW by about 80 %, from 38.89 g O_2 L^{-1} (FEED) to 7.86 g O_2 L^{-1} (RO PERM) at the end of the process. The COD of RO RET was almost 1.4 times greater than in the FEED sample. The filtration treatments did not affect the pH of the samples. pH values were very close to the pH range (5.5-9.5) requested by Italian regulations (*39*) to discharge OMWW in sewer or surface water.

As shown in **Figure 1**, filtration caused a significant reduction of the phenolic content and antioxidant capacity of the permeates. In particular, a significant reduction of TP and a slight decrease of *o*-DPH in UF PERM respect to FEED was recorded; in the last fraction obtained at the end of membrane filtration system (RO PERM), both *o*-DPH and TP were nearly zero. Regarding RO RET, *o*-DPH and TP were two and three times higher than those of FEED sample, respectively. A similar trend was recorded for the antioxidant capacity ($ABTS^{\bullet+}$).

The composition was also evaluated by HPLC analysis and the main simple and complex phenolic compounds were quantified using the external standard method. The quantification was aimed to compare the composition of the different fractions of OMWW collected during the sequential filtrations.

Phenolic compounds were detected at 240, 280 and 330 nm, but quantification and identification were made at 240 or 280 nm at the highest absorption wavelength of each compound; none of the main phenolic components of OMWW showed appreciable absorbance at 330 nm.

The chromatograms of an OMWW sample before filtration treatments, recorded at 240 and 280 nm are shown in **Figure 2A** and **2B,** respectively.

The main 32 compounds detected at 240 and 280 nm were considered for the comparison of the phenolic amounts in the different OMWW permeates and retentates; 19 compounds were evaluated at 240 nm and 13 at 280 nm; their concentrations were expressed as mg kg^{-1} of HYTY, as reported in **Table 2**. The main phenols of Table 2 are also indicated in **Figure 2**.

As can be seen in **Table 2**, RO PERM and RO RET showed, respectively, the lowest and the highest phenolic concentrations, for all compounds evaluated. Additionally, in UF PERM a significant reduction in phenol content in comparison to FEED was detected for the majority of phenols. The most abundant components, reported in bold italic (peaks 2 and 4), were identified as HYTY and TY, as discussed later.

For each sample, the mean percentage content of phenolic compounds quantified in HPLC was compared with the initial content (FEED), which was fixed at 100%, as is shown in **Figure 3**. The UF PERM showed a phenol concentration that was significantly lower than the FEED sample (about 40% lower) at both 240 and 280 nm; in RO PERM, the phenol concentration was about 0.5% of the initial content of FEED at both wavelengths. In the RO RET, however, the concentration of phenolic compounds appeared to be increased by 2.55 and 2.86 times, at 240 and 280 nm respectively, compared to the initial FEED sample.

Based on HPLC analyses at 240 and at 280 nm and on the quantization of the 32 selected phenols, the UF treatment employed did not have a selective behavior on the single and complex phenols considered (about 130 – 600 g mol^{-1}).

The trend of phenols detected by HPLC was similar to the TP and *o*-DHP determined by spectrophotometric analysis; moreover, it was strictly proportional to the antiradical capacity previously evaluated (**Figure 1**). A slight reduction in phenols by UV analysis and antiradical capacity in UF PERM, and their disappearance in RO PERM, were observed.

Of the 32 compounds used for quantitative evaluation, 11 compounds were identified (**Table 3**). The main compounds of OMWW were identified basing on reference standards, mass spectrometry, UV and bibliography data (*6*, *18*). HYTY and TY were the main components with concentrations in FEED samples of 183.9 and 47.0 mg kg^{-1}, respectively. Other compounds, such as EPI, CAT, and FER and OE, not reported in **Table 3**, were detected only at trace levels in RO RET samples. Some phenolic molecules, usually detected in OMWW, as CAF, *p*-COU, CIN and SYR (*6*, *13*) were not found. Their absence was confirmed by the injection of reference standards. OMWW collected at the end of the oil season and during storage may have led hydrolytic processes and microbiological degradation, explaining the high concentration of simple phenols and the absence of the previously cited molecules (*5*). As reported in the Material and Methods section, OMWW were sampled from the storage tank where they were usually stored during seasonal oil production, as during normal handling before disposal. This choice was made to verify the qualitative and quantitative composition of OMWW produced on an industrial scale and the effect of different filtration treatments. The results obtained showed that, even in absence of optimal storage conditions of OMWW, filtration by RO gave a retentate fraction that was rich in phenols and a permeate with concentration of phenolic substances near the limits of detection.

In particular, RO PERM could be recycled and reused in the oil mill, for washing or for other operations, contributing to reduce water consumption and disposal costs of OMWW. For this purpose, the draft guidelines proposed by the Codex Alimentarius Commission (*40*), concerning the hygienic reuse of processing water in food plants, should be followed. For evaluation of microbiological, chemical, physical and sensory properties, specific limits defined by each country should be respected, taking into account the specific category of reuse of OMWW.

The RO RET, which is characterized by a high phenolic content, could be used in animal breeding through direct utilization as animal feed or following protein enrichment (*41*);

OMWW could also be processed and further employed in cosmetic formulations, or used as functional ingredient in pharmaceutical or food products. With reference to the last applications, further studies are necessary to fully characterize the composition of crude OMWW extracts and verify which treatments are necessary to convert raw OMWW to a food or pharmaceutical grade. In addition to the microbiological aspects and the concentration of phenolic substances, it will be necessary to eliminate compounds responsible for off-odor development and their precursors.

In conclusion, TP content, *o*-DHP and antioxidant capacity showed a similar trend, with a progressive reduction of their values in the UF and RO permeates, and a significant increase in the retentate fraction of the RO. HPLC data confirmed these results: the concentration of phenolic compounds in the permeate of UF was about 40% lower compared to the untreated sample; in RO PERM, however, the phenol content approached zero. The RO RET had a phenol content that was 2.6 times higher than that in the untreated sample.

The main components of OMWW were secoiridoid derivatives, namely, hydroxytyrosol, tyrosol and oxidized elenolic acid; however, further studies are necessary to determine the nature of several yet unidentified compounds in OMWW.

All the compounds detected at 240 and 280 nm had a similar behaviour during filtration, showing that neither UF nor RO membranes had a selective effect on the retention of phenols present in OMWW.

The filtration membranes used in this investigation showed a good capacity both to concentrate phenols in the osmotic retentate fraction and to produce a final water waste that was poor in phenolic substances, as indicated by spectrophotometric and chromatographic analyses.

ABBREVIATIONS USED

3-HPA, 3-Hydroxyphenyl acetic acid; CAF, caffeic acid; CAT, (+)-catechin; CIN, cinnamic acid; COD, chemical oxygen demand; DAOA, deacetoxy oleuropein aglycon; DHPG, 3,4-dihydroxyphenylglycol; Di-HBA, Di-hydroxybenzoic acid; EPI, (-)-epicatechin; ESI, electrospray ionization; FA, ferulic acid; GA, gallic acid; HYTY, hydroxytyrosol; LA, 2-(5-ethylidene-2-oxo-tetrahydro-2H-pyran-4-yl) acetic acid; MW, molecular weight; *p*-COUM, *p*-coumaric acid; *o*-DPH, *orto*-diphenol; OMWW, olive mill waste water; OLE, oleuropein; OxEA, oxidized elenolic acid; SYR, syringic acid; TP, total phenol; TY, tyrosol; VA, vanillic acid; VAN, vanillin.

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416 **TABLES**

Table 1. Composition of OMWW Processed by Different Membrane Systems[a]

Parameter	FEED	UF PERM	RO PERM	RO RET
DM[b] (%)	3.02±0.06 b	2.39±0.17 c	0.08±0.04 d	5.61±0.09 a
COD[c] (g O_2 L^{-1})	38.89±0.49 b	20.09±0.12 c	7.86±0.01 d	54.53±0.32 a
pH	4.98±0.02 a	4.80±0.32 ab	4.39±0.01 b	5.14±0.02 a

[a]Mean values (n = 3). Different letters in the same row indicate statistically significant differences ($p < 0.05$). [b]DM Dry matter. [c]COD Chemical oxygen demand.

Table 2. Content of Phenolic Compounds in OMWW Samples[a]

			Concentration (mg HYTY kg^{-1})			
Peak	tr (min)	λ (nm)	FEED	UF PERM	RO PERM	RO RET
1	5.58	280	6.7 ±0.23 b	5.4 ± 0.83 b	0.1 ± 0.02 c	23.7± 0.24 a
2	***15.11***	***280***	***183.9 ± 5.63 b***	***119.3 ± 0.35 c***	***2.0 ± 0.20 d***	***398.7 ± 1.25 a***
3	18.95	280	0.9 ± 0.02 b	0.8 ± 0.26 b	0.0 ± 0.00 c	2.8 ± 0.15 a
4	***21.90***	***280***	***47.0 ± 0,71 b***	***34.1 ± 0.05 c***	***0.5 ± 0.08 d***	***134.0 ± 1.96 a***
5	24.14	240	4.7 ± 0.04 b	3.4 ± 0.09 c	0.0 ± 0.00 d	13.5 ± 0.12 a
6	25.42	280	3.0 ± 0.03 b	2.2 ± 0.16 c	0.0 ± 0.00 d	9.3 ± 0.04 a
7	26.41	280	1.8 ± 0.06 b	1.4 ± 0.05 c	0.0 ± 0.00 d	5.2 ± 0.06 a
8	27.43	280	8.0 ± 0.16 b	5.7 ± 0.03 c	0.0 ± 0.00 d	22.1 ± 0.16 a
9	27.79	240	2.9 ± 0.21 b	1.9 ± 0.26 c	0.0 ± 0.00 d	5.6 ± 0.01 a
10	29.97	280	1.3 ± 0.09 b	1.0 ± 0.03 c	0.0 ± 0.00 d	3.6 ± 0.07 a
11	32.65	240	2.2 ± 0.02 b	1.9 ± 0.67 b	0.0 ± 0.00 c	4.8 ± 0.08 a
12	33.72	280	6.2 ± 0.13 b	4.5 ± 0.17 c	0.0 ± 0.00 d	17.7 ± 0.02 a
13	36.22	240	3.2 ± 0.12 b	2.2 ± 0.19 c	0.0 ± 0.00 d	8.5 ± 0.05 a
14	38.06	240	10.5 ± 3.22 b	4.8 ± 1.97 bc	0.0 ± 0.00 c	34.0 ± 0.81 a
15	38.41	280	21.2 ± 0.5 b	14.7 ± 0.13 c	0.0 ± 0.00 d	58.1 ± 0.22 a
16	39.46	240	34.4 ± 0.3 b	21.9 ± 0.18 c	0.0 ± 0.00 d	74.9 ± 0.73 a
17	42.86	240	1.6 ± 0.09 b	1.0 ± 0.03 c	0.0 ± 0.00 d	4.5 ± 0.01 a
18	47.69	240	5.4 ± 0.26 b	4.2 ± 0.25 b	0.0 ± 0.00 d	14.3 ± 0.63 a
19	48.28	280	3.1 ± 0.43 b	1.9 ± 0.06 c	0.0 ± 0.00 d	8.4 ± 0.08 a
20	51.86	240	8.7 ± 2.25 b	3.6 ± 1.44 bc	0.0 ± 0.00 c	29.6 ± 0.95 a
21	53.33	240	14.8 ± 1.83 ab	11.2 ± 4.27 b	0.0 ± 0.00 c	24.4 ± 1.60 a
22	54.32	240	23.6 ± 0.18 b	14.4 ± 0.37 c	0.0 ± 0.00 d	57.5 ± 0.01 a
23	55.74	240	2.6 ± 0.38 b	2.2 ± 0.25 b	0.0 ± 0.00 b	10.6 ± 1.40 a
24	57.06	280	14.2 ± 0.04 b	10.4 ± 0.18 c	0.0 ± 0.00 d	39.3 ± 1.53 a
25	57.73	240	1.2 ± 0.07 b	0.9 ± 0.10 c	0.0 ± 0.00 d	3.7 ± 0.01 a
26	57.98	240	1.3 ± 0.03 b	0.9 ± 0.05 c	0.0 ± 0.00 d	3.7 ± 0.01 a
27	58.10	240	3.8 ± 0.03 b	2.5 ± 0.02 c	0.0 ± 0.00 d	7.7 ± 0.01 a
28	58.85	240	3.8 ± 0.09 ab	3.0 ± 0.43 b	0.0 ± 0.00 c	3.9 ± 0.11 a
29	58.97	240	7.8 ± 1.15 b	6.2 ± 0.03 b	0.0 ± 0.00 c	16.7 ± 0.08 a
30	59.10	240	3.4 ± 0.31 b	1.7 ± 2.40 b	0.0 ± 0.00 b	12.0 ± 1.54 a
31	59.38	240	13.3 ± 1.84 b	10.7 ± 4.53 b	0.0 ± 0.00 c	23.9 ± 1.45 a
32	59.85	280	0.7 ± 0.07 b	0.5 ± 0.21 bc	0.0 ± 0.00 c	2.1 ± 0.15 a

[a] Mean values (n = 3). Different letters in the same row indicate statistically significant differences ($p < 0.05$). Values are expressed as mg kg^{-1} of HYTY.

Table 3. Retention Times, UV Absorbance Maxima, Molecular Weights (MW), and MS Fragmentation Patterns of the main Phenolic Compounds of OMWW

					Major fragments ESI positive				Major fragments ESI negative		
Peak	Analyte	tr (min)	λ_{max} (nm)	MW	$[M + H]^+$	$[M + Na]^+$	$[M – H_2O + H]^+$	Other fragments	$[M - H]^-$	$[2M - H]^-$	Other fragments
1	3,4-DHPG	5.6	**232**/280	170		193.1 (76.9)	153.1 (100)	275.1 (86.0)	169.1 (100)	339.1 (67.9)	205.0 (20) – 232.0 (36.6)
2	HYTY	15.1	236/**280**	154			137.1 (100)	177.1 (24.2) - 251.1 (87.5)	153.0 (100)	307.0 (69.4)	
3	CATHECOL	18.9	230/**276**	110					109.1 (100)	219.1 (24.9)	155$[M+HCOO^-]^-$ (10.2); 187.1 (6.8); 459.5 (7.1)
4	TY	21.9	**234**/278	138			121.1 (100)	193.1 (13.2)			
5	Di-HBA	24.1	220/**256**/295	154	155.1 (100)		137.1 (14.3)				
6	3-HPA	25.4	**230**/275	152	153.1 (100.0)			107.1 (42.9)- 193.1 (57.5) - 251.1 (38.8) -283.1 (41.2)	151.1 (100)		107.1 (14.4)
9	VA	27.4	230/**261**/292	168	169.1 (100)		151.1 (47.4)		167.1 (100)		
10	3,4,5 TMBA	36.2	**233**/274	212	213.1 (100)	235.1 (20.3)	195.1 (8.8)	447.1 (7.0) $[2M+Na]^+$			
15	unknown	38.4	228/**273**	166		189.0 (7.8)	149.1 (100)	186.0 (30.3) - 251.1 (75.1) - 371.0 (25.2) $[2M+K]^+$	165.1 (33.0)	331.1 (100)	211.1 (83.6) $[M+HCOO^-]^-$
16	unknown	39.5	**240**	244	245.1 (50.1)	267.0 (30.2)	227.1 (80.6)	213.1 (100)-386.1 (50.5)	243.1 (100)		197.1 (6.4)
20	OxEA	51.9	**242**	258	259.1 (70.1)	281.1 (23.8)		213.1 (100) $[M-COOH]^+$ 241.1 (68.9) $[M-OH]^+$ - 407.1 (38.3) -539.2 (12.9)$[2M+Na]^+$ - 555.2 (5.8) $[2M+K]^+$	257.1 (100)		381.0 (13.6) - 444.1 (19.5)
21	unknown	53.3	**240**					195.1 (12.5) - 227.1 (100) /249.1 (12.5) /359.1 (5.9) /475.1 (9.3)			
22	OxEA	54.3	**240**	258	259.1 (100)	281.1 (17.2)		213.1 (61.2) $[M-COOH]^+$ - 241.1 (36.2) $[M-OH]^+$ - 407.0 (6.0) - 539.2 (9.7) $[2M+Na]^+$	257.1 (100)		113.0 (5.3) – 723.5 (11.5) – 740.5 (30.2)
24	4-HBA / 3,4-hydroxybenzaldehyde	57.1	**230**/282	138	139.1 (43.2)			227.1 (100) – 259.1(36.9) – 281.0 (28.6)	137.1 (100)	275.1 (12.7)	113.0 (5.5) - 183.1 (11.1) $[M+HCOO^-]^-$
27	unknown	58.1	**240**/282	188	189.2 (20.3)	211.1 (15.5)	171.1 (100) 153.1 (25.6)	143.1 (42.1) $[M-COOH]^+$- 208.1 (26.4) – 227.1 (5.1) $[M + K]^+$- 363.1 (9.8) $[2M + Na]^+$ - 415.2 (17.6) $[M + K]^+$	187.1 (100)		269.1 (36.3) - 379.1 (15.4) - 442.0 (25.4)
29	DAOA	59.0	**232**/282	320	321.1 (54.3)	343.1 (81.1)		137.1 (100) [loss acidic group] - 200.6 (21.7) - 221.0 (20.9) - 359.0 (10.4) $[M+K]^+$ - 500.1 (25.9)	319.1 (100)	639.2 (24.5)	355.0 (10.4) - 382.1 (59.5)-433.0 (8.4)
31	unknown	59.4	**240**	240	241.1 (100)	263.1 (10.4)		503.1 (5.2) $[2M + Na]^+$			

FIGURES

Figure 1. Content of phenolic compounds (*o*-DPH, TP) and corresponding antiradical activity ($ABTS^{\bullet+}$) of OMWW. TP and *o*-DPH, expressed as mg GA mL^{-1} OMWW are reported on the left; $ABTS^{\bullet+}$, expressed as mmol Trolox L^{-1} OMWW is reported on the right .

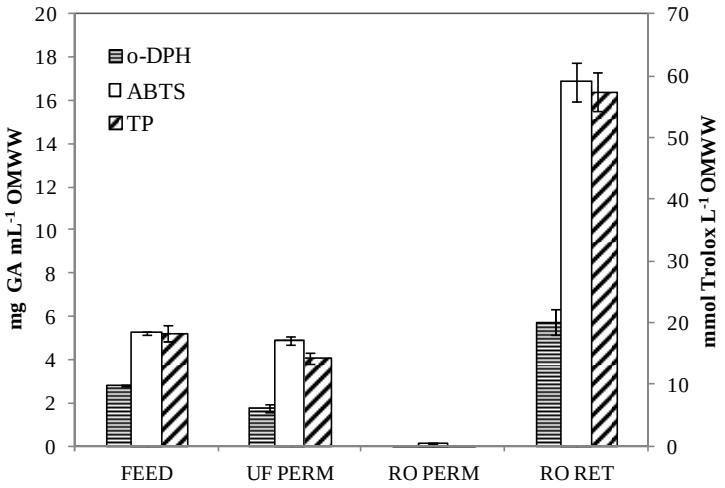


Figure 2. UV chromatograms of an OMWW (FEED) sample recorded at 240 nm (**A**) and 280 nm (**B**).

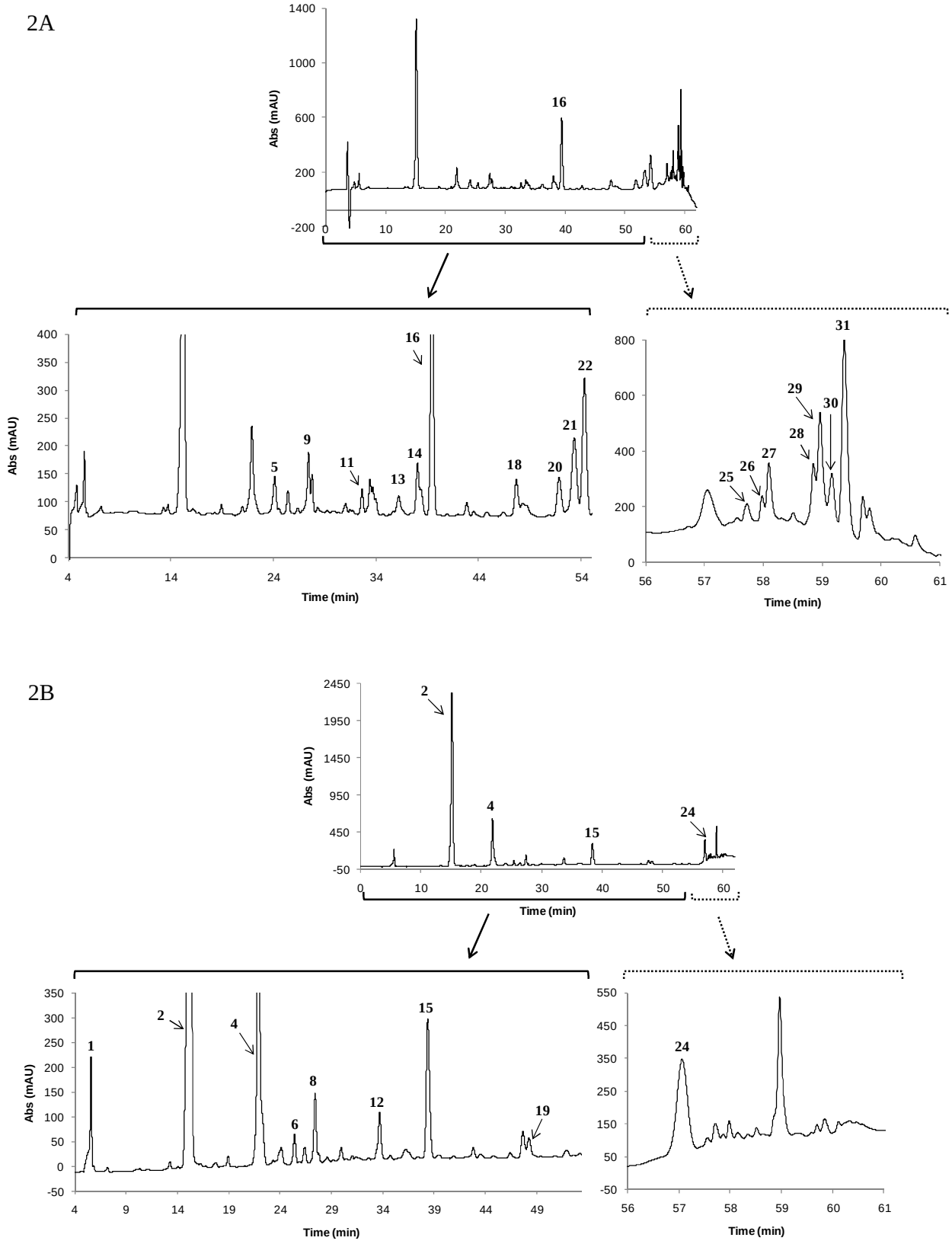
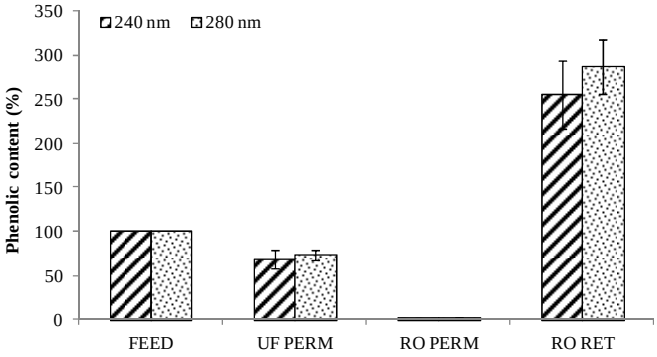


Figure 3. HPLC quantification of phenols at 240 and 280 nm. Results are expressed in percentages; the phenol content of the FEED sample was considered 100%.



4.3 PHENOLIC COMPOSITION OF CHESTNUT BARK EXTRACT

Vegetable tannins have been defined as water-soluble polyphenolic compounds having relative molecular mass between 500 and 3000 and besides giving the natural, usual phenol reactions, they have some special properties such as the ability to precipitate alkaloids, gelatine, and other proteins from solution (Tang *et al.*, 1992).

Basing on the behaviour with base or acid treatment, and on spectral and chromatographic data, tannins have been classified into three groups: condensed tannins (proanthocyanidins), which have the general polymeric flavan-3-ol structure, hydrolizable tannins which are generally glucose esters of gallic acid and hexahydroxydiphenic acid (HHDP) and complex tannins, which possess both condensed and hydrolysable characters (Tang *et al.*, 1992).

The composition of tannins obtained from chestnut wood (Pasch and Pizzi, 2002), bark (Garro-Gálvez *et al.*, 1997) and flesh (Hwang *et al.*, 2001) has been determined and its components mainly belong to the group of hydrolysable tannins (Vázquez *et al.*, 2009). In particular, sweet chestnut contains high amounts of ellagitannins; these molecules produce ellagic acid after hydrolysis.

Gallic acid is a tri-hydroxybenzoic acid and its molecule is characterized by the presence of a carboxyl group substituted on a phenol with 3 hydroxyl substituent (figure 4.3.1).

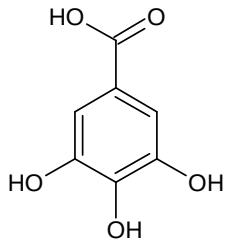
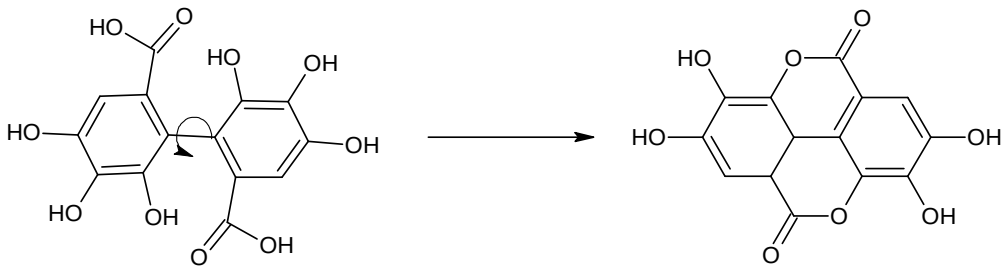


Figure 4.3.1 Gallic acid structure.

Ellagic acid is a dimeric derivative of gallic acid and it mainly exists in higher plants, combined with its precursor, the HHDP (Amakura *et al.*, 2000; Vekiari *et al.*, 2008). Ellagic acid is formed spontaneously from HHDP which, in aqueous solution, undergo to lactonization reaction, as represented in figure 4.3.2.



HHDP Ellagic acid

Figure 4.3.2 Lactonization of HHDP to ellagic acid.

In the past various chromatographic and spectral methods have been developed to analyse vegetable tannins in plant extracts (Tang *et al.*, 1992; Vivas *et al.*, 1993a, 1993b, 1996), food and beverages, but nowadays, reversed-phase HPLC with UV and mass detection is one of the most frequently used (Zywicki *et al.*, 2002).

In research article 4 the composition of chestnut bark extracts was studied through HPLC-DAD/ESI-MS. A complete qualitative and quantitative analysis of the tannin compounds was carried out. A preliminary quantitative tannin estimation was obtained thanks to a Folin Ciocalteau test.

Tannin analysis of chestnut bark extracts (*Castanea sativa* Mill.) by HPLC-DAD-MS

Patrizia Comandini,[*,†] Maria Jesus Lerma-Garcia,[§] Ernesto Francisco Simó-Alfonso,[#] and Tullia Gallina Toschi[†]

[†]Department of Food Science, University of Bologna, Piazza Goidanich 60, I-47521 Cesena, FC, Italy

[§]Department of Analytical Chemistry and Food Technology, University of Castilla – La Mancha, Avda. Camilo José Cela 10, E-13004, Ciudad Real, Spain

[#]Department of Analytical Chemistry, University of Valencia, Doctor Moliner 50, E-46100, Burjassot, Valencia, Spain

Running title: HPLC-DAD-MS tannin analysis

Key words: Chestnut bark extract, Ellagitannins, HPLC, Mass spectrum

[*]Author to whom correspondence should be addressed:

Patrizia COMANDINI, Dipartimento di Scienze degli Alimenti, Università di Bologna

P.zza Goidanich, 60, 47521 Cesena (FC), Italy

Telephone +390547338121, fax +390547382348

e-mail: patrizia.comandini2@unibo.it

Abstract

Numerous kind of commercial tannin extracts are available on the market and an important fraction is obtained from *Castanea sativa* Mill.

Tannin extracts are used for animal feed and by many industries, such as the leather industry, the food industry, and especially in wine and spirit production. Owing to the numerous uses of tannin extracts, a rapid, reliable, and complete characterization of the phenolic and in particular of the tannin fraction of chestnut bark extracts is advisable.

In this investigation an HPLC-DAD/ESI-MS method for the complete analysis of tannin composition of chestnut bark extracts was developed. Seven phenolic compounds (vescalin, castalin, gallic acid, vescalagin, 1-O-galloyl castalagin, castalagin and ellagic acid) were isolated from chestnut bark extracts. 1-O-galloyl castalagin was found for the first time in chestnut bark extracts. The phenolic components of four commercial chestnut bark extracts were quantified and compared.

1. Introduction

Tannins are complex polyphenols synthesized by a wide range of plants and trees (Muller-Harvey, 2001); thanks to their ability to precipitate gelatin and other proteins from solutions (Mehansho, Butler, & Carlson, 1987), they are proposed to play key roles in the chemical defences of the plant species against biological decay. This property influenced all the other characteristics such as taste and toxicity and some pharmacological effects (Vivas, Bourgeois, Vitry, & Glories, 1996).

Based on their structure, tannins are conventionally divided into condensed and hydrolysable tannin molecules. Condensed tannins have a flavonoid core as a basic skeleton, and hydrolysable tannins are esters of a polyol (most often β-D-glucose) with either gallic acid (gallotannins) or hexahydroxydiphenic acid (HHDP, ellagitannins) (Salminen, Ossipov, Loponen, Haukioja, & Pihlaja, 1999; Mämmelä, Savolainen, Lindroos, Kangas, & Vartiainen, 2000). Several species, such as *Acacia*, *Acer*, *Quercus* and *Castanea* sp. are well known for having both condensed and hydrolysable tannins (Muller-Harvey, 2001; Živković, Mujić, Zeković, Nikolić, Vidović, & Mujić, 2009).

Castanea genera belongs to the Fagaceae family and *Castanea sativa* Mill. is one of the most cultivated chestnut species (De Vasconcelos, Bennett, Rosa, & Ferreira Cardoso, 2007).

The composition of tannins obtained from chestnut wood (Pasch & Pizzi, 2002), bark (Garro-Gálvez, Riedl, & Conner, 1997) and flesh (Hwang, Hwang, & Park, 2001) has been determined and the components mainly belong to the group of hydrolysable tannins (Vázquez, González-Alvarez, Santos, Freire, & Antorrena, 2009). In particular, sweet chestnut contains high amounts of ellagitannins and the main structures found are castalin and vescalin (Peng, Scalbert, & Monties, 1991), castalagin and vescalagin (Viriot, Scalbert, Hervé du Penhoat, & Moutounet, 1994), kurigalin, 5-*O*-galloylhamamelose, (3', 5'-dimethoxy-4'-hydroxyphenol)-1-*O*-β-D-(6-*O*-galloyl)glucose, chestanin and acutissimin A (Lampire, Mila, Raminosoa, Michon, Du Penhoat, Faucheur, Laprevote, & Scalbert, 1998; Peng et al., 1991).

Industry uses various plant materials (leaves, fruit, galls, bark and wood) to produce numerous kind of commercial tannin extracts. Several thousand tons of sweet chestnut tannins are produced every year in Europe (Vivas et al., 1996). These commercial tannin extracts are used for animal feed (Muller-Harvey, 2001) and by the leather (Scalbert, Monties, & Janin, 1989) and the food industry, especially in wine and spirit production (Vivas et al., 1996; Sanz, Cadahia, Esteruelas, Munoz, De Simon, Hernández, & Estrella, 2010).

One of the first contributions to the analysis of commercial tannin extracts was provided by Tang, Hancock, & Covington (1992) who studied the composition and the structure of commercial chestnut tanning agents. In particular castalagin and vescalagin were isolated by thin layer and column chromatography; then their structure was established by means of nuclear magnetic resonance and fast atom bombardment mass spectroscopy. The authors also found some other compounds, but their structures were not defined.

In 1993 Vivas and collaborators (Vivas, Chauvet, Glories, & Sudraud, 1993a; Vivas, Chauvet, Sudraud, & Glories, 1993b; Vivas et al., 1996) realized a study on the major commercial tannin extracts with the aim of determining their botanical origin by the analysis of specific species-markers (phenolic acids and coumarins) of the plant sources and the extraction solvents used. They also developed a method in liquid secondary ion mass spectrometry for the determination of the qualitative composition of commercial tannin extracts (Vivas et al., 1996). In 2002 Zywicki, Reemtsma, & Jekel analysed hydrolysable and condensed tannins in commercial vegetable tanning agents and in tannery wastewaters by reversed-phase liquid chromatography-electrospray ionization-tandem mass spectrometry.

Chestnut bark extracts are widely used by industry and at the best of our knowledge there are only limited information on the qualitative and quantitative characterization of the phenolic fraction.

In this investigation a rapid HPLC-DAD/ESI-MS method for the complete analysis of tannin composition of chestnut bark extracts was developed. In order to have a preliminary quantitative tannin estimation, a colorimetric assay for the determination of the total phenol content was made. Folin-Ciocalteau test was chosen thanks to its ability to react with all kind of tannins, both condensed and hydrolizable.

2. Experimental

2.1. Chemicals and samples

Methanol (p.a.), monohydrate gallic acid (assay 99.1 %) and ellagic acid (assay ≥ 96 %) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (gradient grade, for HPLC) was from VWR (Milano, Italy), formic acid (assay 98-100%) was from Merck (Darmstadt, Germany). Deionized water was obtained from an Elix 10 water purification system from Millipore (Bedford, MA, USA). Sodium molybdate dihydrate was from Carlo Erba (Rodano,

Milano, Italy). Na_2CO_3 was from BDH AnalaR (Poole, U.K.). Folin−Ciocalteu reagent was purchased from Merck.

Four different chestnut bark extracts, reported as TAN1, TAN2, TAN3 and TAN4, have been analysed. All samples were bought from local markets in Emila-Romagna region (Italy).

2.2. Sample preparation

Several solvents have been compared in order to obtain the best tannin extraction.

According to the method described by Vekiari, Gordon, García-Macías, and Labrinea (2008) and Bianco, Handaji, and Savolainen (1999), an amount of 350 mg of chestnut bark extract was weighted and dissolved in 20 mL of methanol. The mixture was vortexed for 1 min and kept at ambient temperature for 30 min and then it was sonicated for 30 min. The extract was filtered on cellulose acetate filters (0.45 µm) and diluted 1:2 with water.

The same extraction procedure was repeated by using different solvents: water, a mixture of methanol/water (50/50, v/v) and a mixture of acetone/water (70/30, v/v) were compared.

The extraction procedures were tested on sample TAN1 in order to choose the best method of dissolution. The samples were stored at -18°C until analysis.

2.3. Colorimetric determination of total phenol (TP) content

The total phenol (TP) content of the extracts was determined by the Folin–Ciocalteau method at 750 nm (Singleton & Rossi, 1965), using a Shimadzu Spectrophotometer UV-VIS 1204 (Kyoto, Japan). TPs were calculated as gallic acid equivalent (GAE) from the calibration curve of gallic acid standard solutions ($r^2 = 0.9998$) and expressed as g GAE/100 g of extract (on a dry basis). The analyses were done in triplicate; and the mean values and the standard deviations were calculated.

2.4. HPLC–DAD-MS equipment

HPLC analysis were carried out on an HP 1100 Series (Agilent Technologies, Palo Alto, CA, USA), equipped with a binary pump delivery system, a degasser, an autosampler, a HP diode-array UV–Vis detector and a HP mass spectrometer. A C18 Luna column 5-µm particle size,

25 cm×3.00 mm I.D. (Phenomenex, Torrance, CA, USA) was used. All solvents were filtered through a 0.45-μm filter disk (Millipore). MS analyses were carried out using an electrospray (ESI) interface operating both in positive and in negative mode.

2.4. HPLC–DAD-MS analysis

Two elution gradients were compared:

1. According to Vekiari et al. (2008) the first linear elution gradient tested was: from 0 to 8 min 7% B; from 8 to 25 min, 7 to 32% B; from 25 to 30 min, 32 to 35% B; from 30 to 35 min, 35 to 7% B, followed by a re-equilibration of the column for 5 min in the initial conditions.
2. According to Sandhu & Gu (2010) the second linear elution gradient tested was: from 0 to 2 min, 5% B; from 2 to 10 min, 5 to 20% B; from 10 to 15 min, 20 to 30% B; from 15 to 20 min, 30 to 35% B; from 20 to 60 min, 35 to 80 %B; from 60 to 65 min, 80 to 85% B; from 65 to 70 min, 85 to 5% B, followed by a re-equilibration of the column for 5 min in the initial conditions.

In both cases the following solvent system was used: mobile phase A, water–formic acid (99.5:0.5, v/v); mobile phase B, acetonitrile. All solvent used were of HPLC grade. The flow-rate was 0.5 mL/min. The injection volumes was 10 μL. All the analyses were carried out at room temperature.

The following conditions of ESI interface were used: drying gas flow, 9.0 L/min; nebulizer pressure, 35 psig; gas drying temperature, 350 °C; capillary voltage, 3000 V; fragmentor voltage, 60 V.

Phenolic and tannin compounds were identified comparing retention times, UV and MS spectra of the detected peaks with those of commercial standards (gallic and ellagic acid); if reference compounds were not available a tentative identification was made by analyzing and comparing elution order, spectroscopic and spectrometric information with literature data. The quantification of each compound was performed using eight-point regression curves obtained using gallic (r^2 = 0.9993) or ellagic acid (r^2 = 0.9992). Gallic acid amount was calculated at 280 nm with gallic acid as reference standard; ellagic acid and ellagitannins (vescalin, castalin, vescalagin, castalagin and 1-*O*-galloyl-vescalagin) amounts were quantified at 254 nm, using the ellagic acid calibration curve. For vescalin and castalin, a correction of molecular weight with a multiplication factor of 632/302 was applied; for

158 vescalagin and castalagin a multiplication factor of 934/302 was used; finally for 1-*O*-galloyl
159 castalagin the correction of molecular weight with a multiplicative factor of 1086/302 was
160 calculated.

161

162 *2.5. Statistical analysis*

163 The analytical results was evaluated by the software Statistica 8.0 (Statsoft Inc.,Tulsa, OK).
164 Analysis of variance (ANOVA) was used to determine if significant differences existed at a
165 level of confidence of p <0.05 (Honestly Significant Differences or Tukey's HSD multiple
166 comparison).

167

168 **3. Results and discussion**

169 *3.1 Sample dissolution tests*

170 As reported in Section 2.2, different solvents have been compared, in order to obtain the best
171 dissolution and the highest spectrophotometric response of samples. In particular the sample
172 TAN 1 was dissolved with methanol, water, methanol/water (50/50), and acetone/water
173 (70/30) mixtures.

174 The methanolic sample had some precipitate at the bottom of the flask; with the others
175 solvents the tannin extract was completely dissolved, but different clearness levels were
176 obtained. When the tannin extract was dissolved in methanol and acetone/water mixture it
177 was most cloudy, instead when water was used, the sample obtained was clear and well
178 dissolved.

179 A preliminary spectrophotometric evaluation of the different dissolution procedures was
180 made. The unitary net absorbance of each solvent tested were calculated by using the
181 following formula:

182 $$A_{un} = (As-Ab)/Ws$$

183

184 Where A_{un}: unitary net absorbance (AU/g)

185 As: sample absorbance (AU)

Ab: blank absorbance (AU)

Ws: sample weight (g)

The blank was obtained by reading the absorbance of the pure solvent or of the mixture used for the dissolution. As reported in table 1, the unitary net absorbance obtained by using methanol was almost double respect to water, and it was, respectively, 75% and 45% higher than methanol/water and acetone/water mixtures.

By considering the dissolution test and the spectrophotometric results, the following analytical determinations were made on chestnut bark samples dissolved with methanol.

3.2 Spectrophotometric determination of the total phenol (TP) content

A very large number of hydrolysable tannins exist in nature and many structural variations among them are caused by oxidative coupling reactions of acid units or by oxidation of aromatic rings. Numerous colorimetric tests have been proposed for the analysis of hydrolysable tannins, such as those based on the KIO_3, rhodanine, $NaNO_2$ reagents, but most of them can only detect the galloyl or the HHDP groups, without considering the more complex oxidation products. As a consequence many hydrolysable tannins might not be quantified through colorimetric test (Muller-Harvey, 2001).

In this investigation it was decided to make a spectrophotometric test in order to have an evaluation of the content of total phenols of different chestnut bark extracts and to verify if a preliminary discrimination of the sample was possible. Table 2 reports the total phenol content of the chestnut bark tannin extracts analysed.

TP content of chestnut bark extracts ranged from 23.9 and 56.1 g GAE/100 g dry extract. TAN 1 and TAN 4 resulted the samples with the highest TP content. The mean concentration of TPs in commercial samples was quite uniform, with the exception of TAN 3, whose content was about 47% of the other samples analysed.

3.3 Gradient development and HPLC-DAD-MS analysis

Two elution gradients were tested and the HPLC chromatograms obtained are reported on Figure 1. In both cases it was possible to separate 7 main compounds, numerated in the Figure. The best separation was obtained with the gradient adapted by Sandhu & Gu (2010) (Figure 1 B), moreover this elution gradient enabled to reduce the separation time of about 7 minutes, so it was adopted for the following analysis.

In table 3 are reported the spectroscopic and spectrometric information on the compounds separated and their identification. The main peak in the mass spectra of chestnut bark extract tannins, obtained in the negative ion ESI-MS mode, was the deprotonated molecule $[M-H]^-$ and the ion $[M-2H]^{2-}$, as previously reported for other hydrolysable tannins (Salminen et al., 1999; Juang, Sheu, & Lin, 2004).

Vescalin and castalin isomers (peaks 1 and 2) provided $[M-H]^-$ ions at m/z 631 and peaks of m/z 331 and 481 corresponding respectively to the monogalloyl-glucose ($[Galloyl-glu-H]^-$) and HHDP-glucose ($[HHDP-glu-H]^-$) produced during the hydrolysis of the molecules. Moreover a low intensity peak of m/z of 301, was obtained for both compounds, corresponding to the liberation of ellagic acid $[EA-H]^-$.

Peak 3 was assigned to gallic acid thanks to both molecular ion $[M-H]^-$ and the ion at m/z 125.0 generated from the loss of a CO_2 group from the carboxylic acid moiety $[M-H-CO_2]^-$. The presence of gallic acid in chestnut bark tannin extracts might be quite questionable (Canas, Leandro, Spranger, & Belchior, 1999). In fact the occurrence of gallotannins in wood can not be excluded (Seikel, Hostettler, & Niemann, 1971; Vivas et al., 1993b), but it has never been confirmed. An acceptable hypothesis (Canas et al., 1999) suggests that gallic acid has derived from the hydrolysis of some galloyl esters associated with the parietal composites of the cells (Viriot et al., 1994).

In the case of vescalagin and castalagin (peak 4 and 6), that differ only in the stereochemistry in position C_6, the molecular ions at m/z 933 were detected, together with the fragments at m/z 466, associated to the pattern $[M-2H]^{2-}$.

Peak 5 was assimilated to an ellagitannin, based on the similarity to the UV-visible spectra, characterized by a maximum at about 245 nm and a shoulder at 280 nm, as showed in figure 2. Analyzing the mass spectrum, it was deducted that this molecule could be originated from the esterification of castalagin or vescalagin with a gallic acid residue, giving a molecule with a galloyl-HHDP-glucose structure. In particular it was tentatively identified as 1-*O*-galloyl-

castalagin, an hydrolysable tannin with molecular weight of 1086, previously identified in *Eugenia grandis* (Nonaka, Ishimaru, Watanabe, Nishioka, Yamauchi, & Wan, 1987). As for the other ellagitannins, the main fragments detected were the molecular ion, with m/z 1085 and the residue with m/z 542.1 corresponding to the pattern $[M-2H]^{-2}$.

Peak 7, with retention time of 18.9 min, was ellagic acid as its mass spectrum had only one major peak with m/z 301; its UV spectrum, showed a maximum absorbance at 254 nm.

The identification made were confirmed also by the analysis in ESI positive mode (data not shown).

Gallic and ellagic acid gave $[M+H]^+$ fragment. While castalin, vescalin, castalagin, 1-*O*-galloyl-castalagin and vescalagin identifications were characterised by the presence of the fragments $[M+H]^+$ and $[M+H_2O]^+$.

Although chestnut bark extracts are classified as elagitannin extracts they may, nevertheless, contain gallotannins, because ellagitannins are biologically formed from pentagalloyl-glucose (gallotannin) (Zywicki et al., 2002).

Besides the seven compounds previously identified, other mass spectra of minor compounds have been detected in the chestnut bark extracts. The m/z data obtained in ESI negative mode showed that all these components belonged to the gallotannin class, with the exception of roburine E/grandinin (table 4). These compounds were detected at a trace level, and were not always quantifiable in the chestnut extracts, for this reason the quantitative analysis was made on the seven compounds initially separated.

In table 5 are reported the concentrations of each compound and the total concentration of tannins in each extract.

In all samples analysed castalagin and vescalagin isomers, in free or hydrolysed form, were the main components. In particular, in TAN 1 and TAN 2 vescalagin and castalagin were the most abundant compounds, followed by 1-*O*-galloyl castalagin; in TAN 3 and TAN 4, instead, 1-*O*-galloyl castalagin was present with a concentration superior to the other compounds.

The global amount of castalin and vescalin, was always lower than the sum of castalagin and vescalagin, however the proportion between castalin and vescalin isomers were different in the extracts analysed.

In each extract the concentration of gallic acid was higher than ellagic acid and resulted always the superior to castalin and vescalin amounts.

Analysing the global amount of tannins in each sample it is possible to note that the concentration of tannins and phenolic compounds in the sweet chestnut bark extracts analysed was quite wide, ranging from 4.75 to 16.73 g/100 g dry extract.

4. Conclusions

A rapid HPLC-DAD/MS method for the analysis of tannin components of chestnut bark extracts was developed. Four commercial chestnut bark extracts were analysed and seven compounds (vescalin, castalin, gallic acid, vescalagin, 1-*O*-galloyl castalagin, castalagin and ellagic acid) were isolated and quantified. 1-*O*-galloyl castalagin was for the first time found in chestnut bark exctracts.

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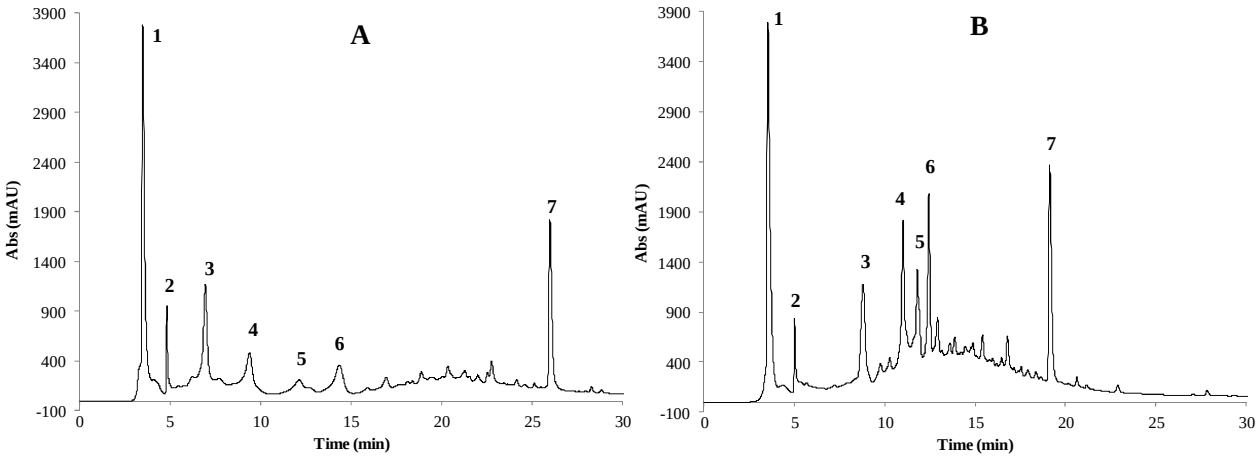
375 **Figures**

376

377 **Figure 1**. Chromatograms obtained with gradient 1 (A) and 2 (B). See text for further details.
378 The compounds separated are indicated on Table 3.

379



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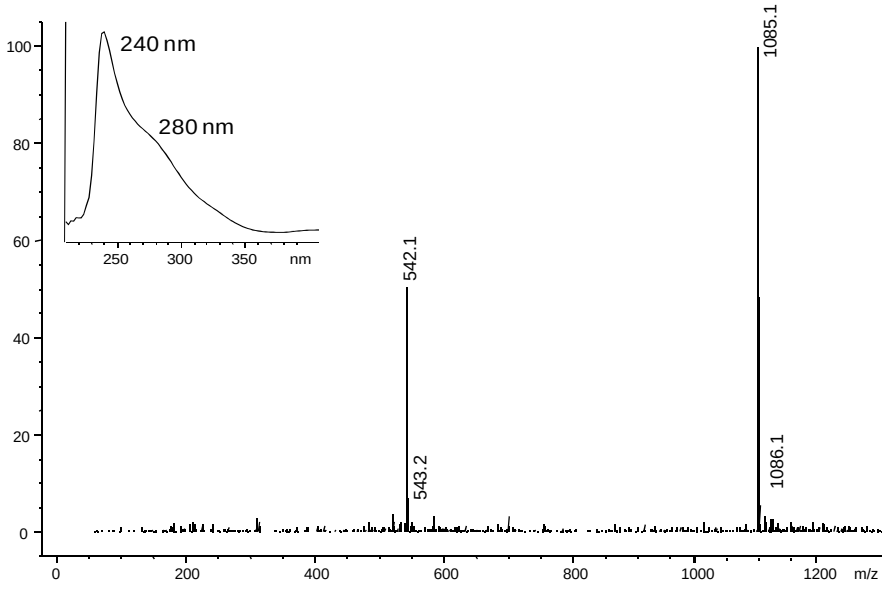
381

382 **Figure 2.** UV and mass spectra of peak 5, identified as 1-*O*-galloyl castalagin.

383



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Tables

Table 1. Unitary net absorbance (AU/g) obtained with different solvents. Values are means ± standard deviations (n=3). Different letters in the same row indicate statistically significant differences ($p < 0.05$).

Solvent tested	**Unitary net absorbance (AU/g)**
Methanol	$5.94^{a} \pm 0.13$
Water	$3.04^{d} \pm 0.03$
Methanol/water (50/50)	$3.38^{c} \pm 0.05$
Acetone/water (70/30)	$4.08^{b} \pm 0.06$

Table 2. TP content (g GAE/100 g dry extract) of the commercial tannin extracts analyzed. Values are means ± standard deviations (n=3). Different letters in the same row indicate statistically significant differences ($p < 0.05$).

Sample	**TP content (g GAE/100 g dry extract)**
TAN 1	$54.9^{a} \pm 3.2$
TAN 2	$43.2^{b} \pm 1.4$
TAN 3	$23.9^{c} \pm 0.9$
TAN 4	$56.1^{a} \pm 2.9$

Table 3. Retention times, spectral characteristics (maximum absorption wavelength), ESI negative mass fragmentation patterns and identification of the components of chestnut bark extracts separated by HPLC-DAD-MS.

Peak No.	**tr (min)**	**λ_{max} (nm)**	**MW**	**Major fragments ESI negative**		**Identification**
				$[M - H]^-$	**Other fragments**	
1	2.5	245/275sh	632	631.0	331.0 $[Galloyl\text{-}glu\text{-}H]^-$/481.0 $[HHDP\text{-}glu\text{-}H]^-$	Vescalin
2	3.6	246/280sh	632	631.1	331.0 $[Galloyl\text{-}glu\text{-}H]^-$/481.0 $[HHDP\text{-}glu\text{-}H]^-$	Castalin
3	6.6	232/272	170	169.0	125.0 $[M\text{-}H\text{-}CO_2]^-$	Gallic acid
4	9.5	245/280sh	934	933.0	466.0 $[M\text{-}2H]^{2-}$	Vescalagin
5	10.7	240/280sh	1086	1085.1	520.2/542.1 $[M\text{-}2H]^{2-}$	1-*O*-Galloyl castalagin
6	11.1	248/280sh	934	933.0	181.1/466.0$[M\text{-}2H]^{2-}$/996.0	Castalagin
7	18.9	254/302/368	302	301.0	-	Ellagic acid

sh, shoulder.

Table 4. m/z fragments of minor compounds detected in ESI negative mode.

Analyte	**m/z**
Monogalloyl-glucose	331.0
Roburine E/grandinin	1065.1
Digalloyl-glucose	483.0
Digalloyl-HHDP-glucose	785.2
Digallic acid	321.0
Trigalloyl-glucose/kurigalin	635.0
Trigalloyl-HHDP-glucose	937.3
Tetragalloyl-glucose	787.2

Table 5. Concentration of tannins and phenolic compounds in sweet chestnut bark extracts, expressed as g/100 g dry extract. Values are means ± standard deviations (n=3). Different small letters in the same row indicate statistically significantly differences (Honestly Significant Differences or HSD by Tukey $p<0.05$). Different capital letters in the same column indicate statistically significantly differences (Honestly Significant Differences or HSD by Tukey $p<0.05$).

	g/100 g dry extract			
	TAN1	**TAN2**	**TAN3**	**TAN4**
Vescalin	1.19 $^{a\,E}$ ± 0.06	1.05 $^{b\,D}$ ± 0.09	0.44 $^{c\,D}$ ± 0.01	1.22 $^{a\,D}$ ± 0.02
Castalin	0.73 $^{b\,F}$ ± 0.05	0.67 $^{b\,E}$ ± 0.02	0.31 $^{c\,E}$ ± 0.01	1.00 $^{a\,E}$ ± 0.03
Gallic acid	2.80 $^{a\,D}$ ± 0.09	1.56 $^{c\,C}$ ± 0.02	0.65 $^{d\,C}$ ± 0.01	1.80 $^{b\,C}$ ± 0.03
Vescalgin	4.08 $^{a\,A}$ ± 0.03	3.46 $^{b\,A}$ ± 0.19	0.29 $^{d\,E}$ ± 0.01	0.56 $^{c\,G}$ ± 0.01
1-*O*-Galloyl castalagin	3.20 $^{b\,C}$ ± 0.12	2.46 $^{c\,B}$ ± 0.18	1.58 $^{d\,A}$ ± 0.03	5.39 $^{a\,A}$ ± 0.06
Castalgin	3.80 $^{a\,B}$ ± 0.16	3.41 $^{b\,A}$ ± 0.06	1.05 $^{d\,B}$ ± 0.09	2.20 $^{c\,B}$ ± 0.11
Ellagic acid	0.93 $^{a\,F}$ ± 0.02	0.61 $^{c\,E}$ ± 0.04	0.43 $^{d\,D}$ ± 0.01	0.80 $^{b\,F}$ ± 0.02
Total	**16.73 ^{a} ± 0.43**	**13.22 ^{b} ± 0.52**	**4.75 ^{c} ± 0.05**	**12.96 ^{b} ± 0.27**

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5. SENSORY ANALYSIS: STUDY OF VOLATILE PROFILE OF POTATOES

5.1 POTATO FLAVOUR: BACKGROUND AND UPDATES OF LITERATURE

Potato flavour, which is defined as the combined perception of aroma, taste and mouthfeel sensations, has a great importance because it is one of the main qualitative criteria in assigning different potato varieties to a fresh or processed food market (Petersen *et al.*, 1998).

The volatile profile of potatoes has been widely investigated (Maga, 1994; Dresow and Böhm, 2009; Jansky, 2010). Recent studies gave emphasis on the role of agricultural environments on flavour compounds (Dresow and Böhm, 2009; Jansky, 2010) and on the occurrence, formation and control procedures of volatile and non-volatile flavour components of raw and processed potatoes (Maga, 1994).

A mini-review concerning the sensory and instrumental analysis of the volatile fraction of raw and processed potatoes has been written (research article 5). This investigation aims to update the information on the volatile components of potato tubers, giving particular attention to the cooking or processing method.

This study takes into account both the main extraction (simultaneous distillation and extraction (SDE), solvent and direct solvent extraction techniques, headspace analysis, 4. solid-phase microextraction (SPME)) and analytical techniques (gas chromatography - mass spectrometry (GC-MS), gas chromatography/olfactometry (GC/O) or gas chromatography-FID/olfactometry (GC-FID/O) gas chromatography-MS/olfactometry (GC-MS/O)) used in potato flavour analysis and the sensory analysis of potatoes, with particular attention to the findings concerned with the volatile profile.

As regards the sensory evaluation of a food, it can be made through discriminative, descriptive or affective tests. Discriminative tests investigate whether there is a sensory difference between samples (Stone and Sidel 1992), such as the triangle test, the duo-trio test and the paired comparison test.

Descriptive tests involve the detection and description of both qualitative and quantitative sensory components of a product by trained panels. The main methods of descriptive analysis are the Flavour Profile and the Quantitative Descriptive Analysis (QDA®).

The affective tests have the primary goal to assess the personal response of users or potential users of a product (acceptance, preference, or consumer tests). A large number of individuals are required to take part in a sensory acceptance test (> 100). Preference can be measured directly by comparing two or more products with each other, or indirectly by determining which product is the most appreciated in a multiproduct test. The two most widely used methods to measure preference and acceptance are the paired comparison and the 9-point hedonic scale tests.

With reference to the evaluation of raw and processed potatoes, the sensory tests most frequently used are the Flavour Profile, the QDA® and the preference tests, as detailed in research article 5.

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Characterization of Potato Flavours: An Overview of Volatile Profiles and Analytical Procedures

Patrizia Comandini • Lorenzo Cerretani* • Giampaolo Blanda • Alessandra Bendini • Tullia Gallina Toschi

Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze degli Alimenti, P.zza Goidanich, 60 – I-47521 – Cesena (FC), Italy

Corresponding author: * **lorenzo.cerretani@unibo.it**

ABSTRACT

Potatoes may be cooked by several methods such as boiling, baking and frying; they are also used as an ingredient for numerous home-made and mass-produced foods like sticks, chips and other snacks. An important factor affecting consumer preferences of these products is their flavour, which is defined as the combined perception of aroma, taste and mouthfeel sensations. Flavour, and in particular the volatile profile of potatoes, has been widely investigated in the last few years, and complex patterns have been found. Although raw potatoes possess little aroma, more than 140 volatile compounds have been identified in boiled potatoes, whereas over 250 have been found in baked potatoes and more than 500 compounds have been isolated in French fries. Among these, many lipid oxidation and Maillard reaction products have been reported, together with smaller amounts of indigenous flavour compounds. Many extraction methods have been developed to characterize the aroma of potatoes, with the goal of reducing analytical detection limits, avoiding formation of artefacts during isolation and reducing analysis cost and time; among these are distillation techniques, solvent and direct solvent extraction techniques, static and dynamic headspace methods and solid-phase microextraction. As regard isolation and quantification of potato volatiles, gas chromatography-mass spectrometry and gas chromatography/olfactometry are frequently used. The analytical approach is often completed with the sensory evaluations. This review describes the flavour profile of the main forms of cooked potatoes, taking into account their mechanism of generation; extraction and analysis procedures are also considered, reporting both conventional and innovative methods.

Keywords: analytical techniques, characterization, extraction, flavour, potatoes

CONTENTS

INTRODUCTION

Potato (*Solanum tuberosum* L.) cultivation is widespread worldwide as a result of its appreciated sensory and nutritional properties, in addition to its adaptability to different climatic conditions. Potatoes may be cooked in many different ways such as boiling, baking or frying; various potato-based products are also produced, including extruded, dehydrated and potato snacks. Recently, the ready-to-use and ready-to-eat market has extensively utilized potato preparations.

One of the most important qualitative criteria in assigning different potato varieties to a fresh or processed food market is the flavour profile. The volatile profile of raw potatoes is weak, but is quite different from that of cooked potatoes. Additionally, the volatiles produced from major cooking procedures differ significantly each other (Whitfield and Last 1991). Therefore, when studying the volatile fraction of potato tubers a distinction must be made between raw (Petersen *et al.* 1998), boiled (Nursten and Sheen 1974; Josephson and Lindsay 1987; Petersen *et al.* 1998; Oruna-Concha *et al.* 2002b), baked (Buttery *et al.* 1973; Coleman and Ho 1980; Coleman *et al.* 1981; Duck-ham *et al.* 2001, 2002), microwaved (Oruna-Concha *et al.* 2002a, 2002b), fried (Carlin *et al.* 1986; Wagner and Grosch 1997, 1998) and manufactured products such as extruded (Majcher and Jelén 2009) and dehydrated (Nissen *et al.* 2002; Laine *et al.* 2006) potatoes.

Sugars, amino acids and lipids are the main precursors of potato volatile compounds (Whitfield and Last 1991);

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Invited Review

Table 1 Current SDE applications in potato volatiles analysis.

Sample	Distillation (t-T)[a]	Extraction solvent	Instrumental technique[b]	Reference
Boiled potatoes	30 min - n.a.	Diethyl ether/pentane (1:1)	GC-MS (DB-WAX; 68.3 min)	Jensen *et al.* 1999
	120 min - 100°C	Dichloromethane	GC-MS (INNO-Wax; 90 min) GC-PND (INNO-Wax; 90 min) GC-O (INNO-Wax; 43.3 min)	Ulrich *et al.* 2000
	n.a. - 60°C	Dichloromethane	GC-MS (FFAP; 70 min)	Blanch *et al.* 2009
Baked potatoes	120 min - n.a.	Pentane/diethyl ether (9:1)	GC-MS (BPX-5; 66.75 min)	Oruna-Concha *et al.* 2001
Extruded potato snacks	120 min - 100°C	Ethyl ether/pentane (1:1)	GC-O and GC-MS (SBP-5; 30 min - Supelcowax 10; 40.5 min)	Majcher and Jelén 2009
Potato flakes	30 min - n.a.	Diethyl ether	GC-MS (n.a.)	Nissen *et al.* 2002

[a] Distillation time (min) and temperature (°C)
[b] Separation and detection technique (column stationary phase; analysis time, min)
n.a. Not available data

their formation can be due to enzymatic or chemical reactions that have been recently overviewed (Dresow and Böhm 2009). The flavour profile of potatoes depends both on the cooking procedure and numerous other factors like cultivar selection, agronomic and storage conditions. Moreover, the extraction technique used during the analysis may affect the nature and the quantity of volatiles isolated.

Potato flavour has been widely investigated in recent reviews by Dresow and Böhm (2009) and by Jansky (2010), with emphasis on the role of agricultural environments on flavour compounds. In 1994, Maga described the occurrence, formation and control procedures of volatile and non-volatile flavour components of raw and processed potatoes.

The present review updates the information on the volatile components of potato tubers, and gives particular attention to the cooking or processing method. In the first part, a brief summary of the main extraction and analytical techniques used in potato flavour analysis is presented. As this study takes into account the sensory analysis of potatoes, and the findings concerned with the volatile profile are reported, a brief explanation of the definitions used should be made. "Flavour" is usually defined as the complex combination of the olfactory (orthonasal and retronasal perceptions), gustatory and trigeminal sensations perceived during tasting. Volatile, non-volatile components and mouthfeel sensations interact to determine food flavour. The term "Odour" refers to the direct olfactory component of flavour (orthonasal perception), while "Aroma" describes the attributes perceptible by the olfactory organ via the back of the nose (International Standard ISO 5492, 2008-10-15). However, these terms are sometimes used with different meanings, e.g. "Flavour" may refer to the volatile profile only or to the retronasal olfactory perception during tasting. For accuracy, in this review focusing on the sensory and instrumental analysis of the volatile fraction of raw and processed potatoes, the terms aroma and flavour are used synonymously, limiting them to the olfactory stimuli, without taking into account taste and mouthfeel sensations.

INSTRUMENTAL ANALYSIS

To determine which compounds are responsible for the flavour of a food product, one crucial step is to select a suitable method for their isolation. This procedure should allow the extraction of all compounds that contribute to flavour of the food product, but not alter the profile of characteristic volatiles, and in particular it should not form artefacts. An additional difficulty in the isolation of volatile compounds is their presence in a wide range of concentrations from ng/kg to mg/kg, and their odour thresholds, which are often below detection limits using conventional GC detectors. Therefore, GC-MS and gas chromatography–olfactometry (GC–O) are usually used to characterize the aroma profile of a food product. Several extraction methods for isolating crucial compounds of potatoes have been used; among these are distillation techniques like simultaneous distillation and extraction (SDE) (Nickerson and Likens 1966), solvent extraction techniques such as solvent-assisted flavour evaporation (SAFE) (Engel *et al.* 1999), headspace methods such as static and dynamic headspace and solid-phase microextraction (SPME) (Pawliszyn 1997).

Extraction and concentration techniques

1. Simultaneous distillation and extraction (SDE)

The SDE method, developed by Nickerson and Likens in 1966, was essentially based on steam distillation of volatile compounds at high temperatures for extended times, but it has been performed with numerous variations from the original version (Buttery *et al.* 1970; Nursten and Sheen 1974; Mutti and Grosch 1999; Ulrich *et al.* 2000).

Due to the analytical conditions required, this process may lead to the creation of new aromatic substances, especially during extended treatments. This extraction is performed with dedicated equipment and assures good detection limits. **Table 1** reports recent SDE applications in potato analysis.

2. Solvent and direct solvent extraction techniques

Solvent extraction is a simple and efficient technique for aroma isolation. The major limitation of this method is that if the food contains lipids, they will also be extracted along with the aroma constituents, and consequently they must be removed prior to further analysis. The separation of aroma components from extracts containing lipids can be performed via molecular distillation, steam distillation and dynamic headspace. Despite bias added by further distillation procedures, this combination (solvent extraction followed by distillation) has been widely applied due to its efficiency at isolating a broad range of volatiles. Engel *et al.* (1999) developed a much more rapid and yet highly efficient Solvent Assisted Flavour Evaporation (SAFE) distillation head, which is now widely used.

The extraction procedure has been employed in several investigations by Petersen *et al.* (1998, 1999, 2003) to explore the volatile fraction of raw shredded and boiled potatoes. They developed a mild extraction procedure in which a large quantity of sample (from 150 to 280 g of food matrix) was homogenized with variable amounts of tap water to ensure sufficiently low viscosity prior to extraction with diethyl ether/pentane (1: 1). The sample was stirred until an emulsion was created, frozen and non-frozen organic phases were discarded. After drying by adding Na_2SO_4, the sample was concentrated and finally analyzed by GC-MS and GC-O.

In order to reduce the interference of starch and oils and increase the concentration of the extract, Petersen (1999) also evaluated the vacuum distillation of volatiles. In this case, a large quantity of boiled potatoes (333 g) was mixed with water and the suspension was distilled at 36-39°C at a vacuum pressure of 20 mbar. The distillate was extracted with ether/pentane under magnetic stirring, the phases were separated using a funnel and the organic phase dried and concentrated by blowing nitrogen on the surface. A similar

Table 2 Current DH applications in potato volatiles analysis.

Sample	Ext. gas[a]	Ext. time[b]	Adsorption polymer	Trap dimensions	Desorption conditions	Instrumental technique[c]	Reference
Boiled potatoes	120 mL/min	20 min	Tenax TA (85 mg)	105 mm - 3 mm	10 min - 260°C	GC-MS (CP-SIL 8 CB low bleed; 70.5 min)	Oruna-Concha *et al.* 2002b
	200 mL/min	60 min	Tenax (100 mg)	n.a.	n.a.	GC-MS (J&W DB-Wax; 68.3 min)	Thybo *et al.* 2006
Baked potatoes	120 mL/min	20 min	Tenax TA (85 mg)	105 mm - 3 mm	10 min - 260°C	GC-MS (CP-SIL 8 CB low bleed; 70.5 min)	Duckhman *et al.* 2001, 2002; Oruna-Concha *et al.* 2002a, 2002b
Fried potatoes	50 mL/min	30 min	Tenax TA (100 mg)	100 mm - 3 mm	5 min - 245°C	GC-FID, GC-MS, GC-O (MDN-5S; 66.5 min)	Van Loon *et al.* 2005
	40 mL/min	60 min	Tenax TA (85 mg)	155 mm - 3 mm	5 min - 280°C	GC-MS (Cp-Sil8; 62 min)	Martin and Ames 2001
Potato flour	40 mL/min	45 min	Tenax TA (85 mg)	3.5 in. - 0.25 in.	10 min - 300°C	GC-MS (DB-5; VF-WAXms; 54.5 min)	Elmore *et al.* 2010

[a] Flow of the extraction gas (mL/min)
[b] Extraction time (min)
[c] Separation and detection technique (column stationary phase; analysis time, min)

procedure was used by Majcher and Jelén (2009) who applied the SAFE technique to extruded and dried potato snacks by using a small amount of sample to extract volatile compounds (20 g).

Direct solvent extraction is a very simple and convenient technique, and it is frequently carried out with a Soxhlet extractor. It has been applied to isolate potent odorant from both boiled potatoes (Mutti and Grosch 1999) and French fries (Wagner and Grosch 1997, 1998).

Samples were dried, ground finely, placed in a Soxhlet thimble and extracted with dichloromethane (Wagner and Grosch 1998; Mutti and Grosch 1999) or diethyl ether (Wagner and Grosch 1997). The extract was concentrated by distilling off the solvent; the aromatic fraction and the solvent were purified by distillation under high vacuum, using the apparatus reported by Sen *et al.* (1991) and Jung *et al.* (1992). Next, volatiles were separated into neutral/basic and acidic fractions before their identification on HRGC/MS and HRGC/O equipments.

3. Headspace analysis

The original headspace procedure, named Static Headspace (SH), involves static recovery in which the sample is equilibrated in a sealed container at a controlled temperature; however, low sensitivities are usually obtained (Sides *et al.* 2000). The Dynamic Headspace (DH) technique, in contrast, is based on the stripping of volatile components with a flow of inert gas (e.g. N_2, He), their subsequent adsorption by polymers and desorption in GC. Quantitative extraction is granted by high temperatures, such as those employed by Salinas *et al.* (1994), who extracted the aromatic compounds from cooked and reconstituted dehydrated potatoes at 100°C for 1 h, or by extended treatments such as those described by Josephson and Lindsay (1987) who performed extraction for 15 h at 21°C. Under these conditions, enzymatic reactions may take place and synthesize *ex novo* aromatic components that were not present before the extraction. The DH is extensively used in volatile extraction from potato samples and current applications are shown in **Table 2**. In all applications, the carrier gas used is nitrogen, and the flask containing the sample was frequently held in a water bath at 37°C during extraction.

Only three applications of SH have been recently reported in potato flavour analysis. Limbo and Piergiovanni (2007) used a static headspace analyser to extract volatiles from raw potatoes; Wagner and Grosch (1998), moreover, applied the SH analysis to isolate methylpropanal, 2,3-butanedione and methanethiol from frozen French fries; a similar technique was applied to investigate perceivable odours in fresh and stored French fries (Wagner and Grosch 1997).

4. Solid-Phase Microextraction (SPME)

In 1990, solid-phase microextraction (SPME) was developed by Arthur and Pawliszyn as a sample pre-concentration method, as an alternative to DH, before chromatogramphic analysis. In this technique, an inert fibre, coated with a stationary phase, is placed in the headspace of the sample (Headspace Solid-Phase Microextraction, HS-SPME) or inside the sample itself if the liquid (Direct Immersion Solid-Phase Microextraction, DI-SPME) allows volatile adsorption. The loaded fibre is thermally desorbed into a GC carrier gas flow, and the volatiles released are analyzed (Reineccius 2006). The optimization of solid-phase microextraction conditions includes, in addition to the selection of the operative mode (HS-SPME and DI-SPME) and the fibre coatings, the equilibration, adsorption and desorption conditions (temperature and duration). With regards to the fibre coatings, different stationary phases are available, including polydimethylsiloxane (PDMS) carbowax/divinylbenzene (CW/DVB), divinylbenzene/carboxen/polydimethylosiloxane (DVB/CAR/PDMS), carboxen/polydimethylsiloxane (CAR/PDMS) and polydimethylsiloxane/divinylbenzene (PDMS/DVB).

This technique has been applied to various food flavour and off-flavour analyses (vegetables and fruits, beverages, dairy products, oils and other food), pesticides, agrochemicals and food contaminants (Kataoka *et al.* 2000). To the best of our knowledge, only the headspace operative mode (HS-SPME) has been used in volatile analysis of potatoes, as summarized in **Table 3**.

Separation and identification techniques

1. Gas Chromatography - Mass Spectrometry (GC-MS)

Mass spectrometry is used to either determine the identity of an unknown volatile compound or can also act as a mass-selective GC detector. It is advisable that MS identifications are supported by other data such as GC retention data, infrared spectroscopy or nuclear magnetic resonance. MS can be operated in selected ion detection mode (SIM), multiple-ion mode (MIM) or full scan mode. In the SIM or MIM mode, the MS measures only selected ions at very short time intervals throughout a GC run, leading to greater sensitivity and a larger number of scans than full scan detection mode.

The magnetic sector or quadrupole requires significant time to scan a typical mass range, while ion trap (GC-ITMS) and time-of-flight (GC-TOFMS) MS detectors, in contrast, can collect spectra much faster (ion trap about 10–15 spectra/sec and TOF up to 500 spectra/sec). The TOF instrument can take a large number of spectra across a GC peak and reduce noise, thereby improving both sensitivity and detection limits. Another advantage is the deconvolution of mixed spectra, i.e. the resolution of the MS data of

Table 3 Current HS-SPME applications in potato volatiles analysis.

Sample	Fiber	Equilibration (t-T)[a]	Extraction (t-T)[b]	Desorption (t-T)[c]	Instrumental technique[d]	Reference
Raw potatoes	DVB/CAR/PDMS (50/30 μm)	5 min - 80°C	20 min - 60°C	5 min - 250°C	GC-MS (Rtx-1; 86.7 min)	Longobardi *et al.* 2010
Boiled potatoes	DVB/CAR/PDMS (50/30 μm)	10 min - 37°C	30 min - 37°C	3 min - 250°C	GC-MS (ZB-WAX; 63.3 min)	Blanda *et al.* 2010
Steamed potatoes	PDMS (85 μm)	n.a.	20 min - 50°C	2 min - 280°C	GC-MS (DB1701; 42 min)	Morris *et al.* 2010
Potato chips	PDMS (100 μm)	5 min - 30°C	60 min - 30°C	5 min - 250°C	GC-ITMS (HP-VOC fused silica; 41 min)	Lojzova *et al.* 2009
	CW/DVB (65 μm)					
	DVB/CAR/PDMS (30/50 μm)			2 min - 250°C	GC-TOFMS (HP-VOC fused silica; 38 min)	
	PDMS/DVB (65 μm)				GC x GC-TOFMS (HP-VOC fused silica and Supelcowax 10; 38 min + 38 min)	
	DVB/CAR/PDSM (50/30 μm)	n.a.	15 min - 60°C	3 min - 250°C	GC-FID (SP2330 fused silica; 35 min)	Pangloli *et al.* 2002
	PDMS/DVB (65 μm)					
Potato crisps	DVB/CAR/PDSM (50/30 μm)	5 min - 70°C	20 min - 70°C	5 min - 250°C	GC-MS (DB-5; 22 min)	Sanches-Silva *et al.* 2004
	CAR/PDMS (75 μm)	5 min - 70°C	20 min - 70°C	3 min - 260°C	GC-MS (DB-5; 22 min)	Sanches-Silva *et al.* 2005
	PDMS/DVB (65 μm)					
	DVB/CAR/PDMS (50/30 μm)					
Extruded potato snacks	PDMS	10 min - 50°C	30 min - 50°C	5 min	GC-O and GC-MS (SBP-5; 30 min - Supelcowax 10; 40.5 min)	Majcher and Jelén 2009
	CW/DVB					
	DVB/CAR/PDMS					
	PDMS/DVB					
	CAR/PDMS					
Potato flakes	PDMS	10 min - 35°C	60 min - 35°C	270°C	GC-MS (CP-WAX 52 CB; 35.5 min)	Laine *et al.* 2006
	CAR/PDMS/DVB					
	PDMS/DVB					
	CAR/PDMS					

[a] Equilibration conditions: time (min) and temperature (°C)
[b] Extraction conditions: time (min) and temperature (°C)
[c] Desorption conditions: time (min) and temperature (°C)
[d] Separation and detection technique (column stationary phase; analysis time, min)

one compound from a mixture of compounds that co-elute. The deconvolution process may also be implemented using two-dimensional GC, which involves collecting part of a GC run and re-chromatographing it on a different chromatographic phase. These systems typically permit the collection of a selected part of several GC runs, improving sensitivity (Reineccius 2006). All these techniques have been used for analysis of raw and processed potato volatiles, as shown in **Tables 1-3**.

2. Gas Chromatography/Olfactometry (GC/O), Gas Chromatography-FID/Olfactometry (GC-FID/O), Gas Chromatography-MS/Olfactometry (GC-MS/O)

In GC/O, the human nose is used as a selective and sensitive detector of volatile compounds, and the odour character of GC peaks is shown in an aroma profile. The effluent from the GC column is mixed with air and water vapour and is perceived by human assessors who identify the odours of compounds eluting from the column. Several parameters have to be considered in the optimisation process, but usually the most significant error factors are those that affect the perception of aromas by sensory panellists (Reid 2003). The GC column effluent can be split in two portions, one going to a sniffing port and the remainder going to a flame ionization (FID) or a mass (MS) detector. In alternative, the GC run may be made by passing all of the GC column effluent to the nose at one time: the column is then connected to the instrument detector, and a second run made (Reineccius 2006).

Although a large number of volatile compounds are present in foods, not all contribute to aroma. Patton and Josephson (1957) proposed to estimate the importance of an aroma compound in defining the sensory character of a food by calculating the ratio of the concentration of the compound to its sensory threshold in that food. This ratio is known as the odour activity value (OAV) (also referred to as odour value, odour unit, flavour unit, or aroma value). Only compounds present above their sensory threshold concentrations in a food are likely to be significant contributors to aroma.

The major screening procedures for determining the key odorants in food are based on Aroma Extract Dilution Analysis (AEDA), developed by Ullrich and Grosch (1987), Aroma Extract Concentration Analysis (AECA), described by Kerscher and Grosch (1997), and CHARM Analysis developed by Acree and Barnard (1984). Diluted (or concentrated) samples, prepared by using one of the extraction techniques previously described, are evaluated by GC/O. The occurrence of an aroma (its retention time or Kovats index) is recorded in each dilution, and a greater number of dilutions in which an odorant is detected, is reflected in a higher CHARM or Dilution Value. AEDA has been used to identify the major odorants from boiled potatoes (Mutti and Grosch 1999) and French fries (Wagner and Grosch 1997, 1998).

SENSORY ANALYSIS

The sensory evaluation of a food can be made through discriminative, descriptive or affective tests. Discriminative tests investigate whether there is a sensory difference between samples (Stone and Sidel 1992). The most common are the triangle test, duo-trio test and paired comparison test.

Descriptive tests involve the detection and description of both qualitative and quantitative sensory components of a product by trained panels. Descriptive tests can establish relationships between descriptive sensory and instrumental or consumer preference measurements. There are several different methods of descriptive analysis, such as Flavour Profile and the Quantitative Descriptive Analysis (QDA).

The affective tests have the primary objective to assess

the personal response from users or potential users of a product (acceptance, preference, or consumer tests). A large number of individuals are required to take part in a sensory acceptance test (> 100). Preference can be measured directly by comparing two or more products with each other, or indirectly by determining which product is the most appreciated in a multiproduct test. The two most widely used methods to measure preference and acceptance are the paired comparison and the 9-point hedonic scale tests.

The sensory tests most frequently used for the evaluation of raw and processed potatoes are the Flavour Profile, QDA and the preference tests, as detailed in the following paragraphs.

VOLATILE COMPOUNDS IN RAW POTATOES

About 159 volatile compounds have been identified in raw potatoes (Dresow and Böhm 2009), but their aromatic profile has not been widely studied. Most investigations have focused on cut or sliced potatoes, and the main volatiles identified were those derived from oxidation and enzymatic activity. In particular, many compounds such as aldehydes, ketones and alcohols, derived from the lipoxygenase activity on unsaturated fatty acids were detected (Mazza and Pietrzak 1990; Maga 1994; Petersen *et al.* 1998). Significant products of potato lipid oxidation are hexanal, octenal and isomeric forms of 2,4-decadienal (Maga 1994). Some alcohols, like 2-methyl and 3-methylbutanol derived from leucine and isoleucine metabolism (Drawert *et al.* 1975). Other compounds identified in raw potatoes, and responsible for vegetable-like odours are methoxypyrazines. Their mechanism of formation has been investigated, and the biosynthetic pathway in potato tubers, or production from microflora present in soil or on the potato surface, have been hypothesized (Maga 1994; Dresow and Böhm 2009).

Longobardi *et al.* (2011) carried out HS-SPME/GC-MS on three potato cultivars ('Arinda', 'Sieglinde', and 'Red Cetica') produced in three different locations in Italy (Sicily, Apulia, Tuscany). 32 volatile compounds were identified and a discriminant function analysis (DFA) was applied on normalized data. The complete separation of potato samples of different geographical origin was achieved and the recognition ability was 100% for each class. The prediction ability was 91.7% and among 36 samples analysed, only four samples were incorrectly classified. The same classification results were obtained applying these statistical methods on the complete data set, including also isotopic data.

Recently, increasing attention has been given to ready-to-eat and ready-to-use vegetables with the specific aim of increasing the shelf life of these products since manufacturing operations promote the development of enzymatic browning and microbial growth (Beltrán *et al.* 2005). Modified atmospheres, in particular high oxygen partial pressures (10, 55 and 100 kPa O_2) in combination with ascorbic and citric acid dipping, have been applied to potato slices; the accumulation of volatile compounds (ethanol, acetaldehyde and hexanal) has been studied after 3, 7 and 10 days of storage at 5°C (Limbo *et al.* 2007). The higher pressures applied (55 and 100 kPa) had an inhibitory effect on the production of anaerobic volatiles (acetaldehyde and ethanol). In contrast, the lowest hexanal accumulation was obtained at 10 kPa O_2, and a substantial increase was recorded in potatoes that were not submitted to the treatment solution and stored at 100 kPa.

The sensory quality of fresh cut potatoes was investigated also by Beltrán *et al.* (2005) who evaluated the effect of traditional and non-traditional sanitizers on potato strips stored under modified atmosphere and vacuum packaging. Sodium hypochlorite, sodium sulphite, peroxyacetic acid and ozone were used either alone or in combination. The aroma of the strips was evaluated by an expert panel after 5, 11 and 14 days of storage. The best sensory characteristics, were obtained with vacuum packaging. When the modified atmosphere was used, the application of sodium sulphite prevented browning, but it conferred off-odours to potato strips. When the dipping process was carried out in ozonated water and in ozone plus peroxyacetic acid solutions, potato strips stored under vacuum conditions maintained the typical full aroma even after storage for 14 days at 4°C; the authors concluded that the latter treatment was optimal as it could also preserve the microbial quality of the potato strips.

VOLATILE COMPOUNDS IN PROCESSED POTATOES

The sensory profile of processed potatoes is related to the way of cooking but also cultivar selection has an important role, as reported in the following investigations. Sensory properties of different potato varieties have been evaluated by Pardo *et al.* (2000) through the assessment of satisfaction on a verbal hedonic scale. The authors compared 7 varieties ('Bartina', 'Caesar', 'Desirée', 'Agria', 'Edzina', 'Monalisa' and 'Victoria') and found that 'Bartina' was preferred for the flavour in fried products, while 'Victoria' and 'Desirée' were best in terms of flavour for boiled potatoes. These different scores, depending on frying or boiling, suggest a specific use for each potato variety.

Seefeldt *et al.* (2011a) investigated visual, texture, taste and flavour attributes of 11 potato varieties ('Asparges', 'Ballerina', 'Bintje', 'Ditta', 'Folva', 'Hamlet', 'Liva', 'Spunta', 'Sava', 'Saturna' and 'Vivi') grown in loamy and sandy locations and used for three culinary preparations (mashed, oven-fried and boiled potatoes). They found that texture and appearance were the most important attributes for the sensory evaluation of the different culinary preparations, whereas flavour played a minor role for describing potato quality. Also the effect of soil type on flavour and taste was relatively low for all preparations.

Relevant investigations carried out after 1995 and concerning the volatile profile of boiled, baked, fried, dehydrated and extruded potato products are presented. In **Table 4** are reported new volatile compounds detected in boiled potatoes respect those summarized by Dresow and Böhm (2009); advances in the aromatic profile of fried (chips, French fries and crisps), dehydrated and extruded potatoes are also shown, updating the results reported by Maga (1994).

Boiled potatoes

The aroma of boiled potatoes is weak, although it is distinct and very different from the aroma of raw potatoes. Several mechanisms are responsible for the thermal formation of aroma compounds in boiled potatoes, including lipoxygenase-initiated reactions of unsaturated fatty acids that take place after disruption of cells and create large amounts of 2,4-decadienal, (*E*)-2-octenal and hexanal; the autoxidation reactions are responsible for pentanal generation and the Maillard and Strecker reactions lead to components like pyrazines, phenylacetaldehyde and methional (Maga 1994).

Petersen *et al.* (1998) compared the aroma of raw and boiled potatoes of the 'Bintje' variety using a mild extraction technique to ensure major preservation of the more labile compounds of potato aroma. 29 and 25 compounds were identified in raw and boiled extracts, respectively, by GC-MS. The results were in agreement with those previously reported by Josephson and Lindsay (1987), who found that raw shredded potatoes contained relatively high amounts of 2,4-decadienal, (*E*)-2-octenal and hexanal. After boiling, the concentration of the first two compounds decreased, while hexanal increased to become the dominant volatile. Moreover, GC odour profiling of raw and boiled potatoes was performed by evaluating the odour quality and intensity of potato extracts after separation on GC column. 33 odour impressions were detected in boiled potatoes: 8 of them were identified by GC-MS (2-ethyl furan, hexanal, heptanal, (*E*)-2-heptenal, acetic acid, methional, (*E,Z*)-2,6-nonadienal and phenylacetaldehyde) and 4 by the retention index and quality odour ((Z)-4-heptenal, 2-heptanol, 2-

Table 4 New volatile compounds identified in processed potatoes.

	Boiled[a]	Fried[b]	Ext-De[c]		Boiled[a]	Fried[b]	Ext-De[c]
Hydrocarbons				Undecanal	9		
Propylcyclopentane		5		2-Undecenal	9		
Butylcyclopentane		5		4-Ethylbenzaldehyde	9		
Propylcyclohexane		5		2-Hydroxybenzaldehyde		5	
2,2,4,6,6-Pentamethyl heptane			3	4-Hydroxy-3-methoxybenzaldehyde		7	
Bicyclo-2,2,2-1-methyloctane		11		5-Ethyl-1-cyclopentene-1-carboxaldehyde		5	
Hexadecane	9			**Ketones**			
3-Ethyl-2-methyl-1,3-hexadiene	9			2,3-Butanedione			4
1-Heptene		5		6-Methyl-5-penten-2-one	6		
6-Methyl-1-heptene		11		2-Methyl-3-hexanone		5	
1-Octene		5		1-Octen-3-one		7;8	
(E)-2-Octene		5		3-Octen-2-one	9		3
(Z)-2-Octene		5		*(E)*-3-Octen-2-one		11	
Styrene		5		1,5-Octadien-3-one			4
α-Ionone			3	*(Z)*-1,5-Octadien-3-one		7	
α-Curcumene			3	3,5-Octadien-2-one			3
Alcohols				3-Nonen-2-one		11	
Ethanol			4	6-Undecanone	9		
2-Propanol		5		6-Dodecanone	9		
1-Undecanol		5		β-Damascenone			4
Dodecanol	6			**Esters**			
1-Dodecen-3-ol	9			Pentyl methanoate		11	
Tetradecanol	6			Methyl acetate		5	
1,5-Heptadiene-3,4-diol		11		*n*-Hexyl acetate	9		
Acids				Methyl 2-propenoate		5	
2-Octenoic acid		11		Hexyl-propanoate	9		
Phenyletanoic acid		7		Methyl butanoate	9		
Aldehydes				*(E)*-Methyl 2-butenoate		5	
Acetaldehyde			1	Butyl butanoate	9		
Propanal			1	Methyl 3-methylbutanoate		5	
Methylpropanal		2		Methylbutyl butanoate	9		
2-Methyl propanal			3	Hexyl-butanoate	9		
Butanal			4	Butyl hexanoate	9		
2-Methyl butanal			3	Pentyl hexanoate		11	
3-Methyl butanal			3	Hexyl hexanoate	9		
(E)-2-Ethyl-2-butenal		5		Ethyl octanoate	9		
Pentanal	6		3	**Lactones**			
Hexanal			1;3;4	γ-Octalactone		8	
(E)-2-Hexenal			4	γ-Nonalactone		8	
Heptanal			4	γ-Decalactone		8	
2-Heptenal			3	δ-Decalactone		8	
(E,Z)-2,4-Heptadienal		5		4-Hydroxynonanoic acid lactone		7	
Octanal			4	4-Hydroxy-2-nonenoic acid lactone		7	
(E)-2-Octenal			4	**Pyrrole compounds**			
Nonanal			4	Pyrrole		5;10	
(Z)-3-Nonenal		7		2-Acetylpyrrole		10	
(E,E)-2,4-Nonadienal			1	3-Acetyl-1-methylpyrrole		10	
(E,Z)-2,6-Nonadienal		7		2-Methylpyrrole		5	
2,6-Nonadienal			4	2-Methyl-1(*H*)-pyrrole		10	
trans-4,5-Epoxy-*(E)*-2-nonenal		7		3-Methyl-1(*H*)-pyrrole		10	
(E)-2-Decenal		7		1-Ethylpyrrole		5	
(E)-4,5-Epoxy-*(E)*-2-decenal		7;8		1-Ethyl-1(*H*)-pyrrole		10	
(E,E)-2,4-Decadienal			1	2-Ethyl-1(*H*)-pyrrole		10	
1-Butyl-1-(*H*)-pyrrole		10		2-Acetylpyrazine		7	4
1-Pentyl-1(*H*)-pyrrole		10		2-Acetyl-6-methylpyrazine		10	
2-Pyrrolidinone		10		2-Butyl-3-methylpyrazine		10	
1-Methyl-2-pyrrolidinone		5;10		2-Ethenyl-3-ethyl-5-methylpyrazine		7;8	
2-Acetyl-1-pyrroline			4	2-Ethenyl-5-methylpyrazine		10	
1-(*H*)-pyrrole-2-carboxaldehyde		10		2-Ethenyl-6-methylpyrazine		10	
1-Methyl-1(*H*)-pyrrole-2-carboxaldehyde		10		2-Isobutyl-3-methoxypyrazyne		7	
1-Pyrrolidinecarboxaldehyde		10		2-Methyl-3,5-diethylpyrazine			4
Oxazoles				2-Methyl-5-(1-propenyl)-pyrazine		10	
4,5-Dimethyloxazole		5		2-Vinyl-6-methylpyrazine		2	
Indols				3,5-Diethyl-2-methyl-pyrazine		10	
2,3-Dihydroindole		5		3,5-Dimethyl-2-isobutyl-pyrazine		10	
Furan compounds				3-Isobutyl-2-methoxypyrazine		8	
2-Methylfuran		5		5-Ethyl-2,3-dimethylpyrazine		5	
2-Ethylfuran		5		5-Methyl-2,3-diethylpyrazine			4
2-Ethyl-5-methylfuran		5		5-Methyl-5(*H*)-cyclopentan-pyrazine		10	
2-Vinylfuran		5		Dimethylisobutylpyrazine isomer		5	
2,5-Dihydro-3,4-dimethylfuran		5		Ethenylpyrazine		10	

Table 4 (Cont.)

	Boiled[a]	Fried[b]	Ext-De[c]
Tetrahydrofuran		5	
Tetrahydro-2-methyl furan		11	
5-Ethyl-dihydro-2(3)-furanone		11	
5-Pentyl-2(5)-furanone		11	
5-Hexylhydro-2(3)-furanone		11	
3-Hydroxy-4,5-dimethyl-2(*5H*)-furanone		7;8	4
4-Hydroxy-2,5-dimethyl-3(*2H*)-furanone		7;8	4
2,5-Furandione		5	
2-Furfurylthiol			4
2,5-Dimetyl-3-furanthiol			4
Pyrane compounds			
trans-Tetrahydro-5,6-dimethyl-2(*H*)-2-pyranone		11	
3-Hydroxy-2-methylpyran-4-one		7	
Pyridine compounds			
Pyridine		10	
2-Methyl pyridine		10	
3-Methyl pyridine		10	
2,6-Dimethyl pyridine		10	
3-Ethyl pyridine		10	
Acetyl pyridine		10	
n-Acetyl-4(*H*)-pyridine		10	
1-Acetyl-1,2,3,4-tetrahydro-pyridine		10	
1-(2-Pyridinyl)-1-propanone		10	
2-Pyridinecarboxaldehyde		10	
Pyrazines			
(1-Methylethenyl)-pyrazine		10	
2-(*n*-Propyl)-pyrazine		10	
2,3,5,6-Tetramethylpyrazine		10	
2,3-Diethylpyrazine		5;10	
Ethylpyrazine		2;5;10	
Isopropenylpyrazine		5	
Tetramethylpyrazine		10	
Vinylpyrazine		2	
5,6,7,8-Tetrahydroquinoxaline		10	
Sulphur compounds			
Methanethiol			3;4
Phenyl methanethiol			4
Dimethyl disulfide			3
Dimethyl trisulfide			4
2-Methylthiophene		5	
3-Methylthiophene		5	

[a] New volatiles in boiled potatoes respect Dresow and Böhm (2009)
[b] New volatiles in chips, French fries and crisps respect Maga (1994)
[c] New volatiles in extruded an dehydrated potato products respect to Maga (1994)
1 Nissen *et al.* 2002
2 Martin and Ames 2001
3 Laine *et al.* 2006
4 Majcher and Jelén 2009
5 Van Loon *et al.* 2005
6 Blanch *et al.* 2009
7 Wagner and Grosch 1997
8 Wagner and Grosch 1998
9 Blanda *et al.* 2010
10 Lojzova *et al.* 2009
11 Sanches-Silva *et al.* 2005

methoxy-3-isopropylpyrazyne and 2-methoxy-3-isobutylpyrazine). The remaining odours were not found because they were present at concentrations below the detection limits of GC-MS.

In 1999, Mutti and Grosch evaluated the potent odorants of boiled potatoes (variety 'Sieglinde') by aroma extract dilution analysis, aroma extract concentration analysis and GCO of static headspace samples. The volatiles isolated from potato samples were separated into neutral/basic and acidic fractions. In the neutral/basic fraction, 29 odorants were detected and the most odour active compounds were found to be methional (boiled potato odour) and 4,5-epoxy-(*E*)-2-decenal (metallic odour), evaluated on the basis of the flavour dilution (FD) factor. In the acidic fraction, the highest FD factor was shown by vanillin.

Ulrich *et al.* (2000) used sensory techniques and instrumental analysis to illustrate the differences in aroma among 3 German varieties ('Adretta', 'Likaria' and the breeding clone St 1,365) of potato after boiling. The off-flavours components contributed more than the positive ones to differentiate the aroma of the varieties tested, and the main compounds implicated in these differences included (*E*)-2-pentenal, 2-methylbutanol, 2-pentylfuran, pyrrole and different dienals. Essential aroma compounds, similar between the three varieties, were methional, diacetyl and 5 substituted pyrazines, in agreement with former investigations (Salinas *et al.* 1994; Ulrich *et al.* 1997, 1998).

A sensory profiling of several Danish potato varieties grown in different locations has recently been carried out (Kreutzmann *et al*. 2011). A tailor-made sensory profile was developed for different cooking procedures: boiled, mashed, oven-fried and oven-cooked potatoes. The study showed that flavour and taste attributes were significantly correlated and they had a great importance in describing the variations between potato cultivars. In particular, the flavour of boiled potatoes was correlated to the bitterness attribute, while in mashed potatoes potato the flavour was found to be inversely correlated to graininess. The relevance of using sensory descriptors to define appropriateness of potato cultivars for different culinary preparations has been discussed also by Seefeldt *et al*. (2011b).

Currently, several investigations have been carried out concerning off-flavour development in potatoes, and mostly related their development during storage of raw potatoes, and dehydrated potato products (Maga 1994). Moreover, it was noted that when boiled potatoes are stored they rapidly develop off-flavours, one of the most important of which is described as a cardboard-like note. Petersen *et al.* (1998) performed a sensory evaluation of freshly boiled and boiled stored potatoes, followed by GC-MS and GC-sniffing to identify and quantify the compounds responsible for potato off-flavours (POF). Eight compounds (pentanal, hexanal, nonanal, (*E*)-2-octenal, 2,4-heptadienal, (*E*)-2-nonenal, (*E,E*)-2,4-nonedienal and 2,4-decadienal) were identified as potential contributors to POF.

The authors assumed that such potato off-flavours, mainly represented by aldehydes and some alcohols, were produced during 24 h storage from the breakdown of hydroperoxides, resulted from lipoxygenase initiated oxidation of linoleic and linolenic acid during boiling.

To better explain the mechanism of formation of off-flavours in boiled potatoes, with particular emphasis to lipoxygenase activity, Petersen *et al.* (2003) monitored lipoxygenase activity and the content of volatile compounds mainly responsible for the formation of off-flavour (pentanal, hexanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal) in potatoes during winter storage. Aroma compounds were determined at 3, 4 and 7 months after harvest in raw, freshly boiled and in boiled potatoes refrigerated for 24 h. It was found that lipoxygenase activity increased during long-term storage of raw potatoes, starting from 4 months after harvest. However, the increasing lipoxygenase activity during winter storage was accompanied by a decrease in production of the off-flavour compounds when potatoes were stored after boiling. The production of off-flavours during storage of boiled potatoes could not be explained by changes in lipoxygenase activity, and the authors highlighted the needing for further investigations on the availability of substrates leading to production of important aroma compounds in boiled potatoes.

Conventionally, potatoes are stored for long periods after harvest in order to provide a yearlong supply for industry and final consumers. Storage conditions before cooking is an important factor in determining the composition of sensory characteristics of boiled potatoes. In fact, several modifications occur in tuber composition during storage: fatty acids, sugars and amino acids are particularly involved in these changes.

Blanch *et al.* (2009) studied the effect of storage temperature before cooking on boiled potato lipid and sugar-derived volatile constituents by comparing 2 genotypes of *S. phureja* and one of *S. tuberosum*, stored at 4 and 8°C. It was

found that the storage temperature affected lipid-derived volatile components, on the basis of the variety studied, but in general the sugar-derived volatile constituents increased when lower temperatures were used, probably due to a slow metabolism. These conditions inhibited lipid oxidation and lowered the levels of lipid-derived compounds in the final product. The authors recommended to store potato tubers at 8°C since the formation of Maillard products was minimised and no signal of sprouts was observed.

Several agronomic factors may influence the sensory quality of boiled potatoes in addition to variety, including the type of fertilizer and method of application, soil type and climatic factors. Thybo *et al.* (2002) investigated the effect of 6 different organic treatments (cattle slurry and cattle deep litter applied in three ways, corresponding to an equal supply of total nitrogen) on the chemical, rheological and sensory quality of cooled potatoes. Regarding sensory quality, minor differences were found among the organic treatments investigated. Despite such small differences, statistical analysis showed that compared with deep litter, slurry increased the off-odour perception and decreased the typical potato odour and flavour, probably due to the delay in maturation retarding the production of the flavour components. Moreover, potatoes matured with slurry had slightly higher off-odour and off-flavour, but the differences were extremely small.

Minimally-processed potatoes have been studied in recent years with regards to sensory quality and chemical components, some applications of which include raw pre-peeled and precooked vacuum-packed potatoes. Thybo *et al.* (2006) compared 6 different cultivars ('Berber', 'Arkula', 'Marabel', 'Sava', 'Folva' and 'Agria') and evaluated their suitability to be processed as pre-peeled potatoes, taking into account the effects of wound healing and storage time. Concerning the volatile profile, variations in several aroma components were found, such as methional, linalool, *p*-cymene, nonanal and decanal, mainly caused by the effects of cultivar and storage. The highest concentrations of nonanal and decanal were found in the 'Marabel' and 'Berber' cultivars, which showed a high intensity of rancidness and a low intensity in potato flavour. Owing to the high moistness and the low firmness, the 'Marabel' variety seemed to be a less appropriate cultivar for this type of product.

Jensen *et al.* (1999) used precooked vacuum-packed potatoes to evaluate the development of potato off-flavours (POF) in 4 varieties ('Jutlandia', 'Bintje', 'Sava' and 'Dali') grown in two different locations in Denmark. They found statistically significant differences in the content of POF compounds between the growing location (mainly for 'Jutlandia' and 'Sava') among some of the varieties. The growing location effect can be explained by the environmental conditions throughout the period of growing, harvest and storage. The most potent POF compounds, evaluated on the basis of their aroma values (Rothe and Thomas 1963), were (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal, followed by hexanal, (*E*)-2-octenal and (*E*)-2-nonenal. The results of this study showed that agronomic conditions can influence POF formation in precooked vacuum-packed potatoes.

Off-flavour development in boiled potato slices has also been studied by Blanda *et al.* (2010). The authors performed a sensory evaluation system, using a quantitative-descriptive analysis (QDA) scheme, and defined the odour, flavour and texture features of boiled potato slices. A HS-SPME-GC-MS method was developed to determine the volatile components in boiled potatoes, and investigation of the mechanism of generation of off-odours and off-flavours during storage showed that they did not increase linearly with time, but reached a maximum value after 6 h of storage, further decreasing after 8 and 10 h and finally increasing again after 24 h of storage. This trend was explained by a kinetic mechanism involving the formation of hydroperoxides during the first hours of storage. POF formation was strongly correlated with a high content of aldehydes such as 2-penthylfuran, 2-pentenal, 2-hexenal, 2-heptenal and 2-decenal, and good agreement between the sensory evaluation and the HS-SPME/GC-MS analysis was found. Treatment of potato slices with several food additives (ascorbic acid, citric acid, sodium acid pyrophosphate and meta-bisulphite) after cooking was also investigated. Interestingly, ascorbic acid and citric acid did not prevent the formation of POF, but actually enhanced it; although potassium meta-bisulphite prevented POF formation, it caused the formation of other off-flavours. The best additive was sodium pyrophosphate, which did not change the flavour of potato slices during storage.

The impact of volatile and non-volatile metabolites on potato flavour attributes was investigated by Morris *et al.* (2010). Tubers (*S. tuberosum* group Phureja and *S. tuberosum* group Tuberosum) were sampled at harvest and following 3 months' storage. Quantitative descriptive analysis (QDA) was carried out on boiled potatoes by a trained panel and aroma related attributes were evaluated. Moreover the cooked tuber volatile profile was analysed by SPME/GC-MS.

The authors found that hexanal and 2-methylbutanoic acid methyl ester were strongly negatively correlated with aroma intensity but positively correlated with flavour intensity, creaminess and savouriness. Conversely, metabolites positively associated with aroma intensity such as 2-methylbutanal, 3-methylbutanal, and furan were strongly negatively correlated with flavour intensity, savouriness and creaminess. Significant changes in flavour were related to storage: several aldehydes were found at higher levels after storage.

Baked potatoes

One of the most popular ways to cook fresh potatoes is by baking (Lin and Yen 2004). However, unlike boiled potatoes, which have been thoroughly investigated, baked potato flavour has been somewhat neglected, and up to 1994, only 11 publications have been reported (Maga 1994).

Volatiles from baked potatoes are usually classified based on the mechanism of formation. Fatty acids, sugars and amino acids are the main precursors of the compounds responsible for the flavour of baked potatoes (Whitfield and Last 1991). A high proportion of the compounds identified came from lipid oxidation, and many volatiles are formed from the Maillard reaction, with or without the involvement of sulphur-containing amino acids. Smaller amounts of indigenous flavour compounds such as terpenes and methoxypyrazines have also been identified.

Recent studies on baked potatoes have investigated the different flavour profile of skin and flesh potatoes, as well as the effect of storage, varietal and environmental factors on final aroma. Additionally, new cooking methods such as microwave baking have been studied and compared to traditional ones.

Oruna-Concha *et al.* (2001) reported the volatile flavour compounds of 4 different potato cultivars ('Cara', 'Marfona', 'Fianna' and 'Nadine') after baking and separately studied the volatile composition of skin and flesh. It was reported that their composition varied quantitatively and qualitatively among cultivars grown at different sites. Sugar degradation and/or the Maillard reaction were the major sources of volatiles in skin, largely due to pyrazines, in 'Cara', 'Marfona' and 'Fianna' cultivars. Solavetivone was the major volatile in 'Nadine' skins, suggesting that tubers of this cultivar were under stress during storage. Pyrazines, including 2,5- and/or 2,6-dimethylpyrazine, were the most abundant representatives in every cultivar.

'Fianna' gave the weakest volatile profile in flesh (85 ng/g), whereas 'Cara' gave the strongest (869 ng/g); lipid degradation was the predominant source of volatiles in 'Cara' (93% of the total volatiles, corresponding to 810 ng/g), and a major source in 'Fianna' (75% corresponding to 64 ng/g), but accounted only for 15% (14 ng/g) and 19% (21 ng/g), respectively, of the total volatiles in 'Nadine' and 'Marfona'. Levels of volatiles from sugar degradation and/or the Maillard reaction were similar (14-58 ng/g) in

the analysed cultivars.

The odour unit values were taken into account to select the key aroma compounds of skin and flesh baked potatoes. For skin, 2-isopropyl-3-methoxypyrazine has the highest odour unit value and has an important contribution to aroma only in 'Marfona', clearly distinguishing this cultivar from others. In flesh, (*E,E*)-2,4-decadienal appeared to be the most important contributor to aroma in 'Cara' and 'Fianna'. 'Marfona' was distinguished from the other cultivars by the contribution of dimethyl disulphide.

A varietal study was also carried out by Duckman *et al.* (2001) who examined the volatile flavour components in the flesh of 11 potato cultivars ('Nadine', 'Golden Wonder', 'Fianna', 'Estima', 'Cara', 'Saxon', 'Kerr's Pink', 'Maris Piper', 'Desiree', 'Marfona' and 'Pentland Squire') grown in the same location in Spalding. 81 volatile compounds were identified in this study and semiquantitative results, represented by relative GC peak area units, were reported.

Lipid oxidation and Maillard reaction were found to be the major sources of flavour compounds of baked potato flesh, even though other components (sulphur compounds, methoxypyrazines and terpenes) were also present at lower levels. Abundant representatives of lipid-derived products were hexanal, nonanal and decanal. The most abundant representatives of the Maillard reaction and/or sugar degradation were the Strecker aldehydes of isoleucine and leucine, i.e., 2- and 3-methylbutanal, which were identified in every cultivar and contributed to 75-96% of the volatiles in this category. 3,5-dimethyl-2-(2-methylpropyl)pyrazine was first reported in this study. Methional, considered to be one of the most important contributors to the aroma of baked potatoes (Whitfield and Last 1991), has been identified in only 5 cultivars ('Nadine', 'Desiree', 'Marfona', 'Maris Piper' and 'Pentland Squire'). In contrast, dimethyl disulphide (which can form from methional) was present in all cultivars and dimethyl trisulphide was reported in all except 'Golden Wonder'.

14 terpenes were identified, and 11 (α-pinene, *Z*-ocimene, *E*-ocimene, linalool, isophorone, β-cyclocitral, β-damascenone, α-copaene, geranyl acetone, α-aromadendrene and δ-guaiene) had not previously reported to be components of baked potato aroma.

2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, β-damascenone, dimethyl trisulphide, decanal and 3-methylbutanal were found to be major contributors to flavour in at least one cultivar.

Few studies have examined the effect of storage on flavour development after cooking. An extensive investigation was carried out by Duckham *et al.* (2002), who examined the effects of storage time (2, 3 and 8 months at 4°C) on the amounts of selected volatile flavour components in baked potatoes. Five potato cultivars ('Estima', 'Saxon', 'Golden Wonder', 'Kerr's pink' and 'Desiree') grown in different sites were analysed, and several significant differences were found in the levels of individual compounds, compound classes and total monitored compounds in terms of the individual effects of cultivar and storage time and their two-way interactions. A significant increase in the total amount of compounds between 2 and 3 months and between 3 and 8 months storage was recorded. The compounds derived primarily from lipids increased with storage time, as well as the total levels of Maillard/sugar-derived compounds. Individual terpenes (except 3-carene) and 2-isopropyl-3-methoxypyrazine were significantly higher after 3 months compared to the other storage times. Methional was the only sulphur compound that showed a significant storage time effect, decreasing between 3 and 8 months.

The authors suggested that cultivar, agronomic factors and tuber storage conditions affected the levels of flavour precursors and activities of enzymes that mediated the formation of flavour compounds.

Oruna Concha *et al.* (2002b) investigated the effects of 3 cooking procedures, boiling, conventional baking and microwave baking, on the profiles of flavour compounds of 2 cultivars of potato ('Estima' and 'Maris Piper') and identified 95 flavour compounds. The authors noted that microwave-baked potatoes had the weakest isolates of volatiles compounds among tested procedures. In particular, the total amounts of compounds derived from sugar degradation and/or the Maillard reaction, largely represented by 2- and 3-methylbutanal, were highest for conventionally-baked potatoes. However, the lipid-derived compounds were 1.2-1.5-fold higher with microwave baking. Sulphur compounds, such as terpenes and methoxypyrazine, showed no significant differences between conventional and microwave baking. The quantitative and qualitative differences for the flavour compounds were explained by variations in heat and mass transfer processes.

Oruna-Concha *et al.* (2002a) evaluated the effect of cultivar on volatile flavour compounds in potato baked in a microwave oven. The flavour components of the flesh of 8 cultivars ('Marfona', 'Desiree', 'King Edward', 'Fianna', 'Nadine', 'Pentland Squire', 'Saxon' and 'Cara') were isolated by headspace trapping onto Tenax and analysed by GC-MS. Each potato cultivar possessed a unique profile of volatile compounds. Cara had the lowest overall total amount of all categories of compounds, while King Edward had the highest. 80 compounds were identified in this study: 60 were lipid-derived, in contrast to 33 reported by the same authors from conventionally-baked potatoes (Oruna-Concha *et al.* 2001). Seven terpenes (one monoterpene and 6 sesquiterpenes), which were tentatively identified, had not been previously reported as volatile components of potatoes. No alkylpyrazines were identified in the microwave-baked potatoes, since they were more favoured by the conditions encountered during conventional baking of potato tubers. The authors suggested that total levels of compounds and variations among their profiles could be attributed to differences in the activities of lipid enzymes and levels of flavour precursors considering the range of cultivars investigated. Moreover, they recommended sensory analysis to identify the best cultivar for microwave baking.

Jansky (2008) evaluated the contributions of genotype and environment on the sensory properties of baked potatoes, including "potato-like" flavour and off-flavour intensities. Moreover, the relationship between the individual flavour components and the overall quality perception was determined. A trained panel evaluated 16 potato cultivars (russets, whites, reds and specialty clones) grown in different locations and stored for 2 years. Several differences among cultivars and production environments were found. Stored potatoes received higher quality perception scores than fresh potatoes. Potato-like flavour intensity was positively associated with quality perception, and a strong negative association between off-flavour and quality perception was also detected.

The sensory properties of organically farmed and conventionally produced potatoes have been recently investigated by Gilsenan *et al.* (2010), Hajšlová *et al.* (2005) and Wszelaki *et al.* (2005). No significant differences between organic and conventional cooked potatoes for aroma attributes were found.

Potato chips and French fries

Deep-fat frying is one of the oldest processes of food preparation, and consists in the immersion of food pieces in hot oil. The high temperature causes the evaporation of water, which moves away from the food into the surrounding oil that replaces some of the lost water. The aim of deep-fat frying is to seal the food by immersing it in hot oil so that all flavours and juices are retained by the crisp crust (Moreira *et al.* 1995).

The flavour of potato chips is influenced not only by potato tuber cultivar, but also by frying oil composition, temperature and time of frying (Martin and Ames 2001). More than 500 compounds have been identified in the volatile fraction of French fries and potato chips showing a similar aroma.

Wagner and Grosch (1997) identified potent odorants in

French fries by application of both aroma extract dilution analysis (AEDA) and GC-O of headspace samples. Potato strips of the Agria variety were fried in palm oil, and a total of 48 odorants were revealed; 23 components were reported for the first time as components of fried potatoes, also due to a difference in the analytical strategy employed, which enabled them to identify odorants that were not visible as peaks in the gas chromatogram. Among the odorants showing higher (FD) factors, methional, 2,3-diethyl-5-methylpyrazine, (*E,E*)-2,4-decadienal, 4-hydroxy-2,5-dimethyl-3 (2H)-furanone and 3-methylbutanal were used as reference stimuli for flavour profile analysis of French fries. The deep-fried note (caused by (*E,E*)-2,4-decadienal) predominated when French fries were nasally evaluated, whereas the deep-fried and boiled potato-like smells (caused by methional) were mainly perceived in the retronasal test.

In 1998, the same authors (Wagner and Grosch) evaluated the main contributors to the flavour of French fries prepared in palm oil (PO) and coconut fat (CF). The coconut-like note in the flavour profile of CF was mainly stimulated by γ-lactones with 8 and 10 carbon atoms, while the character impact odorants of PO were 2-ethyl-3,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine and 3-isobutyl-2-methoxypyrazine (earthy odour); (*E,Z*)-2,4-decadienal, (*E,E*)-2,4-decadienal and (*E*)-4,5-epoxy-(*E*)-2-decenal (stimulating the deep fried impression); 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (caramel-like note); methylpropanal, 2-methylbutanal and 3-methylbutanal (malty notes); and methanethiol (sulphurous, cabbage-like odour). The odorants showing relatively high OAV were dissolved in sunflower oil to give two model systems, and a sensory study was undertaken. The flavour profile of the model obtained (MPO) was compared to that of the real PO for similarity. Furthermore, changes in the overall flavour of MPO were evaluated after omission of one or more odorants to determine their contributions to the flavour of PO. The absence of methional in MPO was not perceived by the sensory panel, supposing that this molecule did not contribute to the flavour of French fries, while a greater impact on flavour was imparted by methanethiol, another degradation product of methionine.

Martin and Ames (2001) evaluated the effect of frying oils (palmitolein and silicone fluid) on flavour compounds formed in chips. The flavour profile was examined in relation to the heat-transfer process and precursor formation from frying medium. Strecker aldehydes and sulphur compounds did not differ significantly between the frying media. Potatoes were presumed to provide all the precursors required for the formation of these compounds. Although pyrazines were significantly lower when potato slices were fried in silicone fluid, comparing the percentage relative amount of pyrazines in chips fried in palm olein or silicone fluid it was observed that the amount of total pyrazine was similar in the two frying media. The authors suggested that the reaction pathways leading to pyrazine formation in palm olein and silicone fluid were the same, and palm olein did not provide a source of flavour precursors. However, the kinetics of pyrazine formation appeared to be different, probably due to differences in heat transfer in potato slices. With regard to lipid oxidation products, the amounts of 2,4-decadienal were significantly higher in palm olein-fried chips, but there was no significant difference in hexanal levels between samples.

Hawrysh *et al.* (1996) evaluated the quality and storage stability of potato chips deep fried in canola (CO), partially hydrogenated canola (PHCO), soybean (SBO), and cottonseed oils (CSO). Sensory evaluation was made after accelerated (0, 6, and 12 days at 60°C) and practical storage (18 weeks at 23°C). The quality of potato chips was influenced by frying oil and storage conditions. Fresh CO and CSO chips had higher characteristic potato chip odour and lower off odour/flavour than SBO and PHCO chips. During accelerated storage, chips developed off odour/flavour depending on frying oil. At practical storage conditions, CO chips had higher characteristic potato chip odour/flavour and lower off odour/flavour than other chips. The results of this study indicate considerable potential for CO and PHCO as suitable alternative frying oils for snack food manufacture.

Pangloli *et al.* (2002) evaluated the flavour stability of potato chips fried in cottonseed, sunflower oils and palm olein/sunflower oil blends. All the potato chips contained abundant and similar amounts of hexanal and (*E,E*)-2,4-decadienal, deriving from the oxidation of linoleic acid, which was the most abundant fatty acid found in the frying oils. Sensory evaluation showed that the intensity of potato chip flavour was similar among oils and blends and did not change during storage; however oxidative rancidity and off-flavour increased in chips fried in cottonseed oil after 6 weeks storage. This off-flavour was due to 1-decyne, identified by SPME analysis. The authors found that the addition of 20 or 40% of palm olein oil to sunflower oil produced chips more stable to oxidation during storage, without losing the characteristic potato chip flavour.

Warner *et al.* (1997) determined the effects of fatty acid composition of frying oils on intensities of fried-food flavour and off-flavours in potato chips and french-fried potatoes. Cottonseed oil (CSO) and high-oleic sunflower oil (HOSUN) were blended to produce oils with 12 to 55% linoleic acid and 16 to 78% oleic acid. Hexanal, pentanal, 2,4-decadienal, octanal, and nonanal were used to monitor oxidation of the oil during potato chip storage. Volatile compounds were monitored in fresh and aged (6 months at 25°C) potato chips. Analytical sensory panels evaluated french-fried potatoes and pilot plant-processed potato chips; fried-food flavour intensity was the best indicator of overall flavour quality in fresh potato chips. The authors found that the fried-food flavour decreased with decreasing levels of linoleic acid and 2,4-decadienal, a breakdown product of linoleic acid oxidation. HOSUN (78% oleic acid) produced the lowest levels of hexanal and pentanal, indicating greater frying oil stability and oxidative stability of the food. However, fresh potato chips fried in HOSUN had the lowest intensities of fried-food flavour and lowest overall flavour quality. No oil analysis could predict flavour stability of aged potato chips.

Brewer *et al.* (1999) assessed selected volatiles (pentanal, hexanal, (*E*)-2-hexenal, heptanal, (*E*)-2-heptenal, 2-pentylfuran, (*E*)-2-octenal, nonal, (*E, E*)-2,4-decadienal) and sensory characteristics of frying fats (low linolenic acid soybean oil, creamy partially hydrogenated soybean oil, liquid low linolenic acid hydrogenated soybean oil, and liquid partially hydrogenated soybean oil) and of French fries fried in those fats. Odour characteristics of French fries reflected those of the oils in which they were fried. Hexanal in the French fries was an indicator of loss of "positive" odour attributes and development of rancid, grassy, painty and acrolein odours. Hexanal content in French fries was highest for those fried in low linolenic acid soybean oil and lowest for those cooked in low linolenic acid hydrogenated soybean oil.

Van Loon *et al.* (2005) identified odour active compounds in French fries (Agria variety) at mouth conditions, created to mimic release of volatile compounds from the food to the nose epithelia, where odour is sensed. The amount of product in relation to mouth volume, the temperature and the mixing of the product with artificial saliva were taken into account. 122 compounds were identified by GC-MS: 85% of them originated from sugar degradation and/or Maillard reaction. 2-Methylpropanal, 2-methylbutanal, 3-methylbutanal were the main representatives. 26 pyrazines were found of which 5 had not been previously reported from potato (**Table 4**). Fifteen percent of the volatiles were lipid-derived and ethanol, 2-propanol, hexanal, and nonanal showed the highest relative areas of this group. About 50 odour active compounds were responsible for 41 odours perceived by the panel. The compounds with the highest detection frequencies caused strong malty and fried potato notes, combined with caramel/buttery, green, spicy and deep-fried notes. Chemical and sweaty odours were also observed.

Several methods have been developed by Lojzova *et al.* (2009) for the analysis of substituted pyrazines and other aromatic compounds formed during the Maillard reaction in potato chips. The original aim of this study was to find possible volatile markers of acrylamide formation during potato chips preparation, and as previously reported, the release of alkylpyrazines was shown to correlate with acrylamide formation. After HS-SPME, the authors compared 3 different separation/detection approaches: gas chromatography–ion trap mass spectrometry (GC–ITMS), gas chromatography–time-of-flight mass spectrometry (GC–TOFMS) and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOFMS). They identified 13 target alkylpyrazines (**Table 4**). The major problem encountered was the resolution of 3 isomeric pyrazine pairs (2,5/6-dimethylpyrazine, 2-ethyl-5/6-methylpyrazine with 2,3,5-trimethylpyrazine and 2-ethyl-3,5/6-dimethylpyrazine with 2,3-diethylpyrazine). Full chromatographic resolution of all isomeric pairs could not be achieved in any of the systems tested, but the use of GC×GC–TOFMS offered the best solution, mainly because of the lower limits of quantification (LOQs) and better signal-to noise ratio.

In addition to the target pyrazines, another 46 nitrogen-containing heterocyclic compounds (pyrazines, pyrrols, pyridines, pyrrolidinones, and tetrahydropyridines) were tentatively identified in potato chips by GC(x GC)-TOFMS, and only 13 had been previously reported in earlier studies.

The effect of chemical and biological pre-treatments were tested by Anese *et al.* (2009) in order to reduce acrylamide formation and favour the development of the desired sensory properties of deep-fried potatoes. Lactic fermentation in the presence or in the absence of glycine, as well as immersion in an aqueous solution of the amino acid alone, was considered as pre-treatments for potato cubes before deep-frying. The effects of each pre-treatment on deep-fried potatoes were also compared by evaluating sensory attributes and preference. All pre-treatments significantly reduced acrylamide formation in deep-fried potatoes, but lactic acid fermentation in the presence of glycine was the most effective. The dipping treatments did not significantly affect the flavour of deep-fried potatoes; the same result was obtained by a pair comparison preference test carried out on consumers, which showed no differences in preferences between water and chemical or biological dipping.

The sensory effects of different pre-treatments of potato slices (Panda and Desirée varieties) before vacuum and atmospheric frying were also evaluated by Troncoso *et al.* (2009). Control or unblanched slices without pre-drying were analysed; blanched slices in hot water at 85°C for 3.5 min and air-dried at 60°C until a final moisture content of 0.6 kg water/kg dry solid; control slices soaked in a sodium meta-bisulphite solution (pH 3) at 20°C for 3 min. Pre-treated slices were then fried at 120 and 140°C under vacuum conditions (5.37 kPa, absolute pressure) and under atmospheric pressure until they reached a final moisture content of 1.8 kg water/100 kg. Concerning the sensory results, the best flavour was obtained for control potato chips, but no significant differences were found in terms of overall quality between control and chips pre-treated with meta-bisulphite.

Potato crisps

Few investigations have been published on the flavour profile of potato crisps. As for chips, potato crisps also contain a significant amount of frying oil that provides substantial vulnerability to oxidative rancidity. Notable attention has been paid to the study of flavour profile generated by oxidation processes.

Sanches-Silva *et al.* (2005) developed a SPME sampling method for the investigation of volatile compounds released during storage of potato crisps. Crisps were packaged in a transparent film in order to evaluate the changes in the profile of volatiles under accelerated oxidation. After 3 months, 31 compounds were identified. From a quantitative point of view, carboxylic acids were the most important volatiles identified, mainly represented by hexanoic acid. The second most important class of compounds was aldehydes, followed by alcohols, ketones, furans and other compounds that resulted from degradation/rearrangement of lipids and carbohydrates. Hexanal, formed during the oxidation of linoleic acid via the 13-hydroperoxide, was also studied as an indicator of lipid oxidation in potato crisps, stored in darkness or with natural light at room temperature (Sanches-Silva *et al.* 2004). The authors noted that there was a relevant increase of hexanal, starting from 8 days only in samples stored under light conditions.

Another problem arising during the frying of crisps is acrylamide formation. The sensory properties of potato crisps were evaluated when several additives, mitigating acrylamide formation, were added to blanching water (Mestdagh *et al.* 2008). The authors found that some sensory defects occurred when some acrylamide-lowering additives were used, leading to rejection of product by the panel. In particular, citric acid and acetic acid plus L-lysine induced suppression of the regular taste of potato crisps and enhanced sourness and the perception of popcorn-like flavours, respectively, leading to unacceptable final product quality.

Dehydrated potato products

In the potato industry, potato flakes are a crucial by-product obtained with a raw material that can not be used by other means. Unfortunately, non-enzymatic browning reactions occur during processing, and oxidative reactions occurring during storage lead to off-flavour formation (Sapers 1975) with important economic losses. Although potato flakes have a low lipid content, oxidation is important for limiting the deterioration of quality (Löliger and Jent 1983). In fact, the lipid fraction is composed primarily of linoleic and linolenic acids that are quite susceptible to oxidation in presence of air (Buttery *et al.* 1961). Few papers on volatile compound analysis in potato flakes are available, but up to now several aspects have been taken into consideration, such as the detection of non-enzymatic browning and oxidative compounds, the improvement of flavour in dehydrated potatoes, and the evolution of off-flavours during storage.

Laine *et al.* (2006) evaluated the volatile profile of potato flakes (cultivar 'Bintje') by SPME-GC-MS, and in particular, studied off-flavour formation during 6 months of storage. Thirteen volatile compounds were identified at very low levels, and hexanal was the main compound that appeared from the 12th to the 24th week of storage. The non-enzymatic formation of hexanal, mainly derived from linoleic acid hydroperoxide, was demonstrated after the analysis of lipoxygenase activity in potato flakes.

Nissen *et al.* (2002) also evaluated the oxidative status of potato flakes. In particular, they evaluated the potential use of electron spin resonance spectroscopy and investigated the development of oxidation during storage, detecting differences between products protected by different natural antioxidants (i.e. rosemary, green tea, coffee, and grape skin extracts). The oxidative deterioration of dried potato flakes during storage was also monitored by measurement of volatile compounds, at the beginning of storage and after 12 weeks, using headspace GC. Sensory analysis was carried out as a quantitative sensory profiling to evaluate the intensities of a number of defined descriptors for the smell and taste characteristics. Longer chain compounds (e.g. decadienal) decreased during storage, while shorter chain compounds (such as hexanal) derived from breakdown of secondary lipid oxidation products, increased. Sensory evaluation was found to be inconclusive as no significant variations with storage time or treatment were detected; the authors supposed that these results could be due to the oxidative changes in unprotected potato flakes during

storage and to the protection of potato flakes by antioxidants.

Extruded potato products

Much attention has been paid to the formation of flavour compounds via the Maillard reaction during the extrusion process and the potential loss of flavour volatiles during steam distillation after extrusion. The formation of alkylpyrazines in potato flakes, due to the interaction of reducing sugars and free amino acids, was related to this manufacture step (Maga 1994).

Majcher and Jelén (2009) compared the utility of three extraction methods: SPME (solid-phase microextraction), SAFE (solvent-assisted flavour evaporation) and SDE (simultaneous distillation and extraction) for characterization of flavour compounds from extruded potato snacks. Isolated compounds were analyzed using GC–O and GC/MS. The results showed that for GCO analysis the most suitable extraction method was SAFE, which led to identification of 25 most potent odorants out of 30 (identified by mass spectrometry). Due to the low temperature of extraction applied (40° C), SAFE avoided formation of artefacts, in contrast to SDE. The SAFE method also proved to be adequate for identification of flavour components by GC/MS, showing high precision with adequate limits of detection.

SPME was not able to reveal 7 important components at olfactometry port (1-octen-3-ol, 2-ethyl-3,5-dimethylpyrazine, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, 5-methyl-2,3-diethylpyrazine, β-damascenone and an unknown with a flavour of fresh pepper), but it was suitable for the identification of the highest number of volatiles (13 in SPME, compared to 12 and 11 in SAFE and SDE, respectively). In contrast to SDE and SAFE, SPME extraction identified low boiling compounds that co-elute with solvents used in other methods. Additionally, SPME was able to attain very low detection limits (reaching values of 0.2 - 0.3 ppb for hexanal, heptanal 2-ethyl-3,5-dimethyl pyrazines), which made it highly suitable for identification of flavour compounds present at trace levels.

The authors confirmed that SDE should not be used for food products that are rich in carbohydrates, amines or unsaturated fatty acids, which can serve as flavour precursors during long-term heat treatment used in SDE extraction; in this investigation, 2-furfurylthiol, 2,5-dimethyl-3-furanthiol, octanal, (*E*)-2-octenal and nonanal were recognized as artefacts. SPME and SAFE extraction methods were recommended for full characterization of odour-active compounds in extruded potato snacks.

Other potato-based products

Ogunjobi *et al.* (2005) evaluated the sensory properties of Irish potato (*Solanum tuberosum*) slices after fermentation in 2.0% brine solution for 5 days at room temperature. A trained panel of 15 assessors evaluated several sensory traits, including the aroma and overall acceptability, of fermented and fresh potato slices cooked by boiling, frying with palm oil or a different vegetable oil and roasting.

The result of sensory evaluation revealed that the flavour of roasted fermented potato was not different from the fresh control. The flavour and the general acceptability of both fried samples (palm and vegetable oil) were preferred by the panel over controls. Boiled fermented potatoes, in contrast, had the lowest scores.

Elmore *et al.* (2010) studied the effect of sulphur deprivation on the formation of acrylamide and volatile compounds in cooked potato flour. Potato flour was heated at 180°C for 20 min and volatile compounds of three varieties ('King Edward', 'Prairie' and 'Maris Piper'), grown with and without sulphur fertilizer, were compared.

49 compounds were present in at least one of the headspace extracts of the heated flour. 41 compounds were affected by sulphur treatment and 42 compounds were affected by variety. For freshly-harvested potatoes, sulphur deprivation during cultivation resulted in reduced acrylamide formation in cooked tuber flour and an overall increase in aroma volatiles. Many of such compounds were Strecker aldehydes and molecules formed from their condensation, whereas benzaldehyde was found at higher concentrations in the sulphur-sufficient flour, as acrylamide.

PERSPECTIVES

The aroma profile of food products is a key factor for the determination of consumer preference. The volatile profile of raw and processed potatoes has been widely investigated with several analytical techniques, but a detailed characterization of aroma components is difficult to obtain.

The main biochemical components of processed potato flavour have been identified and classified according to their mechanism of formation. However many aromatic molecules are strictly related to a specific culinary preparation; moreover also agronomical measures (varieties, agricultural systems, fertilization and storage conditions) have to be taken into account.

These prefaces highlight the need of further investigation on the factors that can influence the volatile fraction formation, mainly in processed potatoes. The results obtained could led to the use of certain potato cultivars for specific food preparations, owing to their aromatic profile. Investigations on volatile profiles should always be accompanied by sensory analysis in order to take into account the perception of the volatile molecules during tasting and their global effect on product acceptability.

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5.2 INVESTIGATION OF OFF-ODOUR AND OFF-FLAVOUR DEVELOPMENT IN BOILED POTATOES: STUDY ON ITALIAN AND MEXICAN VARIETIES

The flavour of boiled potatoes is quite little and more than 140 volatile compounds have been identified. The typical aroma of boiled potatoes is mainly due to the presence of essential compounds such as methional and various pyrazines produced by the Maillard reaction and Strecker degradation (Ulrich *et al.*, 2000).

One of the main problems of ready-to-eat or ready-to-cook foodstuffs that contain boiled potatoes is the development of an off-flavour called cardboard-like. Petersen *et al.* (1999) found that the production of cardboard-like off-flavour occurs within a few hours from the preparation of boiled potatoes, and is probably due to lipid oxidation. In fact the production of this off-flavour is dependent upon the oxygen availability and it might be initiated by the lipoxygenase activity (Galliard, 1973; Petersen *et al.*, 1999).

The lipoxidase enzymes (lipoxygenase and lipoperoxidase), released from the disrupted cells during the peeling and the cutting process and during the first time of boiling, convert the unsaturated fatty acids, mainly from the cell membranes, to the corresponding hydroperoxides. These precursors of volatile compounds can slowly break down into volatile carbonyl compounds (for instance aldehydes), even after the heat inactivation of lipoxygenase enzyme. This problem is pronounced in pre-cooked potatoes, but can also arise when freshly boiled potatoes are allowed to stand for some time between cooking and serving (Petersen *et al.*, 1998, 2003).

Research article 6 focused on the development of a QDA® scheme to define the sensory attributes of boiled potato slices. Moreover the volatile components in boiled potatoes were investigated through a HS-SPME–GC–MS technique. Finally, the generation mechanism of off-odours and off-flavours in boiled potatoes and the effects of food additives (ascorbic acid, citric acid, sodium acid pyrophosphate and meta-bisulphite) after cooking were examined.

In this first investigation the Marabel cultivar was used, owing to its marked tendency to develop the cardboard-like off-flavour and rancidity (Thybo *et al.*, 2006).

The development of off-flavours in boiled potatoes depend on various factors, as cultivar (Jensen *et al.* 1999; Thybo *et al.* 2006), agronomic techniques (Thybo *et al.* 2002) and storage conditions before cooking (Blanch *et al.* 2009).

In research article 7 the sensory profiles of three Mexican potato cultivars (Alpha, Chica and Gallo) of boiled potatoes were investigated through a QDA® method; the development of off-odours was also studied during 57-hours refrigerated storage. A different

behaviour was observed with reference to the kind of off-odour developed and its time of appearance.

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Investigation of off-odour and off-flavour development in boiled potatoes

Giampaolo Blanda *, Lorenzo Cerretani *, Patrizia Comandini, Tullia Gallina Toschi, Giovanni Lercker

Dipartimento di Scienze degli Alimenti, Università di Bologna, P.zza Goidanich, 60, 47023 Cesena (FC), Italy

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ABSTRACT

The present study focused on the development of a sensory evaluation system, using a quantitative descriptive analysis (QDA) scheme, to define the sensory attributes of boiled potato slices. A HS-SPME–GC–MS technique for a rapid determination of volatile components in boiled potatoes was also investigated. In addition to the mechanism of generation of off-odours and off-flavours in boiled potatoes (POF), the effects of the use of food additives after cooking were examined. POF formation, analysed by both sensory evaluation and HS-SPME, demonstrated an oscillating mechanism of formation of volatile compounds, probably related to enzymatic lipid oxidation and hydroperoxide generation. In particular, POF were strongly correlated with the presence of 2-pentenal, 2-hexenal, 2-heptenal, 2-pentylfuran and 2-decenal. In all, about 50 compounds were detected by HS-SPME technique. Treatment with ascorbate or citrate, after cooking and before storage, did not prevent the formation of off-flavours, in contrast to sodium pyrophosphate. Potassium meta-bisulphite prevented POF formation, but caused the creation of other off-flavours detected by a trained panel.

1. Introduction

Potatoes (*Solanum tuberosum*), originating from the Andes Mountains about 8000 years ago, have widely spread to the rest of the world as a result of their nutritional properties and their adaptability to different climate conditions. The year 2008 was declared the "International Year of the Potato" to highlight the importance of potatoes as a fundamental food resource and to promote the development of sustainable potato-based systems; such actions aim to ensure food security for the increasing world population and also contribute in protecting natural resources (http://www.potato2008.org/en/index.html).

Potatoes may be served in a variety of ways: fried, steam cooked, baked or boiled, with inter-changeable preferences in different regions. Boiled potatoes have a flavour that is rather weak, but which is typical and clearly distinguishable from that of raw or cooked potatoes, and is one of the most important qualitative criteria in assigning different potato varieties to a fresh or processed food market. Volatile compounds responsible for the flavour of boiled potatoes are created by typical chemical precursors of raw tubers, which are characterised by different flavours (Petersen, Poll, & Larsen, 1998).

More than 140 volatile compounds have been identified in boiled potatoes (Ulrich, Hoberg, Neugebauer, Tiemann, & Darsow, 2000), whereas over 250 have been found in baked potatoes (Whitfield & Last, 1991) due to the high temperature and long cooking time that cause the degradation of the large compounds. Different potato varieties, cultivation techniques, storage and cooking methods, in addition to the extraction and the analytical techniques employed, have identified a broad set of aromatic compounds in boiled potatoes, which are quite variable and not always in agreement in different publications (Petersen, Poll, & Larsen, 1999, 2003; Petersen et al., 1998; Ulrich et al., 2000).

The typical aroma of boiled potatoes is mainly due to the presence of essential compounds such as methional and various pyrazines (Ulrich et al., 2000) produced by the Maillard reaction and Strecker degradation. Moreover, lipoxidase (lipoxygenase and lipoperoxidase) enzymes oxidise the fatty acids in boiled potatoes. Palmitic, linoleic and linolenic acids represent more than 90% of the total fatty acids in potatoes (Galliard, 1973), thus creating numerous aldehydes (Josephson & Lindsay, 1987; Petersen et al., 1998).

As reported by Petersen et al. (1999), during storage of boiled potatoes there is production of cardboard-like off-flavours within a few hours from preparation, that is due to lipid oxidation; such compounds, in fact, are strictly related to the presence of oxygen. This problem is particularly significant for the production of potato-based foodstuffs that are stored in modified atmosphere. Under these conditions, the creation of off-flavours slows but does not stop completely. Moreover, the production of volatile components also varies as a function of the amount of oxygen inside the packaging and the permeability of the latter to atmospheric gases. Such reactions may reduce the shelf life of ready-to-eat or ready-to-cook products that contain boiled potatoes.

* Corresponding authors. Tel.: +39 0547 338121; fax: +39 0547 382348.
E-mail addresses: giampaolo.blanda2@unibo.it (G. Blanda), lorenzo.cerretani@unibo.it (L. Cerretani).

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To the best of our knowledge, the extraction techniques widely employed for the separation of aromatic compounds from boiled potatoes are the Lickens-Nickerson method, Dynamic Headspace (DH) and solid–liquid extraction. Over the years, the Lickens-Nickerson method has been performed with numerous variations from the original version (Buttery, Seifert, & Ling, 1970; Mutti & Grosch, 1999; Nursten & Sheen, 1974; Ulrich et al., 2000), but it is essentially based on steam distillation of volatile compounds at high temperatures for extended times. Due to the analytical conditions required, this process may lead to the creation of new aromatic substances, especially during extended treatments. The extraction is performed with dedicated equipment and assures good detection limits.

The DH technique is based on stripping of volatile components with a flow of inert gas (e.g. N_2, He) and their subsequent adsorption by polymers. Quantitative extraction is granted by high temperatures, such as those employed by Salinas, Hartman, Karmas, Lech, and Rosen (1994), which extracted the aromatic compounds from cooked and reconstituted dehydrated potatoes at 100 °C for 1 h, or by extended treatments such as those of Josephson and Lindsay (1987) who performed an extraction for 15 h at 21 °C. Under these conditions, enzymatic reactions may take place and synthesise ex novo aromatic components that were not present before the extraction.

The extraction procedure has been employed in several works by Petersen et al. (1998, 1999, 2003) to investigate the volatile fraction of potatoes. However, if the aromatic compounds in boiled potatoes are extracted with an organic solvent, a large quantity of sample has to be analysed due to the small concentration of aromas in the food matrix. Moreover, the extraction technique frequently leads to the separation of non-volatile chemical compounds that interfere with the analysis and increase the detection limits.

In 1990, headspace solid-phase microextraction (HS-SPME) has been introduced by Arthur and Pawliszyn as an alternative to the DH technique as a sample preconcentration method prior to chromatographic analysis. In addiction to the analysis of pollutants in water, this method has been applied to various food flavour analyses, and its suitability in qualitative and quantitative analysis of the volatile fraction of virgin olive oil has been frequently reported (Baccouri et al., 2008; Vichi, Pizzale, Conte, Buxaderas, & Pez-Tamames, 2003). It has moreover been used in the analysis of oxidation products of refined vegetable oils (Jelen, Obuchowska, Zavirska-Wojtasiak, & Wasowicz, 2000) and milk, where it demonstrated better precision, accuracy, repeatability and linearity of response than DH (Marsili, 1999).

The objectives of the present research were to perform a sensory evaluation system, using a quantitative descriptive analysis (QDA) scheme, to define the odour, flavour and texture features of boiled potato slices, and to develop a HS-SPME–GC–MS method for a rapid determination of volatile components in boiled potatoes. We also investigated the mechanism of generation of off-odours and off-flavours in boiled potatoes, with particular attention to the effects of treatment with food additives after cooking.

2. Experimental

2.1. Samples

Potatoes (*Solanum tuberosum*) of the Marabel variety were harvested in July and stored for three months at controlled temperature and relative humidity. Potato dry matter was 19.94% at the moment of analysis. From the initial stock of potatoes, only average size tubers (150–180 g) were selected.

All tubers were washed with tap water to remove soil residue before manual peeling. The central portion of each tuber was divided into 5–6 slices (5 mm thickness), and the rest of the tuber was rejected in order to obtain slices with a similar size. About 6.5 kg of potatoes slices were boiled in 24 L of tap water for 12 min. After boiling, slices were split in groups (W, AA, CA, PP, MB) and subjected to various treatments (immersion in hot water or in food additive solutions), as shown in Table 1. Slices were then cooled for 10 min at room temperature (22 °C), put in open PET containers, stored in refrigeration conditions (at a temperature of 5 °C and air exposed) until obtaining thermal equilibrium with

Table 1
Schematic representation of the boiled potato samples analysed.

Samples ID	Post-cooking treatment	Cooling conditions	Storing conditions	SPME extraction temperature (°C)	SPME enzymatic inhibition	QDA analysis	QDA of sample purees before SPME adsorption
WA0i	Water immersion for 1 min at 80 °C	10 min at 22 °C	30 min at 5 °C	70	NaCl	No	Yes
WA0j	Water immersion for 1 min at 80 °C	10 min at 22 °C	30 min at 5 °C	70	–	No	Yes
WA0	Water immersion for 1 min at 80 °C	10 min at 22 °C	30 min at 5 °C	37	NaCl	Yes	Yes
WA2	Water immersion for 1 min at 80 °C	10 min at 22 °C	2 h at 5 °C	37	NaCl	Yes	Yes
WA4	Water immersion for 1 min at 80 °C	10 min at 22 °C	4 h at 5 °C	37	NaCl	Yes	Yes
WA6	Water immersion for 1 min at 80 °C	10 min at 22 °C	6 h at 5 °C	37	NaCl	Yes	Yes
WA8	Water immersion for 1 min at 80 °C	10 min at 22 °C	8 h at 5 °C	37	NaCl	Yes	Yes
WA10	Water immersion for 1 min at 80 °C	10 min at 22 °C	10 h at 5 °C	37	NaCl	Yes	Yes
WA24	Water immersion for 1 min at 80 °C	10 min at 22 °C	24 h at 5 °C	37	NaCl	Yes	Yes
AA24	Ascorbic acid solution immersion(3 g/L) for 1 min at 80 °C	10 min at 22 °C	24 h at 5 °C	37	NaCl	Yes	Yes
CA24	Citric acid solution immersion (3 g/L)for 1 min at 80 °C	10 min at 22 °C	24 h at 5 °C	37	NaCl	Yes	Yes
PP24	Sodium acid pyrophosphate solution immersion (3 g/L) for 1 min at 80 °C	10 min at 22 °C	24 h at 5 °C	37	NaCl	Yes	Yes
MB24	Potassium meta-bisulphite solution immersion (3 g/L) for 1 min at 80 °C	10 min at 22 °C	24 h at 5 °C	37	NaCl	Yes	Yes

the temperature refrigeration (30 min at 5 °C) or for different times (2, 4, 6, 8, 10, 24 h at 5 °C), before extraction and analysis of the volatile fraction.

W samples, after boiling, were immediately immerged in hot water (80 °C) for 1 min (water/potatoes, 3:1).

WA0j: HS-SPME–GC–MS analysis of volatile compounds was carried out at 70 °C, without the addition of an enzymatic inhibitor to the extraction solution.

WA0i: during volatile compound extraction, carried out at 70 °C, NaCl was added to the extraction solution to inhibit enzymatic reactions.

WA0: volatile components extraction was performed at 37 °C with the addition of NaCl to the extraction solution.

WA2, 4, 6, 8, 10, 24 samples were obtained using the same extraction conditions of WA0 (temperature: 37 °C, inhibitor: NaCl), but were stored, respectively, for 2, 4, 6, 8, 10 and 24 h at 5 °C, before analysis.

After boiling, samples AA24, CA24, PP24, MB24 were immediately immerged, respectively, in ascorbic acid, citric acid, sodium acid pyrophosphate and meta-bisulphite solutions (3 g/L) for 1 min at 80 °C (solution/potatoes, 3:1). Each of these food additives was of commercial grade (CHIMAB S.p.A., Padova, Italy). After immersion, all further steps were the same as for sample W24 (storage: 24 h at 5 °C, HS-SPME extraction temperature: 37 °C, enzyme inhibitor: NaCl).

2.2. Sensory analysis

Quantitative Descriptive Analysis (QDA, Stone & Sidel, 1992; Stone, Sidel, Oliver, Woolsey, & Singleton, 1974) was carried out in the laboratory of sensory analysis at the "Campus of Food Science" at the University of Bologna. A panel of 12 judges, with experience in sensory evaluation of different foods, were trained to carry out QDA of boiled potatoes.

The best features for sensory description of boiled potatoes (attributes) were developed during a focus session. Then, additional six training sessions were held to enhance the ability of each panel member to recognise and quantify the descriptors previously stated.

Standard solutions of some descriptors were used to calibrate the panelists, when reference compounds were not available the descriptors learning and alignment procedure was realised without reference standards, as described by Sulmont, Lesschaeve, Sauvageo, and Issanchou (1999).

Based on repeatability and reproducibility of panel results only some attributes were judged during evaluation of potato samples. The descriptors "Other off-odours" and "Other off-flavours" were defined as defected odours and flavours different from the cardboard-like ones. Judges could identify the qualitative nature of the defects perceived and quantify them, using their personal lexicon developed in previous sensory evaluation experiences. The descriptors were evaluated on a continuous scale from 1 to 9 points. A score of 1 indicated no detectable perception, whilst 9 indicated maximum perception. The repeatability of each descriptor was also determined. Only the eight judges with the best analytical capacity (and best repeatability) were selected to perform the sensory evaluation described in Table 1.

A specific and standardised test was performed for sensory evaluation of boiled potatoes: the slices of boiled potato were cut into quarters, and each piece was served to the assessors in plastic dishes. Sensory analysis was made in individual booths equipped with red light to avoid any influence of potato colour on sample judgment; older samples, in fact, could have been identified from fresh samples as they had a paler pulp. After tasting each sample, the judges rinsed their mouth with water. Each sample cited in Table 1, except for WA0j and WA0i, was analysed four times.

A cardboard-like off-odour descriptor was also analysed on all sample purees immediately after HS-SPME extraction of volatile compounds, as described in paragraph 2.3.

Values of the median of sensory data and the robust standard deviation were calculated (Giomo, 2000).

2.3. HS-SPME–GC–MSD analysis

Potatoes slices (about 200 g for each sample) were put in a 1000 mL bottle and homogenised with 500 mL of NaCl 0.30 M using an Ultraturrax. The extraction solution was at a temperature suitable to bring the homogenate to 37 °C.

The bottle was closed with a silicon cap and then introduced in a heating bath at 37 °C for 10 min. After this step, the silicon cap was perforated with the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm, coating 2 cm) fibre holder (Supelco Ltd., Bellefonte, PA, USA) and equilibrated for 10 min, with the heating bath kept at 37 °C. Finally, the fibre was exposed for 30 min and immediately desorbed for 3 min at 250 °C in the gas chromatograph.

After removing the fibre from the bottle, each sample puree (2 repetitions) was immediately smelled by the panel judges directly from the bottle of extraction, and the cardboard-like off-odour perception was evaluated using the same scale of the other descriptors analysed during QDA.

Volatile compounds were identified and peak area was integrated by gas chromatography coupled to quadrupolar mass-selective spectrometry using an Agilent 6890 N Network gas chromatograph and an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated on a ZB-WAX Phenomenex column 30 m × 0.25 mm ID, 1.00 μm film thickness. Column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C min^{-1}. The ion source and the transfer line were set to 175 °C and 280 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionisation energy in the 20–250 amu mass range (2 scan/sec). Volatile compounds present in boiled potatoes were tentatively identified basing on computer matching against commercial libraries (NIST/EPA/NIH Mass Spectral Library 2005) as well as our laboratory-made spectral library of pure substances, Kovats retention indices (KI) and literature data. Retention indices were calculated for each compound using homologous series of C9–C19 n-alkanes (Van Den Dool & Kratz, 1963).

As the aim of the present work was to compare the use of the different additives and storage times, and thus absolute quantification was not necessary, data are reported as peak areas.

2.4. Statistical analysis

Data were analysed using Statistica 7.0 (Statsoft Inc., Tulsa, OK, USA) statistical software. The significance of differences at 5% level amongst means was determined by one-way ANOVA using Tukey's test. The data were also analysed by principal component and classification analysis to determine the correlation between the analyses and demonstrate differences between samples.

3. Results and discussion

Developing a QDA test is particularly complex, owing to intense training of assessors and the availability of artificial standards to calibrate the attributes. In this investigation, thanks to a high number of panel trainings, it was possible to get optimal repeatability of the attributes used, as shown in Table 2. As previously defined, the 10 attributes evaluated were the most repeatable between those developed during the focus session; particular attention was given to off-flavour and off-odour attributes generated during

Table 2
Description of the attributes evaluated during quantitative descriptive analysis of boiled potato slices.

Attribute	Definition	Standard employed to train the panel	Mean CVr% of attribute
Typical odour	Typical fragrance or aroma of boiled potatoes as perceived by the nose from sniffing through the external nares (Lawless and Heymann, 1998)	Solutions of Quest Aroma of boiled potatoes	10.5
Cardboard-like off-odour	Defected odour, characteristic of oxidised milk, perceived by sniffing boiled potatoes slices (Amerine, Pangborn, & Roessler, 1965)	Potatoes at different ageing degree	9.0
Other off-odours	Other defected odours perceived by sniffing boiled potatoes samples.	Not employed	3.7
Hardness	Force required dividing the potato in two parts by the front teeth (Thygesen, Thybo, & Engelsen, 2001)	Potatoes (variety Marabel) at different cooking degree	6.7
Mealiness	How mealy/crumbly the potato is felt in the mouth after chewing (Thygesen et al., 2001)	Potatoes (variety Innovator) at different cooking degree	13.9
Adhesiveness	Force required removing the potato sticking to teeth and palate after chewing (Thygesen et al., 2001)	Not employed	12.7
Sweetness	Sweet taste perceived during chewing of boiled potatoes slices	Sucrose aqueous solutions. Potato samples cooked in solutions at different sucrose concentration	8.7
Typical flavour	Typical boiled potato retronasal smell originated in the mouth via transportation of the stimulus molecules up to the back of the nasopharynx and into the region of the olfactory receptors (Lawless and Heymann, 1998)	Potato samples cooked in solutions at different Quest aroma concentrations.	9.4
Cardboard-like off-flavour	Defected retronasal smell, similar to the characteristic defected odour of oxidised milk, perceived after deglutition of boiled potato slices (Amerine et al., 1965)	Not employed	3.2
Other off-flavours	Other defected retronasal smell perceived after deglutition of boiled potatoes slices	Not employed	2.6

sample ageing, as also described by Petersen et al. (1999). The results of sensory analysis of boiled potato samples is reported on Table 3; PCA of the same data is shown in Fig. 1. For all sensory analyses, the CVr% were less than 20%, and were thus considered acceptable for sensory data (data not shown).

In the present work, the Marabel variety was used due to its marked tendency to develop off-flavours and rancidity (Thybo, Christiansen, Kaak, & Petersen, 2006).

As seen in Table 3, boiled potatoes analysed only a few minutes after cooking (WA0) presented a typical odour that was very high and no cardboard-like off-odour was detected. Hardness, mealiness and adhesiveness were 5.6, 5.5 and 5.1, respectively. Sweetness was 3.3, and typical flavour and off-flavours had a trend similar to the correspondent values of odour attributes.

WA0 and WA2 were very similar (Fig. 1), whilst at increasing storage times (4, 6, 8, 10 and 24 h) typical odour, typical flavour, off-odours and off-flavours changed, whereas the other characteristics evaluated did not vary considerably. Off-odours and off-flavour did not increase linearly during storage: they reached a maximum value after 6 h of storage (WA6), further decreased after 8 and 10 h (WA8 and WA10) and finally increased again after 24 h of storage (WA24). Typical odour and typical flavour have a complementary tendency.

The off-odour and off-flavour concentration of samples WA2,4,6,8,10,24 might be explained by a kinetic mechanism involving the formation of hydroperoxides during the first hours of storage, their increase with time and finally their transformation to yield aldehydes that are responsible for potato off-flavour (POF). Due to air exposure during refrigeration, some volatile components evaporate, and a decrease in POF in samples WA8 and WA10 was observed. The increase of off-odours and off-flavours after 10 h storage, in our view, may be due to further oxidation of the remaining lipid portion.

Volatile compounds are generated by enzymatic and chemical oxidation of the lipid fraction of boiled potatoes, which takes place on the surface of the food in contact with oxygen. Autooxidation reactions of linoleic and linolenic acids create hydroperoxides which are then broken with the formation of volatile compounds responsible for off-flavours. As reported on literature, the generation of hydroperoxides from food fatty acids is not linear during the time, because when a limit concentration is reached, the bimolecular interaction of hydroperoxides and the decomposition of secondary oxidation products leads to the increase of compounds with a great impact on flavour, like aldehydes, esters and other degradation products (Frankel, 1982, 1985).

Hydroperoxides may be cleaved also by enzymatic reactions catalysed by lipoperoxidase creating further off-flavours. Owing to the reproductive functions of the potato tubers, enzymatic activity is very high; its inhibition at elevated temperatures is not immediate, and it proceeds step-by-step and at lower temperatures during the initial stages of cooking. As a result, enzymatic reactions are accelerated. The consequences of these mechanisms

Table 3
Quantitative descriptive analysis results of boiled potato slices. Data presented is the median of 16 values (8 judges and 2 replicates); in brackets CVr% are reported.

Attributes	WA0	WA2	WA4	WA6	WA8	WA10	WA24	AA24	PP24	CA24	MB24
Typical odour	5.0 (7.3)	6.1 (4.5)	3.5 (13.2)	2.2 (12.6)	3.6 (12.7)	4.0 (10.3)	3.5 (7.9)	3.2 (15.3)	5.5 (3.6)	2.0 (13.3)	3.0 (14.9)
Cardboard-like off-odour	1.0 (11.2)	1.0 (9.0)	4.0 (8.3)	4.8 (12.0)	2.5 (11.4)	1.9 (7.5)	4.0 (8.8)	5.0 (12.3)	1.0 (7.3)	4.0 (6.3)	1.0 (5.3)
Other off-odours	1.0 (0.0)	1.0 (8.3)	1.0 (4.7)	1.0 (0.0)	1.0 (2.6)	1.0 (5.7)	1.0 (4.0)	1.0 (7.0)	1.0 (3.3)	1.0 (2.3)	5.0 (2.6)
Hardness	5.6 (11.0)	5.3 (8.0)	6.4 (4.5)	5.6 (5.9)	5.4 (10.5)	4.6 (8.8)	4.5 (7.7)	6.0 (4.8)	8.0 (3.8)	4.0 (2.3)	4.5 (6.7)
Mealiness	5.5 (13.1)	4.8 (9.9)	4.7 (10.9)	4.6 (6.8)	4.6 (15.7)	4.0 (18.7)	6.5 (19.4)	4.5 (6.7)	2.0 (19.9)	6.0 (16.6)	6.5 (14.7)
Sweetness	3.3 (8.1)	3.3 (13.5)	3.3 (17.0)	2.4 (13.5)	3.1 (18.3)	3.4 (12.1)	4.0 (12.1)	2.8 (11.7)	3.0 (8.1)	3.0 (12.7)	3.2 (12.7)
Adhesiveness	5.1 (9.0)	5.1 (6.7)	5.3 (12.4)	5.0 (12.3)	4.4 (5.8)	4.0 (3.9)	5.0 (13.0)	4.2 (11.5)	2.0 (8.5)	6.0 (5.7)	6.0 (7.2)
Typical flavour	5.5 (3.4)	5.9 (16.4)	3.0 (12.7)	1.6 (9.7)	3.6 (4.7)	3.3 (9.4)	5.0 (12.4)	1.8 (7.8)	5.0 (8.9)	2.0 (12.5)	2.0 (5.8)
Cardboard-like off-flavour	1.0 (0.0)	1.0 (2.5)	4.3 (6.5)	5.1 (2.6)	3.4 (7.7)	2.1 (3.9)	2.5 (2.0)	6.2 (0.0)	1.0 (2.3)	4.0 (3.2)	1.0 (4.5)
Others off-flavours	1.0 (2.4)	1.0 (0.0)	1.0 (4.5)	1.0 (0.0)	1.0 (0.0)	1.0 (8.7)	1.0 (0.0)	1.0 (5.6)	1.0 (0.0)	4.0 (2.2)	6.0 (5.6)

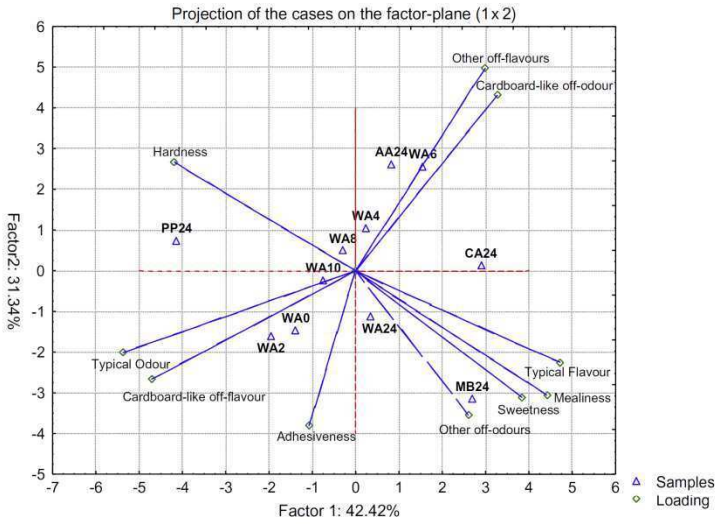

Fig. 1. Principal component analysis of sensory analysis results of boiled potato slices.

are the slow release of volatile components from the food matrix during the successive analytical phases. Another enzymatic mechanism that may be involved is the partial inactivation of potato enzymes, which continue to undergo oxidation even after boiling from atmospheric oxygen (Grosch, 1987; Lercker, Bortolomeazzi, & Pizzale, 1998; Lercker, Capella, & Conte, 1984; Selke, Frankel, & Neff, 1978).

Samples treated with different food additives and stored for 24 h (AA24, CA24, MB24 and PP24) are distant from either WA0 (analysed immediately after cooking) or WA24 (analysed after 24 h of storage without addition of food additives), as shown in the factorial plane of Fig. 1. These results indicate that the additives used do not allow the characteristics of boiled potato slices to remain unchanged, and determine a different evolution of sensorial attributes, with respect to those promoted by different storage times.

AA24 sample has a cardboard-like off-flavour and off-odour that was more intense than the control sample WA24. CA24 sample also differed substantially from WA24, mainly for the presence of other off-flavours defined by the panel judges as "dry", "hay", "biting", which were likely generated by the pH change induced by employing the food additive. These results demonstrate that neither ascorbic acid nor citric acid had any antioxidant effects on the lipid fraction of boiled potatoes, a finding that may be related to their hydrophilic nature, which does not allow them to protect the lipid portion. Moreover, it seems that these acids do not inhibit the activity of oxidative enzymes, which appears higher owing to a pH shift towards more favourable values.

The addition of potassium meta-bisulphite seems to prevent the creation of cardboard-like off-flavour and off-odour. In fact, whilst sample MB24 did not present such attributes, but had other negative characteristics described by the panel members as "beast" and "putrid". These features may be correlated with the capacity of meta-bisulphite to increase the reduction potential and create reduced molecules, with sulphhydryl functional groups.

Sample PP24 was interesting as it had no defects in flavour, odour or taste, but compared to the other samples it was harder and had a lower mealiness. The absence of off-flavours is probably due to the increase of the reduction potential of the system, and it is still unknown if these effects are due to direct inhibition of enzymatic processes. Textural changes in boiled potatoes, in our opinion, are probably due to the creation of a large number of interactions between calcium ions and wall cell pectins, with an increase in calcium pectate and subsequent hardness of boiled potato slices.

These results highlight that further research is needed to explain the reaction mechanisms of additives in boiled potatoes.

During preliminary tests (data not shown), the solid–liquid extraction technique described by Petersen et al. (1999) was applied, but the GC–MS chromatographic traces obtained did not present any significant peaks that were distinguishable from the limits of detection. Differences between our application of the solid–liquid extraction and those reported in literature include the variety of potatoes used (Marabel *vs.* Bintje) and the analysis of slices and not whole tubers. The apparently discrepant results we obtained might be due to the high surface/volume ratio of boiled potatoes, which enable a greater dispersion of volatile compounds, either during the cooking phase or during storage before analysis. However, in our opinion, the solid/liquid extraction technique has the disadvantage of extracting too many interferents that increase the limit of detection. For these reasons, potato slices evaluated by QDA were also analysed by HS-SPME–GC–MS to determine volatile compounds.

HS-SPME extraction was chosen since it is very fast and utilises small amounts of sample. Moreover, it does not extract interferent compounds. Volatile compound analysis by HS-SPME was able to characterise different potatoes samples very well, and in particular those treated with different food additives. Fig. 2 shows the PCA biplot of potato samples and the most representative volatile compounds identified by HS-SPME. Factor analysis was used to determine which variables had greater factor loadings or weights, with those having a value greater than 0.70 being considered significant. Principal components analysis identified two factors that explain 90.69% of the variance: factor 1 explains 63.33% of the variance, whilst factor 2 represents 27.36%. The variables most closely associated with factor 2 were *n*-hexyl acetate, hexyl butanoate and hexyl hexanoate. 2-Pentenal, 2-hexenal, 2,4-heptadienal, 2-heptenal, 2-pentyl furan, 2-nonenal, 2,4 decadienal, and 2-decenal were significantly associated with factor 1.

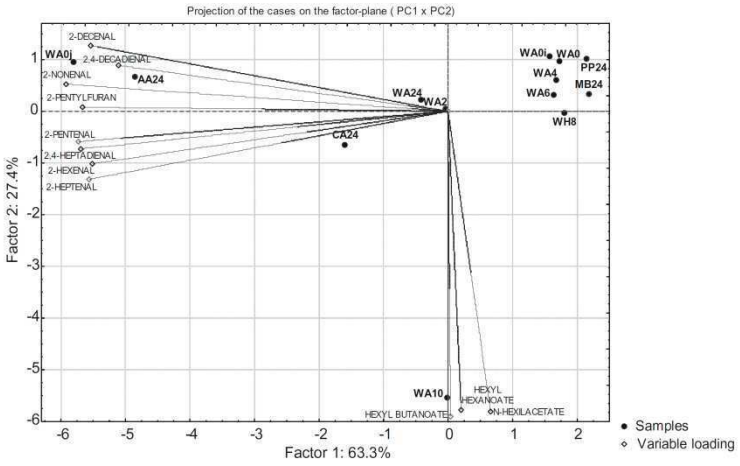

Fig. 2. Principal component analysis of solid-phase microextraction gas chromatography–mass spectrometry analysis results of boiled potato slices.

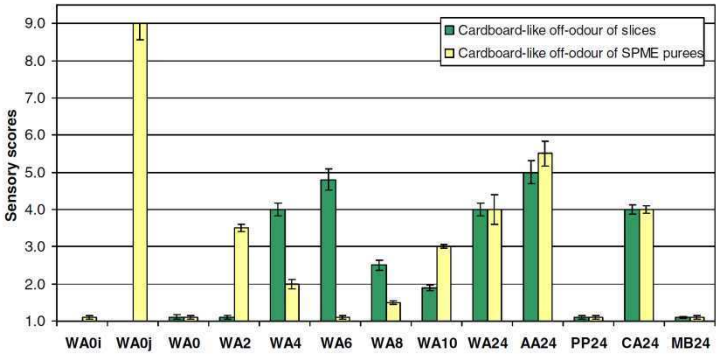

Fig. 3. Quantitative descriptive analysis of cardboard-like off-odour on boiled potato slices and potatoes pureed immediately before solid-phase microextraction analysis.

Samples WA0j and WA0i were analysed to verify the enzymatic production of volatile compounds during the analysis. WA0j, which was extracted with an aqueous solution without enzymatic inhibitor, had an elevated content of aldehydes, probably created by enzymatic processes before and during the adsorption on the fibre. WA0i sample, in contrast, which was extracted with NaCl 0.25 M at the same temperature as WA0j (70 °C), was not substantially different from WA0, which was extracted at 37 °C. In our opinion, this result highlights that temperature does not significantly influence the analysis, and that the oxidation reactions which take place during extraction are exclusively enzymatic. For these reasons, before analysing the volatile fraction in boiled potatoes, it is fundamental to inactivate the enzymatic fraction during the extraction.

In other experiments (data not published), the use of different adsorption temperatures (35, 37, 45, 55 and 70 °C) resulted in aromatic profiles that were very similar, with the same differences in absolute contents of volatile components, but not in the relative ones. In the present work, the temperature selected for absorption of the fibre was 37 °C, which simulated as much as possible that of the human mouth.

In addition, the cardboard-like off-odour attribute was evaluated directly from sample purees after fibre exposure, as described in paragraph 2.2, and the results were compared to those of the same attribute (cardboard-like off-odour) of boiled potatoes slices (Fig. 3). Several differences between slices and purees of the same samples may be noted. WA2 sample slices, which did not have either off-odour or off-flavour, when mashed, showed an increase in these attributes. On the other hand, WA6 had higher POF in slices, which was not detected in the puree. It is likely that this phenomenon was due to the preparation of the puree, which included shredding and homogenising boiled potatoes with NaCl 0.25 M in an aqueous solution at a suitable temperature to bring the system to 37 °C. It is evident that the analyses employed influences the volatile compounds profile.

The presence of cardboard-like off-odour in slices and purees was not seen for all samples, but for slices a trend of this attribute was observed. It did not show linear variations during storage from 2 to 24 h, but there was a maximum of perception at different times for slices and purees that demonstrated peroxide kinetics, as previously assumed.

Good agreement between the sensory evaluation of puree and HS-SPME–GC–MS analysis of volatile compounds was found, as reported below.

As stated by Petersen et al. (1999), the off-flavour of boiled potatoes is mainly due to 8 characteristic aldehydes (reported in thick type in Table 4). In this investigation, we confirmed previously reported results and found a greater number of oxidation products correlated with cardboard-like off-odours and off-flavours. These samples were characterised by the presence of 2-pentenal, 2-hexenal, 2-heptenal, 2-pentylfuran and 2-decenal.

Table 4
Headspace-solid-phase microextraction gas chromatography–mass spectrometry analysis of volatile compounds of potato slices. Different letters in the same row indicate statistically significantly differences (Honestly Significant Differences or HSD by Tukey $p < 0.05$). Values are expressed as peak area integration value (ion abundance x time). Compounds in bold are those identified by Petersen et al. (1999) as responsible for POF generation. Compounds in italics are employed to describe samples in PCA.

Rt (min)	Lib. Acc.	KI	Volatiles	Potato samples																							
				WA0i		WA0j		WA0		WA2		WA4		WA6		WA8		WA10		WA24		AA24		PP24		CA24		MB24	
8.9	91	940.06	2-Ethylfuran	0.0	c	6.2	a	1.1	b	1.9	b	0.0	c	0.6	b	1.0	b	1.6	b	0.0	c	4.9	a	0.0	c	3.5	a	0.0	c
10.5	**90**	**968.64**	**Pentanal**	**0.0**	**c**	**11.5**	**b**	**48.3**	**a**	**39.6**	**a**	**21.2**	**b**	**20.1**	**b**	**14.7**	**b**	**37.5**	**ab**	**37.0**	**ab**	**25.2**	**ab**	**56.6**	**a**	**34.1**	**ab**	**65.7**	**a**
13.3	88	1029.71	1-Penten-3-one	0.0	b	0.0	b	0.0	b	1.7	a	0.8	a	0.0	b	0.0	b	0.0	b	0.0	b	4.2	a	0.0	b	0.0	b	0.0	b
14.7	90	1060.75	2-Butenal	0.0	b	7.0	a	0.0	b	2.2	a	0.0	b	0.0	b	0.0	b	1.3	ab	0.0	b	5.6	a	0.0	b	0.0	b	0.0	b
17.2	**90**	**1116.19**	**Hexanal**	**0.0**	**a**	**19.2**	**a**	**41.3**	**a**	**25.1**	**a**	**25.5**	**a**	**34.9**	**a**	**23.4**	**a**	**26.7**	**ab**	**50.6**	**ab**	**32.3**	**a**	**0.0**	**b**	**33.0**	**a**	**0.0**	**b**
19.6	61	1169.40	Ethylbenzene	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	13.4	a
20.3	94	1184.92	*2-Pentenal*	0.0	c	10.6	a	0.0	c	3.9	b	0.0	c	0.0	c	0.0	c	4.2	b	0.0	c	11.2	a	0.0	c	4.4	b	0.0	c
20.5	36	1189.36	p-Xylene	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	14.6	a
23.4	90	1240.98	Heptanal	0.0	c	3.2	b	1.1	b	3.3	b	0.9	b	1.1	b	0.0	c	2.2	b	3.1	b	8.8	a	0.0	c	3.5	b	0.0	c
23.9	96	1249.45	d-Limonene	4.7	b	2.7	b	0.0	c	9.0	b	0.0	c	0.0	c	4.8	b	19.2	b	0.0	c	0.9	c	2.0	c	210	a	10.3	b
24.6	74	1261.30	Methylbutyl butanoate*	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	6.4	ab	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b
24.9	85	1266.38	Butyl butanoate*	0.0	c	0.0	c	0.0	c	0.0	c	1.8	b	2.9	b	3.4	b	13.9	ab	2.9	b	0.0	c	0.0	c	0.0	c	0.0	c
25.1	78	1269.77	Methyl butanoate*	0.0	b	0.0	b	0.0	b	2.9	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	6.7	a
25.4	98	1274.85	*2-Hexenal*	0.0	b	7.1	a	0.0	b	3.5	a	0.0	b	0.0	b	0.0	b	3.7	ab	5.8	ab	7.7	a	0.0	b	5.8	a	0.0	b
26.0	90	1285.01	*2-Pentylfuran*	40.6	ab	115	a	18.3	b	30.1	b	7.2	b	8.9	b	11.0	b	33.8	b	33.1	b	90.5	a	3.4	b	85.9	a	6.6	b
27.0	86	1301.95	1-Pentanol	0.0	b	3.2	a	0.0	b	1.2	a	0.0	b	0.0	b	0.0	b	0.9	ab	0.0	b	1.4	a	0.0	b	0.0	b	0.0	b
27.2	–	1305.33	Unknown	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.9	a	0.0	b	0.0	b	0.0	b
28.2	92	1322.27	*n-Hexyl acetate*	0.0	c	0.0	c	0.0	c	13.2	b	7.6	b	12.6	b	15.6	b	75.0	ab	12.1	b	1.7	b	0.8	b	21.9	b	25.8	b
29.1	91	1337.51	Octanal	3.4	b	6.6	b	3.8	b	11.2	ab	2.2	b	1.9	b	0.0	c	0.0	c	5.0	b	20.0	a	1.1	b	14.2	a	0.0	c
29.8	57	1349.36	1-Octen-3-one	0.0	c	0.0	c	2.7	b	6.9	ab	1.4	b	1.2	b	0.0	c	6.0	ab	4.1	ab	14.4	a	0.0	c	9.3	ab	0.0	c
30.9	94	1367.99	*2-Heptenal*	7.5	ab	92.4	a	18.0	ab	58.6	a	18.7	ab	18.1	ab	14.1	ab	58.3	ab	62.4	ab	91.4	a	1.6	b	60.8	a	0.0	c
31.4	43	1376.46	Hexyl propanoate	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	7.0	ab	0.0	c	0.0	c	0.0	c	0.0	c	1.1	b
34.2	**98**	**1430.65**	**Nonanal**	**23.4**	**b**	**26.4**	**b**	**10.3**	**b**	**22.0**	**b**	**3.2**	**b**	**3.0**	**b**	**3.7**	**b**	**26.1**	**b**	**20.0**	**b**	**80.8**	**a**	**4.6**	**b**	**28.0**	**b**	**2.3**	**b**
34.9	80	1445.87	Butyl hexanoate*	0.0	c	0.0	c	0.0	c	10.7	b	4.0	b	5.9	b	9.6	b	83.4	ab	9.4	b	0.0	c	0.0	c	30.6	ab	10.5	b
35.0	70	1448.04	3-Octen-2-one	0.0	b	8.5	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	8.7	ab	0.0	b	0.0	b	0.0	b	0.0	b
35.1	83	1450.22	*Hexyl butanoate*	0.0	c	0.0	c	0.0	c	3.9	b	2.1	b	3.1	b	5.2	b	30.9	ab	0.0	c	0.0	c	0.0	c	9.5	ab	0.0	c
35.5	90	1458.91	3-Ethyl-2-methyl-1,3-hexadiene*	0.0	b	14.6	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	13.8	ab	26.7	a	0.0	b	0.0	b	0.0	b
35.9	35	1467.61	Ethyl octanoate	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	1.2	a
36.0	**90**	**1469.78**	**2-Octenal**	**8.3**	**b**	**109**	**ab**	**10.0**	**b**	**43.6**	**b**	**13.9**	**b**	**10.8**	**b**	**11.2**	**b**	**56.3**	**b**	**69.3**	**b**	**181**	**a**	**1.5**	**b**	**103**	**ab**	**0.0**	**c**
36.7	90	1485.00	1-Octen-3-ol	1.7	b	27.5	a	4.3	b	14.2	ab	4.7	b	5.4	b	3.4	b	13.9	ab	16.2	ab	20.7	ab	0.9	b	15.3	ab	0.0	c
37.7	**95**	**1506.74**	***2,4-Heptadienal***	**0.0**	**c**	**15.8**	**a**	**1.3**	**b**	**5.4**	**b**	**3.1**	**b**	**2.5**	**b**	**2.1**	**b**	**7.4**	**ab**	**11.3**	**ab**	**16.5**	**a**	**0.0**	**c**	**11.2**	**ab**	**0.0**	**c**
38.4	–	1521.96	Unknown	0.0	b	0.0	b	1.6	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	3.7	a
38.9	90	1532.83	Decanal	13.2	b	0.0	c	5.8	b	32.4	b	4.4	b	3.0	b	3.4	b	41.5	b	27.9	b	161	a	2.6	b	54.7	b	0.0	c
40.0	71	1556.74	3,5-Octadien-2-one	0.0	b	0.0	b	0.0	b	3.9	a	1.1	a	1.0	a	0.9	a	3.7	ab	7.0	ab	0.0	b	0.0	b	8.8	a	0.0	b
40.1	93	1558.91	6-Undecanone*	0.0	b	19.4	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	18.2	a	0.0	b	0.0	b	0.0	b
40.4	97	1565.43	Benzaldehyde	1.9	b	6.9	a	0.0	c	1.8	b	0.7	b	0.6	b	0.7	b	2.1	b	0.0	c	6.0	a	0.0	c	3.4	ab	0.0	c
40.6	**97**	**1569.78**	***2-Nonenal***	**1.6**	**b**	**25.8**	**a**	**0.5**	**b**	**3.3**	**b**	**1.0**	**b**	**0.8**	**b**	**0.7**	**b**	**4.1**	**ab**	**6.1**	**ab**	**22.0**	**a**	**0.0**	**c**	**8.8**	**ab**	**0.0**	**c**
41.3	90	1585.00	n-Octanol	1.3	b	5.3	ab	0.6	b	2.0	b	0.0	c	0.6	b	0.0	c	2.0	b	0.0	c	7.3	a	0.0	c	4.0	ab	0.0	c
42.4	98	1600.00	Hexadecane	2.4	ab	0.0	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b
43.3	87	1633.21	*Hexyl hexanoate*	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	0.6	b	1.1	b	9.2	ab	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c
43.4	91	1635.74	Undecanal*	0.0	b	8.5	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	7.7	a	0.0	b	0.0	b	0.0	b
44.3	94	1658.56	6-Dodecanone*	0.0	b	12.9	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	4.8	a	0.0	b	0.0	b	0.0	b
45.1	92	1678.83	2-Decenal	1.2	b	15.4	a	0.0	c	1.6	b	0.5	b	0.4	b	0.0	c	0.0	c	0.0	c	16.6	a	0.0	c	2.6	ab	0.0	c
47.5	**91**	**1739.67**	**2,4-Nonadienal**	**0.0**	**c**	**105**	**a**	**0.0**	**c**	**6.2**	**b**	**0.0**	**c**	**0.0**	**c**	**3.1**	**b**	**12.1**	**b**	**24.3**	**ab**	**61.6**	**ab**	**0.0**	**c**	**27.0**	**ab**	**0.0**	**c**
48.0	93	1752.34	4-Ethylbenzaldehyde	0.0	c	24.3	a	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	1.4	b	3.0	b	8.7	ab	0.0	c	2.8	b	0.0	c
49.3	90	1785.30	2-Undecenal	0.0	c	115	a	0.0	c	4.3	b	1.8	b	1.1	b	1.3	b	6.2	b	7.1	b	54.9	a	0.0	c	9.1	b	0.0	c
49.9	**90**	**1800.56**	**2,4-Decadienal**	**0.0**	**c**	**83.9**	**a**	**0.0**	**c**	**3.0**	**b**	**0.0**	**c**	**7.1**	**b**	**1.2**	**b**	**4.7**	**b**	**4.4**	**b**	**28.7**	**ab**	**0.0**	**c**	**7.4**	**b**	**0.0**	**c**
51.9	–	1857.06	Unknown	5.9	ab	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	0.0	c	1.1	b	0.0	c	0.0	c	9.2	a	0.0	c
52.7	83	1879.66	1-Dodecen-3-ol*	19.1	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	3.1	a	0.0	b	0.0	b	0.0	b	0.0	b
53.3	90	1896.61	2-Dodecenal	59.3	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	14.5	a	0.0	b	0.0	b	0.0	b	0.0	b

Lib.Acc.: Library accordance, KI: Kovats Indices.
* Volatile compounds tentatively identified only with mass spectra libraries search.

Whilst other compounds like *n*-hexyl acetate, hexyl butanoate, hexyl hexanoate were formed during storage, they were not however correlated with the perception of oxidised flavour. Their appearance was slow in samples stored for 2, 4, 6 and 8 h, was rapid after 10 h and finally reduced in samples stored for 24 h, even in presence of food additives.

POF in sample WA10 were higher than those in WA2, probably as result of the different volatility of the compounds present. For example, esters are created slowly at low temperatures by condensation of an alcohol with an acid, but since their volatility is very high, their decrease over time is reasonable.

HS-SPME–GC–MS analysis confirmed the efficiency of some food additives in preventing the formation of POF. As an example, sample PP24 did not show significant differences with respect to WA0, whilst CA24 and AA24 samples had a high content of aldehydes that could be responsible for POF. AA24, in particular, had a content of aldehydes that was similar to those of the sample extracted at 70 °C without food additives (WA0j). These results confirm a promoting effect on the formation of aldehydes, in agreement with data obtained by sensory evaluation of boiled potato slices.

Sample MB24, however, did not have cardboard-like off-flavour and, as illustrated on Fig. 2, was not well represented by factor 1. Therefore, it did not contain significant quantities of the aldehydes responsible for cardboard-like off-flavour. By GC analysis, it was shown that this sample did not have a volatile profile that differentiated it from the samples without defects. These results are in contrast with those of sensory evaluation, which identified off-odour and "beast-like" off-flavour. The reason for this discrepancy may be related to the characteristics of HS-SPME analysis as it does not detect these chemical compounds.

Finally, in the samples analysed in the present study, about 50 different compounds were identified, although no pyrazines were detected, in disagreement with previous reports. This is probably due to the low limits of detection of the method used.

4. Conclusions

In this study, it was found that the POF formation could be due to lipoxidase activity and was strongly correlated with a high content of 2-pentenal, 2-hexenal, 2-heptenal, 2-pentylfuran and 2-decenal. During storage of boiled potato slices many other volatiles were produced, and about 50 compounds were detected by the HS-SPME technique in samples treated under different conditions. In particular, the use of some commonly used food additives led to some interesting effects on potato slices: ascorbic acid and citric acid did not prevent the formation of POF, but actually enhanced it. Potassium meta-bisulphite prevented POF formation and caused the formation of other off-flavours. The best additive was sodium pyrophosphate, and potato slice flavour was almost unchanged during storage.

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SENSORY PROFILE OF THREE MEXICAN POTATO CULTIVARS AND OFF-ODORS DEVELOPMENT AFTER BOILING

Short Running title: Sensory profile of Mexican potato cultivars

PATRIZIA COMANDINI[1,3], GIAMPAOLO BLANDA[1], MAYRA CRISTINA SOTO CABALLERO[2], HUGO MUJICA-PAZ[2], AURORA VALDEZ FRAGOSO[2], TULLIA GALLINA TOSCHI[1]

[1] Dipartimento di Scienze degli Alimenti, Università di Bologna, P.zza Goidanich, 60, 47521 Cesena (FC), Italy.

[2] Departamento de Biotecnología y Ingeniería de Alimentos, ITESM, Av. Eugenio Garza Sada, 2501 Sur, 64849 Monterrey, N.L. México.

[3] Corresponding author. TEL: +39 0547 338121; FAX: +39 0547 382348; EMAIL: patrizia.comandini2@unibo.it

ABSTRACT

The flavor of boiled potato is rather weak and changes rapidly during storage; one of the most important off-flavors, described as cardboard-like note, can be generated within a few hours from cooking. Its production depends on different factors and represent a big problem for the production of potato-based foodstuffs. The present study focused on the sensory profiling of boiled potato slices of three Mexican cultivars (Alpha, Chica and Gallo) by a quantitative-descriptive analysis (QDA) and on off-odors produced during their refrigerated storage. The formation of cardboard-like off-odor was detected only in cultivars Alpha and Chica. The appearance of cardboard-like off-odor in cultivar Chica was detected several hours after cooking (24 h) and its level was significantly lower than cultivar Alpha (about 30 % lower after 24 and 33 h of storage). Cultivar Gallo presented different oxidation olfactory perceptions described by the assessors as "burnt" note, detected after 5 h of refrigerated storage.

Key words: Boiling, Mexican cultivars, Potato, Off-odors, Sensory analysis

INTRODUCTION

An important qualitative criteria in assigning a certain potato cultivar to the fresh or processed food market is its aromatic profile.

The flavor of boiled potatoes is rather weak, although more than 140 volatile compounds have been identified (Ulrich *et al.* 2000); they have been especially generated from chemical precursors typical of raw tubers (Petersen *et al.* 1998).

Boiled potato flavor changes rapidly during storage owing to lipid oxidation; one of the most important off-flavors, described as cardboard-like note, is produced within a few hours from cooking (Blanda *et al.* 2010; Petersen *et al.* 1999). This phenomenon is particularly significant when pre-cooked vacuum-packed potatoes are produced, but also for freshly boiled potatoes, when there is some time of standing between cooking and serving (Petersen *et al.* 1999).

Pentanal, hexanal, nonanal, (*E*)-2-octenal, 2,4-heptadienal, (*E*)-2-nonenal, (*E,E*)-2,4-nonedienal and 2,4-decadienal have been identified as potential contributors to potato off-flavors; they probably appeared during boiling as a result of lipoxygenase initiated oxidation reactions of linoleic and linolenic acids, and increased during storage (Petersen *et al.* 1998, 1999).

The formation of boiled potato off-flavors is influenced by several factors as cultivar (Jensen *et al.* 1999; Thybo *et al.* 2006), agronomic techniques (Thybo *et al.* 2002) and storage conditions before cooking (Blanch *et al.* 2009).

In particular, the qualitative and quantitative lipid composition of potatoes, as well as the content of lipid-degrading enzymes (hydrolytic or oxidising) seem to be the major causes of off-flavors formation (Galliard, 1973; Galliard and Matthew 1973). However, up to now a direct correlation between the appearance of oxidation compounds, such as aldehydes and alcohols, during the storage of boiled potatoes and the lipoxygenase activity has not been found (Petersen *et al.* 2003).

The aims of this investigation was to carry out a Quantitative Descriptive Analysis (QDA) of boiled potato slices of three Mexican cultivars (Alpha, Chica and Gallo) and to trace their sensory profiles. Cardboard-like off-odor and other olfactory defects, developed during their refrigerated storage were also investigated.

MATERIALS AND METHODS

Samples

Potatoes (*Solanum tuberosum* L.) of Alpha, Chica and Gallo cultivars were bought at a local market in Chihuahua (Mexico). Potatoes of cv. Marabel, used as reference for the formation of cardboard-like off-odor, where bought in Italy. The tubers had different weights, ranging from 300 to 400 g for cvs. Marabel and Alpha and from 200 to 250 g for cvs. Chica and Gallo.

Potatoes were washed with tap water to remove soil residue before manual peeling. The central portion of each tuber was cut into 5–10 slices (5 mm thickness), depending on its dimensions, and the rest of the tuber was rejected in order to obtain slices of similar sizes. About 200 g of potato slices of each cultivar were boiled separately in 1.5 L of tap water for optimal cooking times (15 min for cvs. Alpha and Chica and 14 min for cv. Gallo cv.).

After boiling, slices were cooled for 15 min at room temperature (24°C). Potato samples were analyzed immediately after equilibration with room temperature and after storing in refrigerated conditions (temperature of 4°C and air exposed) for different times, as detailed in the following paragraph.

Sensory analysis

Quantitative Descriptive Analysis (QDA, Stone and Sidel 1993; Stone *et al.* 1974) was carried out in the laboratory of sensory analysis at the University of Chihuahua (Mexico). A panel of ten judges was specifically trained to carry out QDA of boiled potatoes.

The sensory procedure and the attributes developed by Blanda *et al.* (2010) were modified (Table 1) and used to describe Mexican boiled potatoes. Only significant attributes were used to trace the sensory profile of freshly boiled potatoes, as explained in the Results and discussion session. The descriptors were evaluated on a continuous scale from 1 (no detectable perception) to 9 (maximum perception) points. Four training sessions were held to enhance the ability of each panel member to recognize and quantify the descriptors previously stated.

Potatoes of the cv. Marabel were boiled and stored for 6 hours in refrigerated conditions. These samples were used to calibrate cardboard-like off-odor and flavor, due to their marked tendency to develop cardboard-like note, within a few hours from cooking (Blanda *et al.* 2010; Thybo *et al.* 2006). Typical fragrance of boiled potatoes was calibrated by tasting boiled potatoes freshly cooked (cv. Chica). Potato samples (cv. Chica) cooked for increasing times (from 5 to 18 min) were used to calibrate the panelists for hardness; moreover,

increasing concentrations of sucrose (0 – 3 % w/v) were added to the cooking water of potatoes (cv. Alpha) to calibrate the sweetness attribute. When reference standards were not available, descriptors, learning and alignment procedure were realized as reported by Sulmont *et al.* (1999). After attributes calibration, three complete training sessions were carried out in order to improve panel repeatability.

Sensory evaluation of boiled potatoes was carried out following a specific and standardized test: each potato slice was cut into quarters, and each piece was served to the assessors in plastic dishes. Sensory analysis was made in individual booths. After tasting each sample, the judges rinsed their mouth with water. Each sample was analyzed three times.

As reported in Table 2 a complete sensory analysis (all the significant attributes evaluated) was carried out on freshly boiled potato slices (not stored, 0 h samples) and on potato slices stored for 5 and 9 h, in refrigerated conditions (4°C). Particular attention was paid to the aromatic profile, and the olfactory analysis was carried out also at 24, 33, 48 and 57 h, to determine when aromatic alterations appeared, as detailed in Table 2; Typical odor, Other odors, Cardboard-like off-odor and Other off-odors attributes were evaluated during olfactory analysis.

Statistical analysis

Data were analyzed using PanelCheck software (ver. 1.4.0), following the workflow scheme proposed by Tomic *et al.* (2010) and including mixed model ANOVA, multivariate (Tucker-1 model) and univariate (*F*, *p*, and MSE values) analysis and a consensus approach based on principal component analysis (PCA) for the evaluation of Mexican potato cultivars.

Statistica 7.0 (Statsoft Inc., Tulsa, OK, USA) statistical software was also used.

RESULTS AND DISCUSSION

The first part of this work aimed to trace the sensory profile of boiled potato slices from three Mexican cultivars (Alpha, Chica and Gallo). With this purpose the samples were tasted immediately after cooking. The second part of this investigation dealt with the study of the evolution of boiled potato aromatic profiles after a refrigerated storage. A specific goal was to find the time of appearance of aromatic alterations, with particular attention to the cardboard-like note.

The sensory analysis was performed by training 10 assessors, however only the results of the eight best performers have considered for data computation. The sensory data have been

elaborated following the workflow proposed by Tomic *et al.* (2010), taking into account the panel performance and then the evaluation of the samples.

Assessing the importance of the attributes

A mixed model ANOVA was computed first to assess the importance of attributes. It was decided to use a 3-way ANOVA, modeling samples, assessors, replicates and their interactions, because each replicate was served to the assessors in different sessions. In this way the systematic variations, due to the replicate effect, have been taken into account.

The results are shown in Fig. 1 and over the 12 attributes evaluated, 8 were significant at $p < 0.05$. The attributes: Cardboard-like off-odor, Other off-odors, Cardboard-like off-flavor and Other off-flavors were not detected in any of the samples tasted immediately after cooking. Such attributes were not significant for product effect and it was decided to exclude them from the analysis in the first part of the investigation. The following evaluations related to panel performance have been carried out only on the significant attributes ($p < 0.05$).
By analyzing the replicate effect plot (not shown), it has not been detected any session based variation for all the attributes evaluated.

Panel agreement and discriminative capacity

The multivariate analysis (Tucker-1 test) has been applied in order to get an overview over assessors performances using multiple attributes. The common score and the loading plots have been examined. The sample tasted were well distributed in the multivariate space and the panel could distinguish between them (data not shown).

The correlation loading plot generated by PCA on the unfolded matrix has been used to visualize the performance of individual assessors of the panel. The assessors showed a good agreement in the evaluation of the 8 attributes identified as significant in the samples tasted immediately after cooking (Fig. 1, $p < 0.05$); as shown in Fig. 2, all the judge scores were well clustered at the outer ellipses of the graphs.

Repeatability and discrimination capacity of individual assessors

After the panel agreement, the repeatability and the discrimination capacity were determined following a one-way ANOVA model, as proposed by Naes and Solheim (1991). Three statistical quantities were calculated, F, p and MSE (Mean Square Error) values, the first two providing information on the ability of assessors to discriminate between samples and the third on their reproducibility.

The *F* test showed that each assessors could discriminate the three cultivars, at least for 6 attributes ($p < 0.05$), as reported in Fig. 3a.

Each assessor MSE values were lower than 1.25, for all the attributes tested (Fig. 3b). This indicates a good reproducibility of the analysis. Moreover, for all the 8 attributes identified as significant in the samples tasted immediately after cooking (Fig. 1, $p<0.05$), the CVr% was considered acceptable being always lower than 20% (Giomo 2000).

Analyzing the *F*, MSE and *p**MSE graph (not shown), it was noted that the lowest discrimination power was obtained for the attribute Other odors; in fact only three assessors could discriminate between samples considering this attribute. The reason could be due to the low intensity of Other odors in all the cultivars tested; moreover, its perception was reduced by the higher impact of the Typical odor attribute.

Evaluation of ranking capacity

Profile plots (Fig. 4) showed a good agreement for several of the attributes evaluated. Some disagreement was found for Typical odors and Hardness attributes. The profiles of attributes Mealiness, Adeshiviness and Typical flavor were very alike for most of the assessors, with few exceptions. As an example, for the attribute Mealiness assessor A6 rated cv. Gallo higher than the other assessors. For the attribute Adhesiviness assessor A8 rated cv. Gallo higher than the rest of the panel; assessor A1, instead, for the same attribute rated lower cv. Alpha. Finally, the attribute Typical flavor was rated lower by assessor A8 only for cv. Gallo.

Considering the lacking of previous experience on potato sensory analysis, the panel performance might be considered good and adequate for the following sensory evaluation of boiled Mexican potatoes.

Sensory profiles of Alpha, Chica and Gallo cultivars

After verifying that the panel was trained, a consensus procedure on the raw data was made through a plain PCA analysis. Scores, loadings and explained variances for the first two principal components were evaluated.

Fig. 5 reports the results of PCA. The first axis discriminates between cv. Alpha (on the left side) and cvs. Chica and Gallo (on the right). With reference to PC1 cv. Alpha was characterized by the highest values for textural characteristics as Mealiness and Adhesiviness; however it had the lowest values of Typical flavor, Typical odors and Sweetness that were

higher in cvs. Chica and Gallo. As regard PC2, cv. Gallo was well distinguishable from the other samples thanks to the highest concentration of the attribute Other flavors.

The first two principal components explained 100% of the variance of the data.

A spiderplot illustrating the sensory profiles of the three Mexican potato cultivars is reported in Fig. 6. Cv. Gallo showed high Typical odor and flavor intensities. Besides these typical olfactory and retro-olfactory sensations, assessors detected other odors and flavors, described as hints of raw tuber, green beans and hay notes. In order to quantify these perceptions, the attributes Other odors and Other flavors were added to the sensory score sheet initially used. Cv. Gallo was the sweetest, as opposed to Alpha, which was the least sweet, but with the most defined textural properties (Hardness, Mealiness, Adhesiveness). Cv. Chica had mean values of olfactory and textural attributes.

Study of off-odors development after boiling

The development of the olfactory profile of boiled potato slices was studied during refrigerated storage and a particular attention was paid to the formation of off-odors.

Olfactory tests, in particular the assessment of Cardboard-like off-odor and Other off-odors attributes, were made up to 57 hours, or whenever off-odors were developed in each cultivar, as specified in Table 2.

The performance of the panel in detecting the formation of off-odors in boiled potato slices was evaluated following the same procedure used above for the sensory profiles. Briefly, both attributes were significant at $p < 0.001$ as measured in three-way ANOVA model. F values were respectively 359.51 and 359.84 for Cardboard-like and Other off-odors attributes. The multivariate analysis through Tucker-1 model showed a good separation of the samples tested and a high agreement between the panel assessors in the evaluation of the two attributes. Finally, an univariate approach was carried out to evaluate repeatability, discriminant and ranking capacity. In Table 3 are reported the results of F, p and MSE values, calculated using a one-way ANOVA, highlighting discriminant and repeatability capacity of each assessors.

The profile plots, visualizing sample intensity and rankings for each assessors, showed a good agreement between panel members, as illustrated in Fig. 7.

The formation of Cardboard-like off-odor was detected only in cvs. Alpha and Chica (Table 4). Olfactory analysis of cv. Alpha was stopped at 33 h, when the formation of Cardboard-like off-odor had been clearly detected. The formation of Cardboard-like off-odor in cv. Chica started several hours after cooking (24 h) and its level was significantly lower than in cv. Alpha. These interesting olfactory differences should be investigated in terms of

volatile components produced during refrigerated storage; a low level of off-odors development during storage could be related with a different composition of lipid fraction or of enzymatic patterns. Anyway, considering its sensory profile, the use of cv. Chica as boiled ingredient of ready to eat products can be suggested.

Cv. Gallo presented a different oxidation olfactory perception, described by the assessors as burnt and recorded as Other off-odors attribute. This alteration was significantly detected after 5 hours of refrigerated storage (Table 5) and attribute was not detected in cvs. Alpha and Chica.

CONCLUSIONS

A sensory panel, without previous experience in sensory analysis of boiled potatoes, has been trained and the sensory profiles of three Mexican potato cultivars (Alpha, Chica and Gallo) have been carried out.

Different kinds and levels of off-odors were detected in the samples analyzed during refrigerated storage. The development of Cardboard-like off-odor in cv. Chica started later than in cv. Alpha (24 h *vs.* 9 h respectively). The development of Cardboard-like off-odor in cv. Alpha was more similar to the Italian cv. Marabel previously investigated, where this alteration was perceived after 6 h of refrigerated storage.

Cv. Gallo developed an olfactory alteration, different from the cardboard-like note, identified as “burnt” note, after 5 h of storage.

Further determinations, such as an analytical quantification of the molecules responsible for different odors and off-odors in the three Mexican boiled potato slices, can be useful to complete their characterization and to understand if such results might be related to different metabolic or enzymatic patterns.

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Figures

FIG. 1. PRODUCT EFFECT IN THE THREE-WAY ANOVA MODEL BASED ON 10 ASSESSORS.

Abbreviations: Typ. = Typical; Ot. = Other; Cl = Cardboard-like

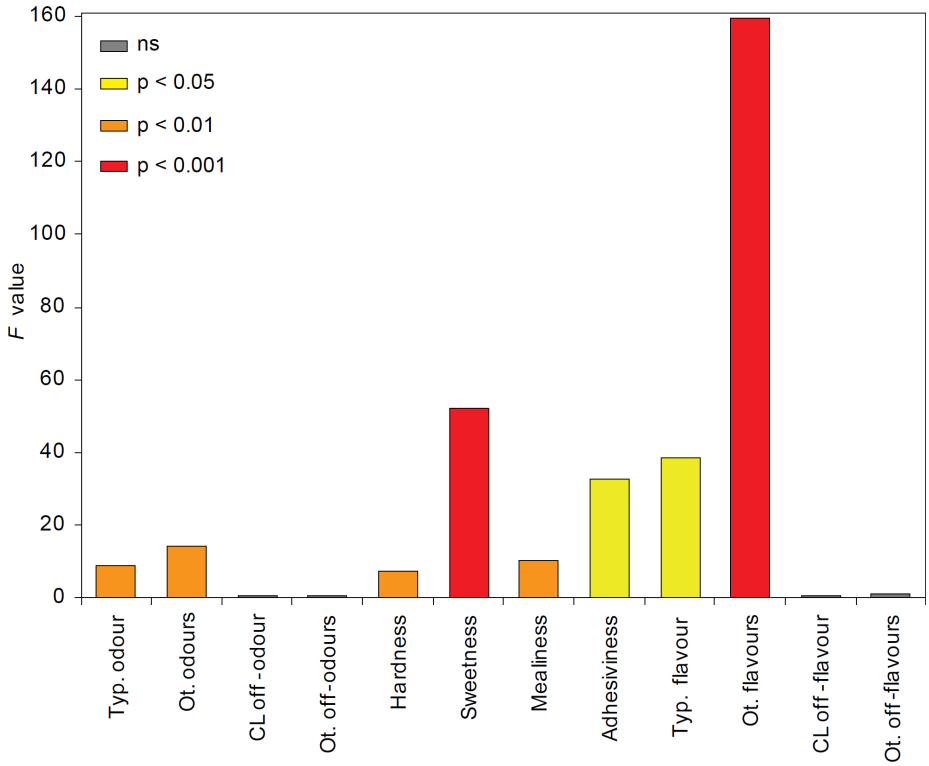
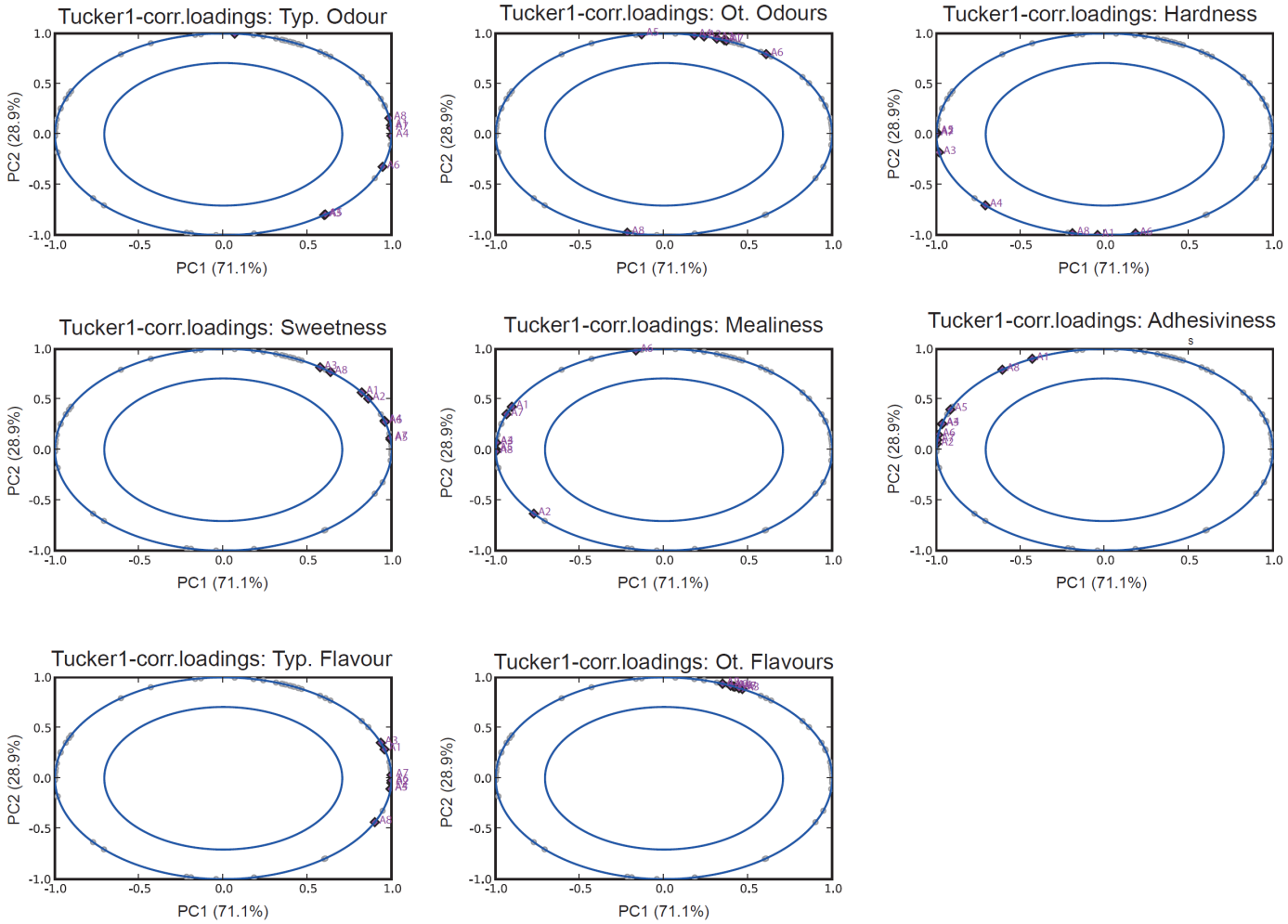


FIG. 2. TUCKER-1 LOADING PLOTS BASED ON SAMPLES AVERAGES. EACH PLOT IS REFERRED TO ONE OF THE EIGHT ATTRIBUTES USED IN THE PROFILING



334 FIG. 3. *F* PLOTS VISUALIZING THE ASSESSORS' ABILITY TO DISCRIMINATE
335 BETWEEN THE TESTED SAMPLES FOR EACH ATTRIBUTE.
336 The horizontal lines indicate *f* values at significance level 1 and 5%
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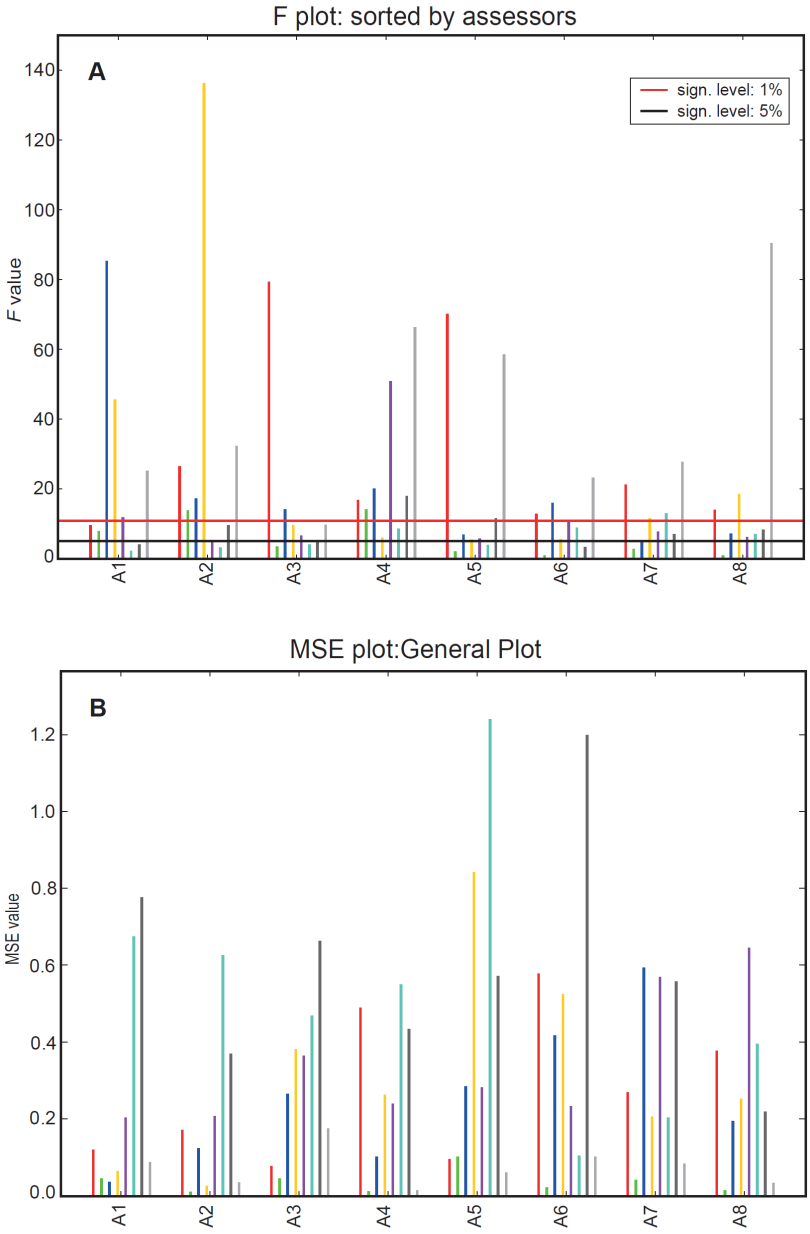

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FIG. 4. PROFILE PLOTS OF THE 8 SIGNIFICANT ATTRIBUTES EVALUATED, VISUALIZING SAMPLE INTENSITY AND RANKINGS FOR EACH ASSESSOR. ON THE VERTICAL AXES ARE REPORTED THE INTENSITY SCORES; ON THE HORIZONTAL AXES THE THREE SAMPLES TESTED, SORTED BY INTENSITY BASED ON CONSENSUS

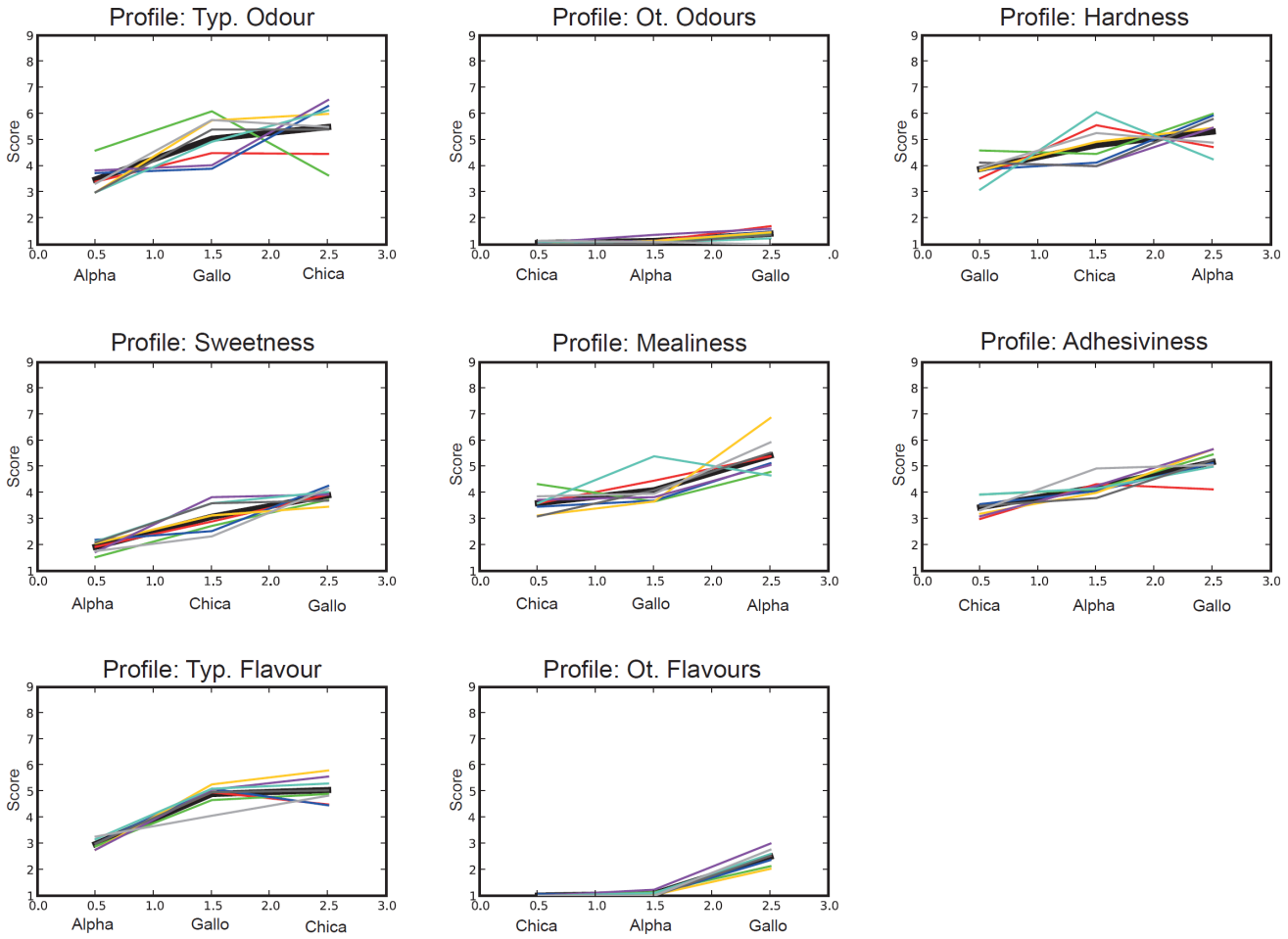


FIG. 5. PRINCIPAL COMPONENT ANALYSIS OF SENSORY ANALYSIS RESULTS OF BOILED POTATO SLICES. SCORES ARE REPORTED IN BOLD ITALIC FORMAT

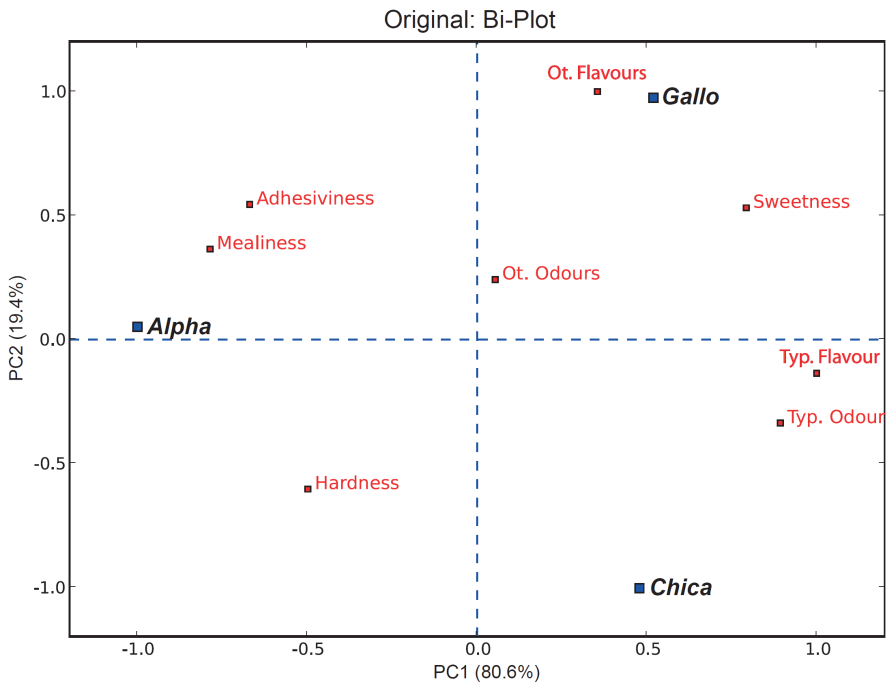


FIG. 6. SENSORY PROFILES OF BOILED POTATO SLICES (CVS. ALPHA, CHICA AND GALLO)

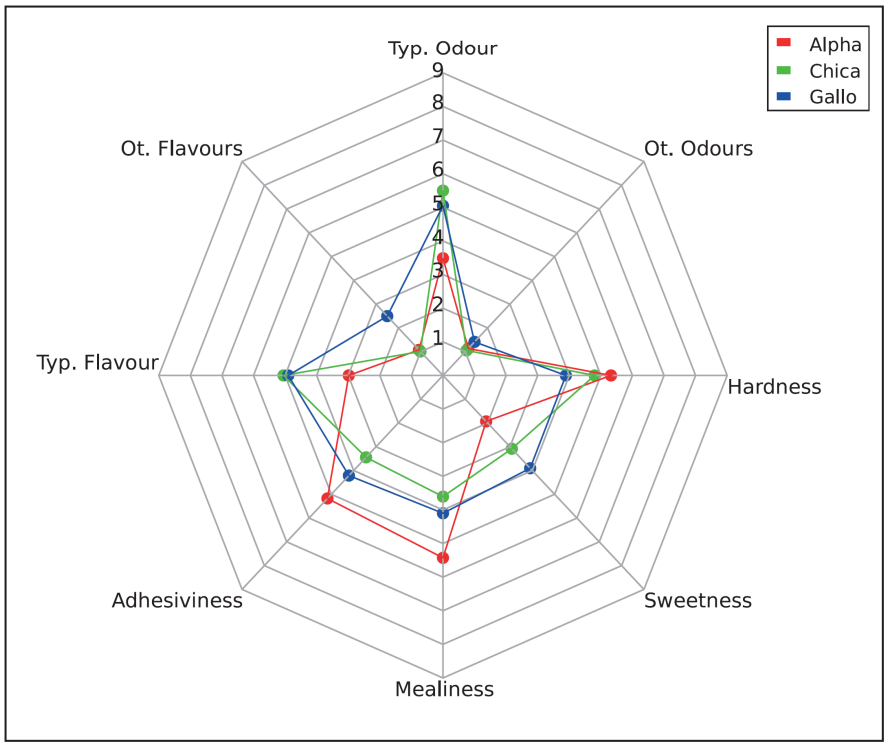
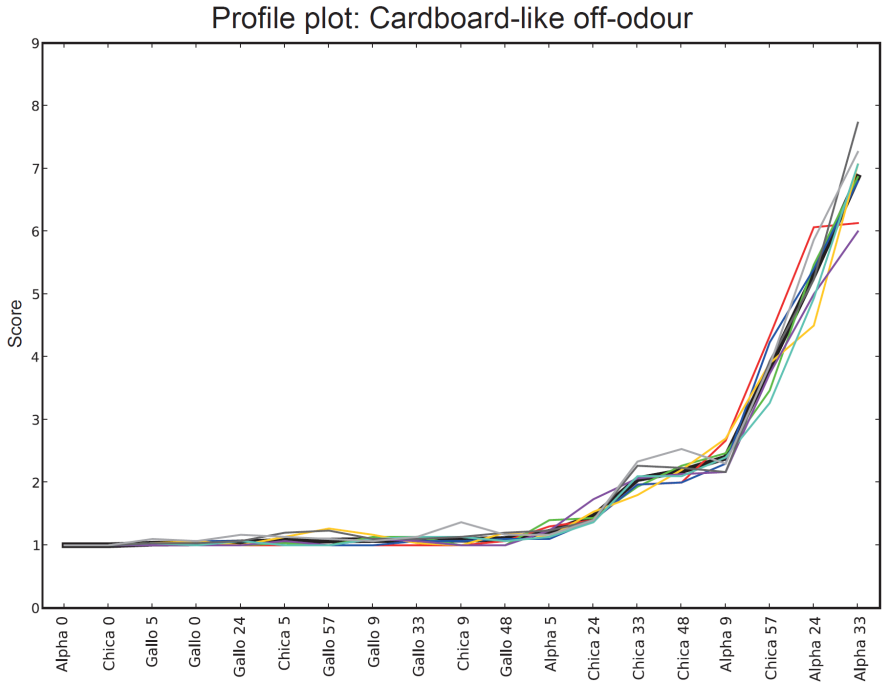
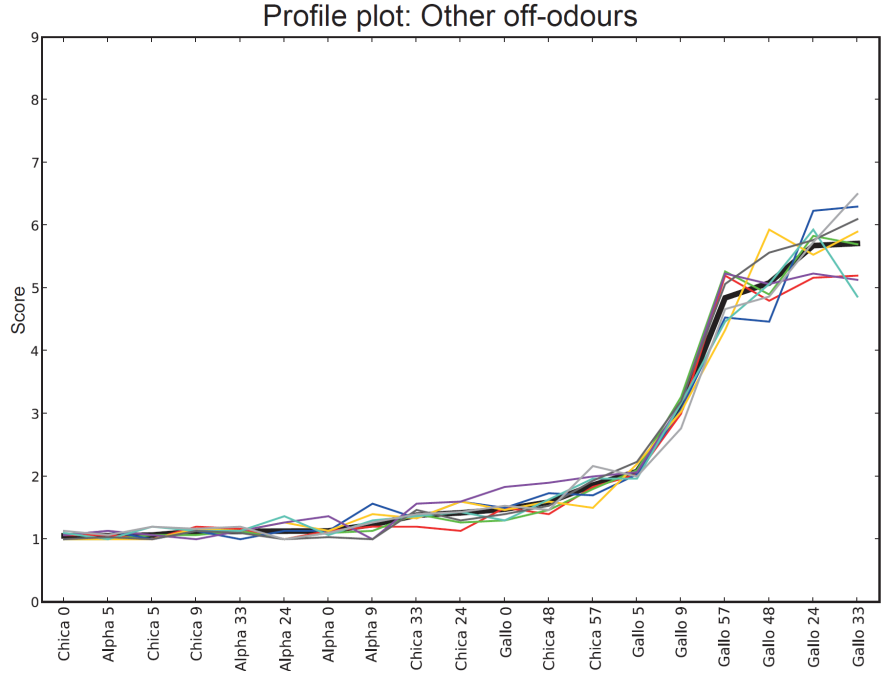


FIG. 7. PROFILE PLOTS OF CARDBOARD-LIKE AND OTHER OFF-ODORS. ON THE VERTICAL AXES ARE REPORTED THE INTENSITY SCORES; ON THE HORIZONTAL AXES ARE INDICATED THE THREE SAMPLES TESTED, SORTED BY INTENSITY BASED ON CONSENSUS





TABLES

TABLE 1.

MODIFIED ATTRIBUTES (BLANDA *ET AL.* 2010) USED FOR PANEL TRAINING AND DURING QUANTITATIVE DESCRIPTIVE ANALYSIS OF BOILED POTATO SLICES. IN ITALICS ARE REPORTED NEW ATTRIBUTES ADDED

Attribute	Definition	Reference employed to train the panel
Typical odor	Typical fragrance or aroma of boiled potatoes perceived by sniffing the sample (Lawless and Heymann 1998)	Fresh boiled potato slices (cv. Chica)
Other odors	*Fragrances of boiled potatoes, different from typical aromas but not related to product deterioration*	*Not employed*
Cardboard-like off-odor	Defected odor, characteristic of oxidized milk, perceived by sniffing boiled potato slices (Amerine *et al.* 1965)	Boiled potatoes (cv. Marabel) stored for different times in refrigerated conditions (4°C)
Other off-odors	Other atypical odors perceived by sniffing boiled potato samples (Blanda *et al.* 2010)	Not employed
Hardness	Force required dividing potato slices in two parts by the front teeth (Thygesen *et al.* 2001)	Potatoes (cv. Chica) at different cooking degree
Mealiness	How mealy/crumbly the potato is felt in mouth after chewing (Thygesen *et al.* 2001)	Not employed
Adhesiveness	Force required removing the potato sticking from teeth and palate after chewing (Thygesen *et al.* 2001)	Not employed
Sweetness	Sweet taste perceived during chewing of boiled potatoes slices (Blanda *et al.* 2010)	Potatoes (cv. Alpha) cooked in sucrose solutions at different concentrations
Typical flavor	Typical boiled potato retronasal smell originated in the mouth via transportation of the stimulus molecules up to the back of the nasopharynx and into the region of the olfactory receptors (Lawless and Heymann 1998)	Fresh boiled potato slices (cvs Alpha, Chica and Gallo)
Other flavors	*Other retronasal odor perceived after the deglutition of boiled potatoes, different from the typical ones but not related to product deterioration*	*Not employed*
Cardboard-like off-flavor	Atypical retronasal odor, similar to the characteristic off-odor of oxidized milk, perceived after deglutition of boiled potato slices (Amerine *et al.* 1965)	Potatoes (cv. Marabel) at different ageing degree
Other off-flavors	Other atypical retronasal odors perceived after deglutition of boiled potato slices (Blanda *et al.* 2010)	Not employed

TABLE 2.
SENSORY EVALUATION PLANNING OF BOILED POTATO SLICES, FROM THE THREE MEXICAN CULTIVARS

Sensory test	Storage time (h)						
	0	5	9	24	33	48	57
Complete QDA	A, C, G	A, C, G	A, C, G	-	-	-	-
Olfactory analysis	-	-	-	A, C, G	C, G	C, G	C, G

Abbreviations: A = cv. Alpha; C = cv. Chica; G = cv. Gallo.

TABLE 3.
F, *P* AND MSE VALUES OF CARDBOARD-LIKE OFF-ODOR AND OTHER OFF-ODORS ATTRIBUTES

Assessors	Cardboard-like off-odor			Other off-odors		
	F value	*p* value	MSE value	*F* value	*p* value	MSE value
A1	86.66	0.000	0.10	49.95	0.000	0.16
A2	42.38	0.000	0.19	41.42	0.000	0.23
A3	61.68	0.000	0.14	44.75	0.000	0.21
A4	36.97	0.000	0.21	34.92	0.000	0.26
A5	28.07	0.000	0.23	49.56	0.000	0.16
A6	70.71	0.000	0.11	32.35	0.000	0.24
A7	52.45	0.000	0.18	31.96	0.000	0.32
A8	62.72	0.000	0.15	40.92	0.000	0.23

The significant level of *F* values at 1% and 5% were respectively 2.45 and 1.89.

TABLE 4.
CARDBOARD-LIKE OFF-ODOR DEVELOPMENT DURING REFRIGERATED STORAGE OF BOILED POTATO SLICES

	Storage time (h)						
	0	5	9	24	33	48	57
Alpha	1.0 (0.0) d	1.2 (13.6) d	2.4 (19.8) c	5.3 (19.3) b	6.9 (12.6) a		
Chica	1.0 (0.0) d	1.1 (15.2) d	1.1 (14.9) d	1.5 (12.6) b	2.1 (17.2) c	2.2 (18.6) c	3.8 (18.0) a
Gallo	1.0 (6.1) a	1.0 (5.2) a	1.1 (10.8) a	1.0 (8.0) a	1.1 (11.4) a	1.1 (12.9) a	1.1 (12.3) a

Olfactory data reported are means of 24 values (8 judges and 3 replicates); in brackets CVr% are reported. Different letters in the same row indicate statistically significantly differences (Honestly Significant Differences or HSD by Tukey $p < 0.01$).

TABLE 5.

OTHER OFF-ODORS DEVELOPMENT DURING REFRIGERATED STORAGE OF BOILED POTATO SLICES

	Storage time (h)						
	0	5	9	24	33	48	57
Alpha	1.1 (17.4) a	1.1 (10.1) a	1.2 (18.8) a	1.1 (21.6) a	1.1 (16.6) a		
Chica	1.1 (9.3) c	1.1 (14.3) c	1.1 (16.2) c	1.4 (19.7) b	1.4 (20.8) b	1.6 (20.0) b	1.9 (21.3) a
Gallo	1.5 (19.4) e	2.1 (17.2) d	3.1 (15.2) c	5.7 (15.2) a	5.7 (15.1) a	5.1 (14.8) b	4.8 (15.7) b

Olfactory analysis results reported are means of 24 values (8 judges and 3 replicates); in brackets CVr% are reported. Different letters in the same row indicate statistically significantly differences (Honestly Significant Differences or HSD by Tukey $p < 0.01$).

5.3 DEVELOPMENT OF A SENSORY LEXICON

The topic of sensory analysis has been deepen with the participation to the European project Ecropolis. This project deals with sensory properties of organic food and its main aim is to collect data about sensory profiles from organic products all over Europe in an interactive multilingual online database, called OSIS (Organic Sensory Information System). OSIS serves as a marketing tool providing sensory information of organic food for consumers, retailers/wholesalers and producers in an easy and traceable way.

The project started in 2009 and partners from France, Germany, Italy, Poland, Switzerland, and The Netherlands are involved in the research activity. To reach the overall goal and objectives of the project and to ensure an efficient and well-timed implementation, the ECROPOLIS project has been divided into six work packages (WP1-6) and one management cluster.

In particular the WP1 produces the necessary information about previous research on sensory characteristics of organic food, the regulatory framework, operators' market needs regarding sensory characteristics, and consumer expectations, perceptions and attitudes about sensory characteristics of organic food. One of the activities of WP1 was the drafting of a sensory glossary including general definitions and specific terms related to organic products. The applicant participated to the sensory glossary writing, that will be published on the Ecropolis website (www.ecropolis.eu). The bibliographic sources used to write the sensory glossary have been published on Aigaion, a shared database of the literature about consumers' expectations, marketing and sensory issues (www.deiagra.unibo.it/ecropolis).

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6. CONCLUSIONS

In the first part of this research project innovative food process technologies have been studied, through laboratory scale systems (ultrasound and *vacuum* technologies) and semi-industrial pilot plants (pulsed electric field).

Ultrasound has been found to be a valuable technique to improve the freezing process of potatoes, anticipating the beginning of the nucleation process, mainly when applied during the supercooling phase. The shorter freezing time obtained thanks to sonication may cause an improving of sensory and texture properties during thawing and cooking. However, in order to obtain such results an efficient removal of heat developed by sonication is required.

Another technique that can be applied to preserve fruit and vegetable liquid food, assuring microbiological safety and preserving their sensory properties is pulsed electric field. In this research project a study of the effects of pulsed electric fields on phenol and enzymatic profile of melon juice has been realized. The statistical treatment of data was carried out through a response surface method, and the experimental design chosen was the central composite design.

Next, impregnation of apple sticks with aroma was investigated. Flavour enrichment has been realized applying different techniques, as atmospheric, *vacuum*, ultrasound technologies and their combinations. The use of the obtained enriched products as ingredient in industrial preparations or as ready-to-eat food has been proposed.

The second section of the thesis deals with the development of analytical methods for the discrimination and quantification of phenol compounds in vegetable matrix, as chestnut bark extracts and olive mill waste water. With reference to the second application, the management of waste disposal in mill sector has been approached with the aim of reducing the amount of waste generated, producing water to be re-used, and at the same time recovering valuable by-products, as phenol concentrates, to be used in different industrial sectors. In particular filtration systems, based on ultrafiltration and reverse osmosis modules have been proposed for the treatment of olive mill waste water.

Finally, the sensory analysis of boiled potatoes has been carried out through the development of a quantitative descriptive procedure for the study and the comparison of Italian and Mexican potato varieties.

An update on flavour development in fresh and cooked potatoes has been realized, through the revision of the scientific literature and the redaction of a mini-review. Last, a

sensory glossary including general and specific definitions related to organic products, used in the European project Ecropolis, has been drafted.