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Quali-quantitative study of the phenolic and polyphenolic compounds and their antioxidant capacity in vegetal matrix processed with different technologies

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1. Preface

En primer lugar, quiero agradecer Hugo y Aurora para invitarme a compartir trabajo y vida con ellos. El respeto que me han demostrado es algo que non olvidarè nunca.

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2. Summary

Food technologies today mean reducing agricultural food waste, improvement of food security, enhancement of food sensory properties, enlargement of food market and food economies. Food technologists must be high-skilled technicians with good scientific knowledge of food hygiene, food chemistry, industrial technologies and food engineering, sensory evaluation experience and analytical chemistry. Their role is to apply the modern vision of science in the field of human nutrition, rising up knowledge in food science.

The present PhD project starts with the aim of studying and improving frozen fruits quality. Freezing process in very powerful in preserve initial raw material characteristics, but pre-treatment before the freezing process are necessary to improve quality, in particular to improve texture and enzymatic activity of frozen foods. Osmotic Dehydration (OD) and Vacuum Impregnation (VI), are useful techniques to modify fruits and vegetables composition and prepare them to freezing process. These techniques permit to introduce cryo-protective agent into the food matrices, without significant changes of the original structure, but cause a slight leaching of important intrinsic compounds. Phenolic and polyphenolic compounds for example in apples and nectarines treated with hypertonic solutions are slightly decreased, but the effect of concentration due to water removal driven out from the osmotic gradient, cause a final content of phenolic compounds similar to that of the raw material. In many experiment, a very important change in fruit composition regard the aroma profile. This occur in strawberries osmo-dehydrated under vacuum condition or under atmospheric pressure condition. The increment of some volatiles, probably due to fermentative metabolism induced by the osmotic stress of hypertonic treatment, induce a sensory profile modification of frozen fruits, that in some way result in a better acceptability of consumer, that prefer treated frozen fruits to untreated frozen fruits. Among different processes used, a very interesting result was obtained with the application of a osmotic pre-treatment driven out at refrigerated temperature for long time. The final quality of frozen strawberries was very high and a peculiar increment of phenolic profile was detected. This interesting phenomenon was probably due to induction of phenolic biological synthesis (for example as reaction to osmotic stress), or to hydrolysis of polymeric phenolic compounds. Aside this investigation in the cryo-stabilization and dehydrofreezing of fruits, deeper investigation in VI techniques were carried out, as studies of changes in vacuum impregnated prickly pear texture, and in use of VI and ultrasound (US) in aroma enrichment of fruit pieces. Moreover, to develop sensory evaluation tools

and analytical chemistry determination (of volatiles and phenolic compounds), some researches were bring off and published in these fields. Specifically dealing with off-flavour development during storage of boiled potato, and capillary zonal electrophoresis (CZE) and high performance liquid chromatography (HPLC) determination of phenolic compounds.

2.Table of Contents

3. List of Abbreviations

OD Osmotic Dehydration

VI Vacuum Impregnation

US Ultrasound

CZE zonal electrophoresis

HPLC high performance liquid chromatography

a_w water activity

T_u eutectic point

T'_g glass transition temperature

T'_m end point of freezing

T_g glass transition temperature of solid,

T'_{gw} glass transition temperature of water

T'_m end point of freezing curve

X'_s solid mass fraction at T'm or (T'g)

ICF Immersion chilling freezing

HDM Hydrodynamic mechanism

DRP deformation and relaxation phenomena

PPO polyphenol oxidase

ABTS+ 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation

TEAC Trolox Equivalent Antioxidant Capacity

PVOD vacuum osmotic dehydration

QDA quantitative descriptive Analysis

Fmax maximum force (N)

Fav average force (N)

Wp, work required to penetrate the samples (Nm*m)

PAC physiologically active compounds

EMD electromigration dispersion

POF potato off-flavor

4. List of Publications

The thesis is based on the experimental works reported in the following nine publications, referred to in the text by **paper 1-9**.

Paper 1 G. Blanda, L. Cerretani, A. Bendini, A. Cardinali, G. Lercker. **"Phenolic Content and Antioxidant Capacity vs Consumer Acceptance of Soaked and Vacuum Impregnated Frozen Nectarines"** Eur. Food Res. Technol. **227**:191–197 (2008) doi: 10.1007/s00217-007-0709-6

Paper 2 G. Blanda, L. Cerretani, A. Bendini, A. Cardinali, A. Scarpellini, G. Lercker. **"Effect of Vacuum Impregnation on the Phenolic Content of Granny Smith and Stark Delicious Frozen Apple cvv."** Eur. Food Res. Technol. **226**: 1229–1237 (2008). doi: 10.1007/s00217-007-0624-x

Paper 3 G. Blanda, L. Cerretani, A. Cardinali, A. Bendini, G. Lercker. **"Effect of Frozen Storage on the Phenolic Content of Vacuum Impregnated Granny Smith and Stark Delicious Apple cvv."** Eur. Food Res. Technol. **227**:961-964 (2008) doi: 10.1007/s00217-007-0801-y

Paper 4 G. Blanda, L. Cerretani, A. Cardinali, S. Barbieri, A. Bendini and G. Lercker. **"Osmotic dehydrofreezing of strawberries: polyphenolic content, volatile profile and consumer acceptance"** LWT-Food Sci. Technol. **42**: 30–36 (2009) doi:10.1016/j.lwt.2008.07.002.

Paper 5 G. Blanda, L. Cerretani, A. Cardinali, E. Boselli, A. Bendini. **"Mass transfer and phenolic profile of strawberries upon refrigerated osmodehydration"** CyTA- Journal of Food. In press (2009).

Paper 6 Valdez-Fragoso A, Soto-Caballero MC, Blanda G, Welti-Chanes J, Mujica-Paz H **"Firmness changes of impregnated whole peeled prickly pear"** J Text Stud. In press (2009).

Paper 7 P. Comandini, G. Blanda, H. Mújica Paz, A. Valdez Fragoso, T. Gallina Toschi. **"Impregnation techniques for aroma enrichment of apples: a preliminary study"** Proceedings 5th International Technical Symposium on Food Processing, Monitoring Technology in Bioprocesses and Food Quality Management, pp. 116 – 121 (2009).

Paper 8 P. Comandini, G. Blanda, A. Cardinali, L. Cerretani, A. Bendini, M.F. Caboni. **"CZE separation of strawberry anthocyanins with acidic buffer and comparison with HPLC".** J. Sep. Scie, **31**: 3257 – 3264 (2008).

Paper 9 G. Blanda , L. Cerretani , P. Comandini, T. Gallina Toschi, G. Lercker. **"Investigation of off-odour and off-flavour development in boiled potatoes"** Food Chemistry **118**: (2010) 283–290

Other publications realized during the PhD course, not discussed in the present thesis:

I. European patent IT/02.04.07/ITA BO20070235. Title: **"Method for preparing cut potatoes fit for cooking and potatoes obtained thereby"**. Date of filing: 31.03.08.

II. G. Blanda, A. Scarpellini, L. Cerretani, A. Cardinali, A. Bendini, G. Lercker. **"Formazione di un panel per l'analisi sensoriale di frutta fresca e trasformata. Utilizzo di uno**

strumento per la visualizzazione della prestazione del panel e degli assaggiatori" Ingredienti Alimentari, anno 6, numero 32, 12-19 (2007).

III. G. Blanda, S. Barbieri, A. Bendini, L. Cerretani, G. Lercker. **"Study of the variation of the phenolic and polyphenolic content and of the antioxidant capacity of extracts obtained from osmotically pre-treated and frozen fruits"** Progr. Nutr. **10**: 153-158 (2008).

IV. M.C. Soto-Caballero, R. García-Gonzáles, G. Blanda, A. Valdez-Fragoso, H. Mujica-Paz. **"Efecto de la aplicación de vacío sobre la actividad de pectinmetilesterasa en sistemas modelo**". In Proc.s of the XXX Encuentro Nacional de la AMIDIQ, Mazatlán, Sinaloa, May 19-22 (2009).

V. M.C. Soto-Caballero, R.R. Gómez-Gonzáles, G. Blanda, H. Mujica-Paz, A. Valdez-Fragoso **"Activation of pectin methylesterase by vacuum pulses in model system"** In Proc.s of the 3rd International Congress on Food and Nutrition, Antalya, Turquey, April 2009.

VI. Soto-Caballero MC, Valdez-Fragoso A, Batista-Ochoa M, Blanda G, Mujica-Paz H (2009) **"Dehydration-impregnation of whole quinces by concentration and pressure gradients"**. In Proc.s of the 3rd International Congress on Food and Nutrition, Antalya, Turquey, April 2009.

VII. Comandini Patrizia, Blanda Giampaolo, Soto Caballero Mayra Cristina, Gallina Toschi Tullia. **"Surgelazione degli alimenti tramite sonocristallizzazione: principi e potenzialità. Atti del VII Convegno AISSA "Agricoltura, Qualità dell'Ambiente e Salute"** - ANCONA 2-4 dicembre 2009 (VIII Sessione Poster: Valorizzazione delle produzioni alimentari), pp. 105-106 (2009).

VIII. G. Blanda. **"Quali-quantitative study of the phenolic and polyphenolic compounds and their antioxidant capacity in vegetal matrix processed with different technologies**" In Proc.s of the 12th Workshop on the Development in the Italian PhD Research on Food Science and Technology, Reggio Calabria 12-14 September, 2007.

IX. G. Blanda. **"Osmotic Dehydrofreezing Applications: Effects on Polyphenolic Profile and Sensory Characteristics of Fruits"** In Proc.s of the 13th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Turin, 10-12 September, 2008.

X. G.Blanda. **"Fruit and vegetables technologies and their influence on product quality"** In Proc.s of the 14th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Sassari, 16-18 September, 2009.

5. Introduction

The last one hundred years have seen a rapid increase in world population due to medical advances and massive increase in agricultural productivity made possible by the Green Revolution. Although in some countries like in Eastern Europe the population growth rate is slowing down rapidly, mainly due to low fertility rates and emigration, the world population growth is exponential as shown in figure 5.A.

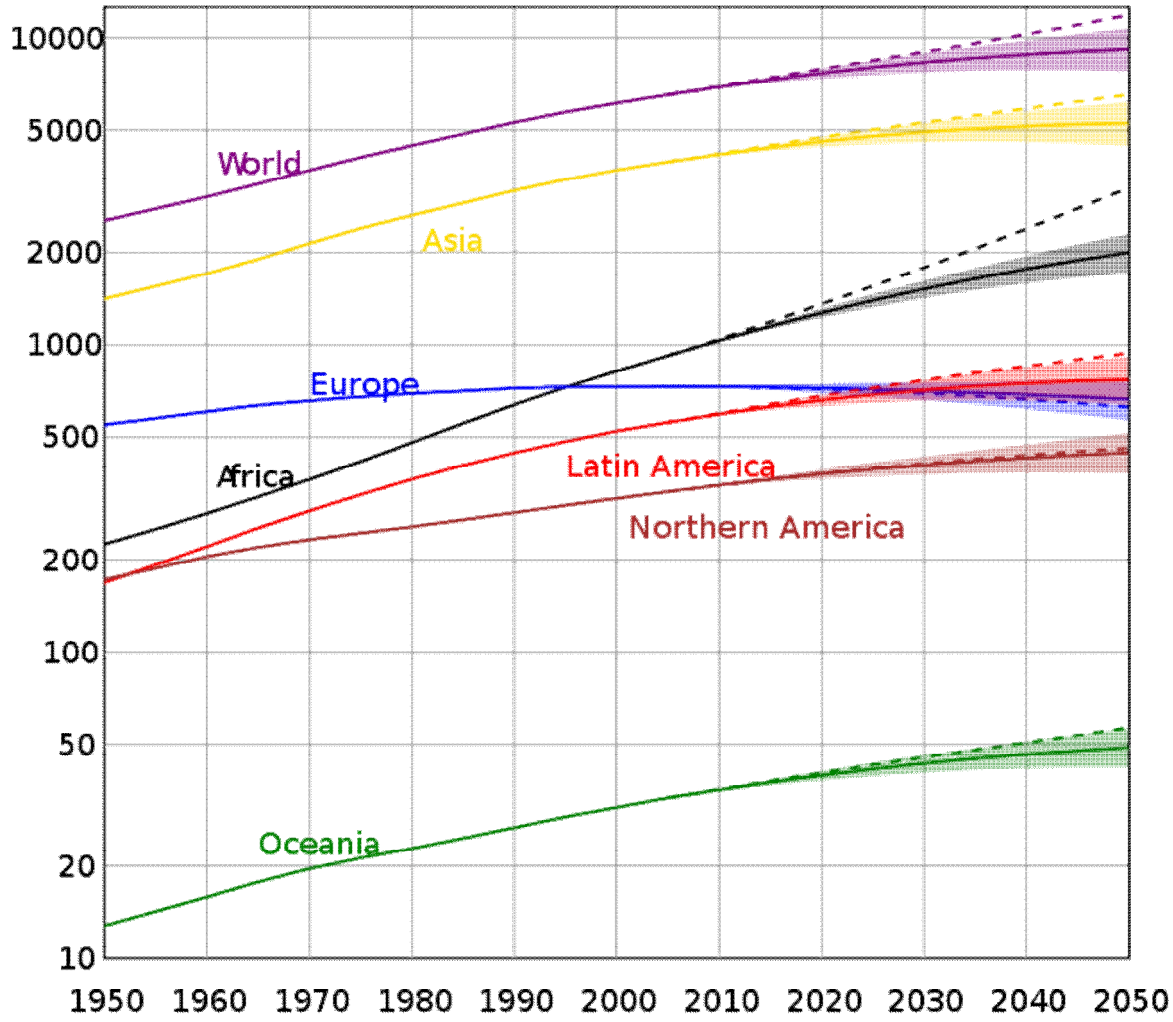

Figure 5.A. Population of the world and its regions (in millions). Data from [1]. Solid line: medium variant. Shaded region: low to high variant. Dashed line: constant-fertility variant. Y-axis is logarithmic scale.

World food production per person has actually been steadily increasing. This means that we have more food per person today than we had decades ago. Then why are there more people hungry and starving today? It is simply because there is both a surplus and a shortage of food, surplus where there is no need for the food and shortage where there is acute need for it. This depends on the availability of land and resources (like water) in places where no need of foods is required [2]. Some people look at genetically modified organism as a

possible solution to the hunger problem in the third world, (a sort of “Second Green Revolution). Some other think that is more important, and easy to realize, to stop food waste in western countries. The primary goal should be to conserve the food we have so it can possibly be distributed. We need to have an efficient distribution plan, so what we produce on the farm eventually reaches the kitchen table. It is generally estimated that 25 to 30% of what we produce is lost in the post-harvest chain and never reaches the consumer’s pot. Food (whether an agricultural or animal product) is highly perishable. In spite of taking all precautions for post-harvest handling, it is impossible to keep all available food in its farm-fresh state. The only way to make it available for later use in distant markets is to convert it to a more stable form. Thus, the major emphasis of food processing is preservation or shelf-life extension by preventing undesirable changes in the wholesomeness, nutritive value, and sensory qualities. This is done by controlling chemical, biochemical, physiological, and microbiological activities. Various technique are used with this purpose:

- removal of heat
- addition of heat
- removal of moisture
- controlling water activity
- addition of preservatives, sugars - salts - acids
- Advanced techniques (irradiation, use of light, pulsed electric field, high pressure, etc.)

Among these technologies, freezing process is a very potent tool to preserve original characteristic of initial raw material.

In the present PhD project the central works have regarded the improvement of sensory and nutritional (polyphenolic content) quality of frozen fruits with the help of some pre-treatment techniques.

5.1 Freezing: positive and negative aspects

Freezing is the unit operation in which the temperature of a food is reduced below its freezing point and a proportion of the water undergoes a change in state to form ice crystals. The immobilization of water to ice and the resulting concentration of dissolved solutes in unfrozen water lower the water activity (a_w). Preservation is achieved by a combination of low temperatures, reduced water activity and, in some foods, pre-treatment by blanching [4]. At -10°C no bacterial growth is possible, and below -18°C no mould or yeast growth is possible. So, the main advantage of freezing is the possibility to stop any microbiological spoilage and to slow down chemical and biochemical reaction. In fact, enzymatic reaction in frozen foods are slowed down significantly, although they are not completely blocked.

As regards the main disadvantage of freezing it regards the volumetric expansion of water during the phase change: volume of ice is 9-10% greater than pure water. As we will see in the following paragraph, water in foods start freezing by initial nucleation and then by ice crystal growth. Ice crystal forming by initial water, expand and cause structure changes, by breaking cell wall, plasma lemma and organelle membranes. The extent of such an effect depends on the way the freezing process is carried on. This structure disruption is particularly evident during thawing down of foods, because enzyme and substrate come in contact and enzymatic reaction occur at very high extent. Food lost its texture and structure, and juice loss occur, in particular in vegetables matrix, where the presence of the cell wall, make the structure rigid, and the ice crystal damage more evident.

5.2 Theory of Freezing foods

5.2.1 Removal of heat and time/temperature diagram

During freezing, sensible heat is first removed to lower the temperature of a food to the freezing point. Most foods contain a large proportion of water, which has a high specific heat (4200 J $kg^{-1}K^{-1}$) and a high latent heat of crystallization (335 kJ kg^{-1}). A substantial amount of energy is therefore needed to remove latent heat, form ice crystals and hence to freeze foods. The latent heat of other components of the food (for example fats) must also be removed before they can solidify but in most foods these other components are present in smaller amounts and removal of a relatively small amount of heat is needed for crystallization to take place. If the temperature is monitored at the thermal centre of a food (the point that cools most slowly) as heat is removed, a characteristic curve is obtained (Fig. 5.B).

The six components of the curve are as follows.

AS - The food is cooled to below its freezing point *F* which, with the exception of pure water, is always below 0ºC. At point *S* the water remains liquid, although the temperature is below the freezing point. This phenomenon is known as super-cooling and may be as much as 10ºC below the freezing point.

SB - The temperature rises rapidly to the freezing point as ice crystals begin to form and latent heat of crystallization is released.

BC - Heat is removed from the food at the same rate as before, but it is latent heat being removed as ice forms and the temperature therefore remains almost constant. The freezing point is gradually depressed by the increase in solute concentration in the unfrozen liquor, and the temperature therefore falls slightly. It is during this stage that the major part of the ice is formed

CD - One of the solutes becomes supersaturated and crystallizes out. The latent heat of crystallization is released and the temperature rises to the eutectic temperature for that solute.

DE - Crystallization of water and solutes continues. The total time t_f taken (the freezing plateau) is determined by the rate at which heat is removed.

EF - The temperature of the ice–water mixture falls to the temperature of the freezer.

A proportion of the water remains unfrozen at the temperatures used in commercial freezing; the amount depends on the type and composition of the food and the temperature

of storage. For example at a storage temperature of 20ºC the percentage of water frozen is 88% in lamb, 91% in fish and 93% in egg albumin.

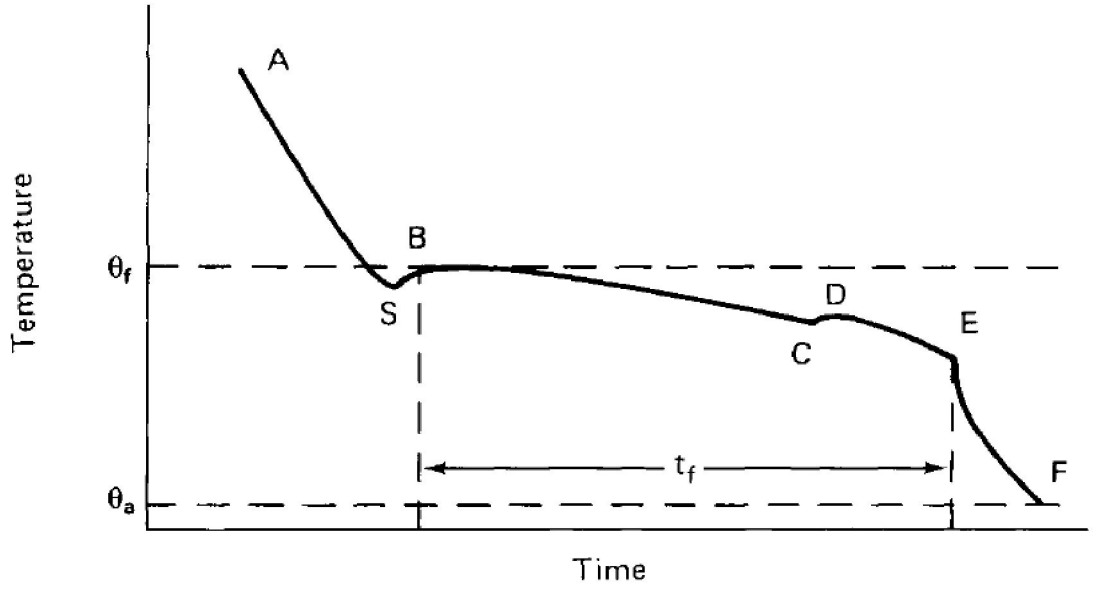

Figure 5.B. Example of Time temperature data during freezing. From [3]

The critical parameters to understand during the freezing process are nucleation, crystallization and ice crystal growth.

Nucleation is the organization of water molecules to form the first "seed" of a ice crystal. Nucleation of water molecules that organize themselves in a crystalline structure is called homogeneous nucleation. In foods it quite doesn't occur, while heterogeneous nucleation, that is the formation of a crystalline structure of water molecules in conjoint with other molecules such as solutes or colloids it's more common. Nucleation is a reversible process: when a nucleus form, if it doesn't reach a critical radius, it melt and doesn't form an ice crystal.

When the critical radius is reached, the initial nucleus begin to growth and form an ice crystal. This process is called **crystallization.** The size and shape of ice crystals depends on different variables such as the food matrix, food composition, freezing rate, etc. In general, the higher the number of initial nuclei, the smallest the ice crystal size in the frozen food, as the amount of water in the food is occupied in a large number of small crystal.

The extent of nucleation and **crystal growth** depend on the extent of super-cooling and the freezing temperature (as shown in figure 5.C). During rapid freezing, heat is removed rapidly from the food, and water molecules migration is negligible with respect with ice

nucleation. In slow freezing, little number of nuclei form, and water molecules migrate to enlarge the ice crystal, that at the end of the process are very big and tend to destroy more the structure.

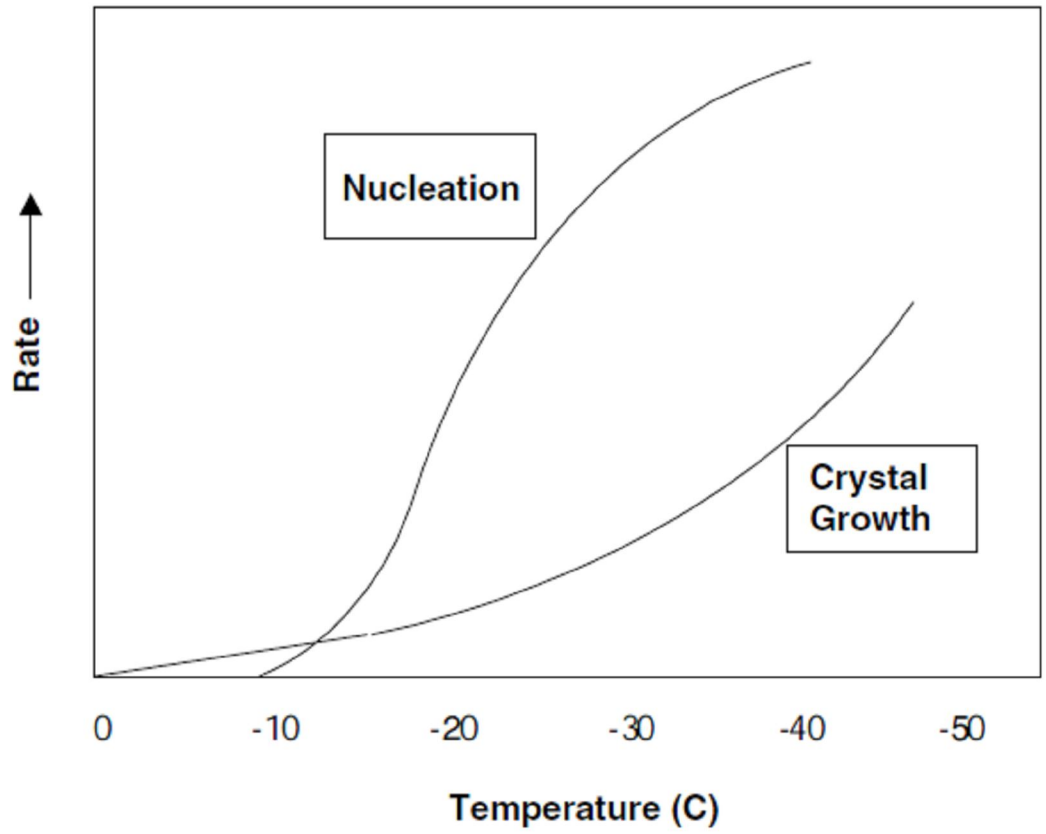

Figure 5.C. Influence of freezing temperature on the rate of nucleation and Crystal Growth. From [2]

5.2.2 State diagram of foods

Frozen foods is a complex matrix where different phases are present. In a simplified way a food could be assimilated to a solution. In figure 5.D. an example of phase diagram of a solution is given. When the food is cooled below its freezing point, two phases are present, the ice phase, and a liquid phase that concentrate more and more with the temperature falling down, as the water molecules pass to the ice phase. The liquid phase reach a very high degree of concentration, until eutectic point is reached, the point at which water and solutes crystallize at the same time. At that point the water remained in a liquid state is a ultra-concentrated solution, that has a high viscosity: this state is called rubber state. Lowering the temperature more and more a second order phase change occur (as described

by P. Ehrenfest) and the rubber phase change in a glassy phase. This transition is better known as glass transition, and is very interesting in understanding stability of frozen food.

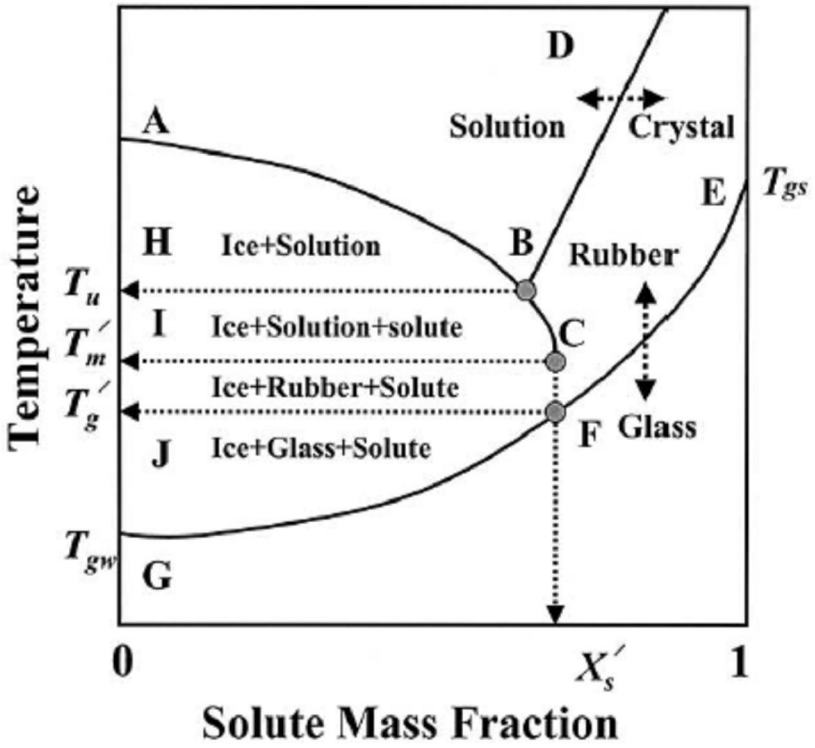

Fig 5.E. A typical state diagram for solution. From [4] ABC: freezing curve, BD: solubility curve, B:eutectic point (T_u), EFG: glass transition line, F:glass transition temperature at the end point of freeze concentration (T'_g), C: end point of freezing (T'_m), CF: line between T'_m and T'_g, T_g: glass transition temperature of solid, T'_{gw} glass transition temperature of water, T'_m: end point of freezing curve, T'_g glass transition at the end point of freezing, X'_s: solid mass fraction at T'_m or (T'_g).

In kinetic term, glass temperature is defined as the temperature ar wich the viscosity of a material reaches 10^{13}-10^{14} Pa*s and the molecular diffusion rate is in the orders of years. The state diagram is commonly used to identify different state boundaries in a material. A state diagram consist of the freezing curve, solubility curve, eutectic point, glass transition line, and condition of end point of freeze concentration (temperature and solid mass fraction). In the state diagram in fig 5.E, the freezing line (ABC) and solubility line (BD) are shown in relation to glass transition line (EFG). The point F (X'_s and T'_g) lower than T'_m (point C) is a characteristic transition in the state diagram. The water content at point F or C is the unfreezable water ($1-X'_s$). In the region AHB, the phases present are ice and solution. Below point B, first crystallization of solute occurs, thus HBCI region transform to three states ice, solution and solute crystal. There is no free water (i.e. able to form ice) exist below point C (T'_m, end point of freezing) and then the solution is transformed to

rubber state. The region ICFJ contains ice, rubber, and solute crystal. The point F is the T'_g, below this point portion of the rubber state is transformed to glass state, thus region JFG contain glass, ice, and solute crystal.

5.2.3 Freezing equipment and frozen storage

The different methods of freezing are generally grouped as:

1. Air freezing

2. Plate freezing

3. Liquid immersion freezing

4. Cryogenic freezing

In each method, a variety of different equipment of different designs, sizes, and modes are available in the marketplace. Some of these are discussed in this section. Air freezing, either by the use of cold air or using pre-cooled media, is by far the most common method employed. In the former, an electricity-based mechanical refrigeration system is used to provide cold air. The term “mechanical refrigeration” generally refers to any system that uses electrical power to produce chilled air. The chilled air is continuously passed over the food product, and in doing so, it removes heat. Mechanical freezing systems are characterized by a large capital investment, a significant ongoing preventive maintenance cost, and a sizeable permanent commitment of plant space. On the other hand, the resulting refrigeration is produced at a fraction of the consumable cost of cryogenic refrigeration. It is widely used technology and present in some form in virtually every food processing plant. In the latter technique, refrigeration is obtained as a pre-cooled substance, such as liquid nitrogen (LN_2) or liquid carbon dioxide (LCO_2), and maintained in storage tanks. Freezing systems that use either of these refrigerants are generally referred to as cryogenic freezing systems. Although, officially, the term “cryogenics” is applied to temperatures below –150°C°F, the term “cryogenic freezing” is widely used in food processing to identify freezing systems using either liquid nitrogen (–198°C) or carbon dioxide (–80°C as a solid). Liquid nitrogen or liquid carbon dioxide is purchased and kept in a pressurized storage vessel. The cryogen is piped as a liquid into the freezer unit and applied directly to the product in a variety of modes, depending on the cryogen, freezer type, or food product. However, the cryogen is a consumable product and, except in very unusual circumstances,

can only be used once. Cryogenic freezing systems are characterized by a moderate capital investment, minimal preventive maintenance costs, and a smaller and more flexible commitment of plant space. However, liquid nitrogen and liquid carbon dioxide pricing and availability vary, based on the geographic location of the processor, and the cost of refrigeration purchased this way can be more than triple the cost of mechanically produced refrigeration.

Frozen foods characteristics are not blocked along years in the frozen state. Chemical and enzymatic reaction continue, although they are slowed down, depending on the storage temperature, and the concentration of unfrozen phase (besides to the rubber or glass state of this phase, that depends on food composition and temperature). So the stability of a frozen food depends on the intrinsic characteristics of the matrix and of pre-treatment used (for example blanching before freezing slow down enzymatic reaction during the frozen storage and during defrosting, along with the condition used during storage. In fact during storage, oscillation around the freezing temperature, for example -18°C cause the **re-crystallization** phenomenon. For example, if temperature rise above -18°C part of water present in the ice crystal melt. As ice crystal of different size are present, it may occur that small crystals melt and disappear completely. If the temperature comes back again to -18°C or below, the liquid water doesn't form new crystal, but it's thermodynamically favored to enjoy ice crystals already present in the food (molecules migrate where chemical potential is lower). This lead to the enlargement of ice crystal size with consequent damage of food structure. Another phenomenon occurring during storage is **freeze-burning**. It is similar to the re-crystallization, as it depends on the temperature oscillations. It is due to water sublimation from the food: Sublimation occurs as water passes directly from the solid state to the vapor state, or from the frozen food product into the atmosphere around the product. Moisture vapor in the atmosphere attempts to reach equilibrium with the materials within a room, as well as with the room itself. The temperature of the freezing coil is always lower than the air in the storage room, so ice will form and accumulate on the coil. Sublimation is a principal contribution to the formation of freezer burn. It increases oxygen contact with the food surface area. This increases oxidative reactions that irreversibly alter color, texture, and flavor. If a product is packed in tight-fitting, water- and vapor-proof material, evaporation cannot take place. The temperature of the packaging material will follow the temperature fluctuations in the room faster than the product itself. As the temperature is lowered, evaporation from the product will form ice on the inside of the packing material,

and when the temperature conditions are reversed, the ice will be deposited on the surface of the product. Glazing, dipping, or spraying a thin layer of ice on the surface of a frozen product helps to prevent drying [2].

5.3 Investigation in frozen foods

There are several possibilities to increase quality of frozen foods and to introduce on the market innovative frozen foods. Investigation in this field is very interesting, as the 17% of innovative product in the world belong to this category and is the second group after the confectionary group (43% of innovative products).

Investigation in freezing technology today means:

- Ultrasound assisted Freezing
- Pressure Freezing
- Use of anti-freeze protein
- Food pretreatment as Osmotic Dehydration (OD), Vacuum Impregnation (VI) or Immersion chilling Freezing (ICF).

5.3.1 Osmotic pre-treatment and vacuum impregnation in cryo-stabilization of food

As just seen, preservation of food by freezing is a good method of ensuring the long-term retention of original characteristics, in almost unchanged state, especially of perishable materials. Freezing of fruit results in various favorable effects with respect to the shelf life and availability throughout the year; nevertheless, various undesirable changes occur because of this process. Freezing destroys cell integrity and compartmentation, thereby increasing the opportunity of undesirable physical, chemical, and biochemical reactions (browning, texture changes, loss of flavor, etc.).

Pre-freezing treatments, selection of the optimum freezing rate, adequate packaging, correct and uniform storage temperature, and rate of subsequent thawing are crucial if the full benefits of food freezing have to be realized and the deteriorative reactions minimized.

Cryo-stabilization technology represents a conceptual approach to a practical industrial technology for the stabilization during processing and storage of frozen foods The key to cryo-protection lies in controlling the physical state of the freeze-concentrated amorphous matrix surrounding the ice crystals in a frozen system, where deteriorative reactions mainly occur. There are two possibilities for achieving an adequate food cryo-protection One is the

reduction in the water content of the product below the content of unfrozen water in the frozen product, allowing its complete vitrification. The technique is termed dehydrofreezing and the concentration step is generally realized by air drying, osmotic dehydration; or a combination of both. Another is the formulation of food with appropriate ingredients to elevate the T_g, thereby enhancing the product stability at the relative freezer temperature.

The specific role of some solutes in protecting cell membranes during cell water loss in drying or cryo-concentration during freezing has been demonstrated. Nevertheless, the possibility of introducing solutes into structured food such as fruit is not easily feasible. Vacuum impregnation technique can offer interesting prospects in developing pretreatments to modify (in a short time) the initial composition of porous fruits, introducing cryo-protective solutes and making them more suitable for resisting damages caused by the frozen-thawing processes. If the impregnation solution is hypertonic, cryo-stabilization is obtained by using the combination of vacuum impregnation and osmotic dehydration. In addition, some benefits from the reduction of the amount of oxygen inside food pores, such as a greater stability against some deteriorative reactions (browning and oxidations), can be obtained [5].

In this PhD thesis, the effectiveness of VI and OD in enhance frozen food quality is discussed (Chapter 6). Besides, as sensory evaluation and analytical tools have been developed to obtain this initial purpose, other works have been carried out to develop and optimize the cited tools.

6 Discussion

6.1 Vacuum Impregnation pre-treatment of frozen apples and nectarines

Osmotic processes prior to freezing are used to produce several kinds of foods that can be stored for lengthy periods, and after thawing, retain favorable texture, color and flavor [6-9]. Due to the mild processing conditions used, osmotic processes have minimal impact on the nutritional and sensory quality of foods [10-12]. Among the developments in osmotic treatments of foods, vacuum impregnation (VI) is a recent application in fruit processing. In fact, VI is a recent technology applied in the food industry and it is possible to rapidly introduce several types of solutions in the porous structure of foods using this technique. As a consequence of this mass transfer, improvements in the physical–chemical, nutritional and sensory properties of foods take place leading to significant advantages such as longer shelf-life, optimization of further processes, and the introduction of innovative products in the food market [13]. VI consists in the exchange of the gases occluded in open pores of the food matrix with treating solutions [14]. The impregnation of solutions in pores is dependent on the hydrodynamic mechanism (HDM) and the deformation and relaxation phenomena (DRP). In the former, the solution is drawn into the pores by capillarity, after gas expulsion during the vacuum step; in the latter, which is less effective than the former, gas expansion due to the pressure changes causes pore deformation and subsequent variation in volume of impregnated solution [15]. Generally, VI is carried out in two steps after immersion of the food matrix in treating solution. In the first step, vacuum pressure (30–120 Mbar) is imposed on the system for a short time (from a few minutes to 45 min), thus promoting the expansion and outflow of the internal gas in the product. The releasing of the gas fills the product pores with the native liquid. In the second step atmospheric pressure is restored and compression leads to a large reduction in volume of the remaining gas in the pores filling the porous structure with liquid [16]. VI permits the direct introduction of specific substances and/or ingredients into food pores, thus maintaining the raw food structure; this effect can be exploited as a pre-treatment step in several food technologies such as osmotic dehydration (OD), freezing, canning, frying, etc. [17-18]. Due to the low temperature applied during VI, the heat damage to tissues is avoided and the color, aroma, and taste of raw materials are highly preserved, especially in vegetables characterized by high porosity. As observed by several researchers [11, 19-20], after VI the polyphenoloxidase (PPO) activity in fruits decreases due to removal of oxygen from the

inner portions of the vegetable matrix. Moreover, as already mentioned, one advantage of VI consists in the possibility to introduce functional substances endowed with specific purposes into the food matrix, i.e., stabilizing agents, antioxidants, antibacterial, etc. In frozen fruits, impregnation with calcium salts can increase fruit texture through interaction with pectin, in order to avoid the tissue breakdown after thawing out [21]. Moreover, compounds with cryo-protective effects such as glycerol, fructose, sucrose and glucose can be introduced [13]. The viability of fruit and vegetables treated by VI depends on the volume of impregnated solution, which is also linked to fruit porosity. The effective porosity depends on many factors but in general apple has the highest porosity, followed by peach, melon and strawberry at similar levels; pear, prune and apricot have a lower effective porosity level [22]. In general, larger fruit porosity permits more effective vacuum treatment.

Fresh fruits contain nutritional and healthful constituents such as minerals, vitamins such as C, E and A, phytochemicals such as folates, glucosinolates, carotenoids, flavonoids and phenolic acids, in addition to lycopene, selenium, and dietary fibers. Recently, polyphenols have received increasing interest as they possess anti-inflammatory, anti-histaminic and anti-tumor activities, and also act as free radical scavengers by protecting against cardiovascular disease [23-26]

In **paper 1** VI was used to pre-treat frozen nectarine slices subsequently frozen. In peaches and nectarines a significant amount of phenolic substances are present [27-29]. Polyphenols are important minor compounds with well-known anti-tumoral and anti-inflammatory properties, and their radical scavenger activity may also prevent cardiovascular disease [25-26]. In vegetables since the PPO activity causes rapid depletion of the phenolic fraction, changes in polyphenols may, therefore, be used as a marker of the oxidative status of foods. The aim of the present investigation is to evaluate the consumer acceptance of VI, soaked and untreated frozen nectarine slices. In particular, a correlation between negative sensory attributes such as darkening, an ‘‘oxidized’’ taste and phenol oxidation and depletion during thawing was previously found. For these reasons, in addition to consumer acceptance, the phenolic fraction has been assessed by both spectrophotometric (*o*-diphenols content) and chromatographic (HPLC) analyses and the antiradical capacity ($ABTS^+$ assay) was also tested.

Table 1 and 2 of paper 1 show how a very short VI treatment could change significantly the composition of nectarines slices: dry matter and refractometric index of VI slices were higher with respect to soaked slice (SK). As the treating solution was composed of 40% fructose, 4%, calcium chloride dihydrate, 2% ascorbic acid (AA) and 0.4% sodium chloride, also the antioxidant capacity of fruits changed significantly (Trolox Equivalent Antioxidant Capacity, TEAC) in a way that VI samples, after defrosting, present a higher antioxidant capacity than initial fresh samples. This effect is due to the high AA content that is possible to introduce till the inner part of the slices, thanks to the hydrodynamic mechanism. This is the main factor that permit to the fruit to slow down phenolic oxidation, and to result very acceptable in the subsequent consumer test which results are shown in table3. In particular, VI samples are comparable with fresh samples as oxidation due to enzymatic reaction during thawing down, are slowed or blocked by ascorbic acid activity even into inside the food. In conclusion in this investigation, this effect was more evident in VI samples than SK samples, and VI was an effective technology for pre-treatment of fruit prior to freezing. Moreover, consumer acceptance of frozen fruit is strongly conditioned by oxidative status. In conclusion, phenolic content and antioxidant capacity appear to be good parameters for determining the acceptance level of frozen nectarine slices. In general, the higher the phenolic content, the higher the acceptance level of samples.

The effectiveness of VI techniques proved in paper 1, and the high protection obtained over the phenolic fraction lead to a study (**paper 2**) on apple polyphenolic fraction depletion after the VI + frozen process. Apples, are rich in phenolic compounds, which are of unquestionable importance due to their contribution to the color, taste and flavor characteristics of both apples and their derived products. In some cases, the major food sources of antioxidants are derived from tea, onions and apples [30]. The most important polyphenolic compounds present in apple are phenolic acids and flavonoids such as flavanols or catechins, flavonols, dihydrocalchones and anthocyanidins, while their relative content depends on several variables [31-33].

Using pulsed vacuum osmotic dehydration (PVOD), Peiró et al. [34-35] found a detectable loss of citric acid, ascorbic acid, galacturonic acid and other components from grapefruit and pineapple in an osmotic solution that had been reutilized. As reported by Andrés et al. [22], in addition to solute leaching, at the end of the vacuum step during VI processes, there is a loss of native liquid contained in the intercellular spaces (pores) due to internal gas expansion in many types of fruits. Moreover, in addition to mass fluxes, structural changes

in tissue such as cell alteration due to deformation and breakage of cellular elements associated with dehydration and gas–liquid exchanges also occur. All these phenomena provoke changes at many levels (reviewed in Chiralt and Talens [36]. With regards to chemical changes of osmotically treated foods, several studies have been performed that have focused mainly on the profile of volatile compounds. Modifications in the aromatic profile depend on osmotic process variables, such as temperature, solute type and concentration, pressure, and the solution/fruit ratio [37]. For example, osmotic treatment results in a greater retention of vitamin C and chlorophyll of frozen kiwifruit slices during storage at –10°C and strawberries treated with sugar solutions can also stabilize the anthocyanins content [38]. It must be considered that in industrial osmotic processes solutions are reused, so that their content in food components reaches equilibrium with the raw material, reducing the lixiviation process [39]. In reality, industrial solution (syrups) management requires frequent supplements and/or thermal treatment in order to maintain a gradient between the two phases. Thus, a more complex theory is necessary to accurately describe food modification during industrial osmotic processes [40]. All these modifications must be studied in order to minimize nutritional depletion of the raw material. To the best of our knowledge, few studies have been performed on modifications of the phenolic profile during osmotic treatment of apple. The primary aims of the present investigation was to evaluate changes in the amount of phenolics in frozen apple slices from two varieties (Granny Smith and Stark Delicious) following VI and to assess the sensory characteristics of slices from the two varieties after VI, cryo-freezing and thawing. Apple slices were analyzed for the phenolic fraction (and other chemical parameters) after the VI + freezing process, in order to understand how these can affect the phenolic fraction. Moreover a QDA (quantitative descriptive Analysis) was carried out on fresh and treated apple slices of the two varieties.

Results of phenolic compounds content in samples are reported both as fresh weight (table 2 of paper 2) and taking into account the concentration effect induced by the VI process lead in hypertonic solution (table 3 of paper 3). Vacuum impregnation prior to freezing preserved the sensory characteristics of apples, resulting in retention of texture and flavour. When compared to fresh raw material, Granny Smith presented a higher retention of texture parameters and was less affected by the VI + freezing process. Attention must be paid to the sweetness perception that increase significantly in treated samples, thus changing the final sensory characteristics of fruits. With regards to the behavior of phenolic compounds

after VI, we found a comparable depletion of more than 20% in Stark and 25% in Granny of total phenols. In reality the phenolic decrease was mostly due to procyanidins and only slightly due to hydroxycinnamic and dihydrochalcones. Notwithstanding, there were differences in the decrease of individual compounds. Indeed, in addition to leaching of solutes in the treatment solution, other reactions could take place such as hydrolysis of sugar residues or other consumption reactions (among which the most important is oxidation). In these experiments, oxidation of phenolics was prevented by using 1.0% ascorbic acid in the treatment solution. Nonetheless, it was noticed that the phenolic leaching occurring during the VI treatment is compensated by the concentration effect due to the loss of water at the end of the process. In fact, 100 g of VI frozen samples presents nearly the same content of antioxidants as 100 g of fresh sample.

In **paper 3** we have investigated the behavior of polyphenolic compounds in both untreated and vacuum impregnated frozen apple slices from two varieties (Granny Smith and Stark Delicious studied in paper 2) after 12 months of storage at -18°C. In table 1 results of phenolic compounds analysis are presented. It should be noted that a similar increase in total phenols occurred in VI samples in both Granny Smith and Stark Delicious varieties. In control samples there was a different behavior: in fact, control Granny Smith slices presented the highest increase in phenolics, while control Stark slices showed no such increase. The increase in total phenolics followed the same trend as the increase in flavan-3-ols in all samples. In particular (+)-catechin presented the greatest increase compared to (-)-epicatechin and other procyanidins. This could be explained by hypothesizing that hydrolysis of polymeric procyanidins occurred during the frozen storage. It is known that enzyme activity is critically impaired at -18 °C, although residual activity could be present in the unfrozen rubbery-state water fraction. Thus in frozen foods, both enzymatic and hydrolytic reactions could take place in this fraction. In particular lyases could convert polyglycosylated phenols to tri-di-mono glycosylated phenols or could liberate the aglycon moiety. In conclusion, during the frozen storage of apple slices there is an apparent increase in many phenolic substances, but this is likely due to hydrolysis of polymeric phenols. This increase seems to be independent of the VI treatment applied, and is better correlated with pH and titrimetric acidity values.

6.2. Osmotic dehydrofreezing and vacuum impregnation of strawberry with different techniques

Strawberries are very popular fruits available during the spring–summer period, although they are highly perishable and susceptible to bruises and fungal attacks. A good way to preserve strawberries (and fruits in general) is through the use of freezing technologies that combine low temperature and water activity (a_w) reduction associated with the cryoconcentration of the fruit liquid phase during ice crystal formation. However, because of the high freezable water content of strawberries, freezing leads to significant cellular damage, and several chemical–physical and organoleptical deteriorations take place, especially when fruits are thawed, with subsequent loss of product quality. **In paper 4**, we pre-treated strawberry fruits using different processes, and after freezing we evaluated polyphenolic content, volatile profile and consumer acceptance with respect to fresh raw material. Consumer acceptance was also assessed after thawing. The samples were treated as in the following:

-Fresh strawberries (*FR* sample): whole fruits were washed in tap water, drained and immediately analyzed.

- Fresh frozen strawberries (*TQ* sample): whole fruits were washed in tap water, drained and immediately frozen in a freezing chamber in direct contact with dry ice pellets. After 30 min, fruits were completely frozen and they were then stored in a conventional freezer at -18 °C for 1 month until analysis as described below.

- Immediately chilled-frozen strawberries (*ICF* sample): washed and drained fruits were submerged in a sucrose solution (69 g of sucrose in 100 g of solution) (which remained liquid at the temperature used) kept at constant temperature of -19°C for 24 h. The fruit:syrup ratio was 1:5 (w/w). At the end of the process, strawberries were accurately and rapidly washed with cold water and stored at -18°C in a conventional freezer for 1 month.

- Osmo-dehydrated strawberries (OD30 sample): whole fruits were washed in tap water, drained and placed in an impregnating chamber. Sucrose syrup (50 g of sucrose in 100 g of solution) was then added and fruits were kept submerged using a grid. The conditions were adapted from Escriche et al. (2000); briefly, the fruit:syrup ratio was 1:5 (w/w), and the syrup temperature was kept constant during the processing (30°C). Osmotic dehydration was continued for 4 h. At the end of the process, fruits were accurately drained from

residual syrup and quickly frozen as described previously. They were then stored for 1 month at -18 °C in a conventional freezer.

- Osmo-dehydrated strawberries (*OD5* sample): fruits were processed as sample *OD30*, but the process temperature was 5°C (kept constant during the processing in a controlled refrigerator) with a processing time of 24 h. At the end of the process, fruits were accurately drained from residual syrup, quickly frozen as described previously and stored for 1 month at -18°C in a conventional freezer.

Vacuum impregnated osmo-dehydrated strawberries (*VOD* sample): whole fruits were washed in tap water, drained and put in a vacuum chamber connected to a vacuum pump. Sucrose syrup (50 g of sucrose in 100 g of solution) was then added and fruits were kept submerged using a grid. Conditions used were adapted from Escriche et al. [11] the fruit:syrup ratio was 1:5 (w/w) and the syrup temperature was kept constant during processing (30°C). A vacuum step at 100 mbar was applied for 5 min. After this, osmotic dehydration was continued for 4 h. At the end of the process, fruits were accurately drained from residual syrup and quickly frozen as described and stored for 1 month at -18°C in a conventional freezer until analysis.

By examining data in table 3 (table have been reported below), the TP content in the *OD30* and *VOD* samples was lower than in the *TQ* samples. Thus demonstrating a significant decrease in polyphenolic compounds during osmotic processes primarily due to phenolic losses in the treating solution (in fact, the solution became pink-coloured during processing) In the case of sample *OD5*, due to the lower temperature applied during the process major phenolic retention may have occurred with respect with the other samples. This higher content may be in part due to the neo-formation of phenolic monomeric compounds from hydrolysis of polymerized substances as previously observed in apples, or could be related to induction of metabolic synthesis due to osmotic stress [41]. This effect was even more evident in *OD5* samples due to the long osmo-dehydrating time that permitted these chemical changes. In Table 4 (table have been reported below), the aroma profiles of samples are reported, and data are expressed as units of chromatogram area (ion abundance *vs* time). As observed by other authors in treated fruits [42-43], there was a strong increase in acetaldehyde and ethanol in treated samples due to the anaerobic processing conditions used. Similarly, other volatile compounds (e.g. compound 12) tended to drastically augment in osmotically treated samples. This effect cannot be explained by the concentration effect

just described, and probably depends either on metabolic pathways related to fermentation or on the transfer of these compounds from syrup to fruits. Other compounds (compounds 10, 13, 14, 19, 22) present in fresh samples tended to decrease or disappear altogether in treated or frozen samples (*ICF* and *TQ*). These changes positively affected the sensory acceptance level of fruit odour thus resulting in a high sensory acceptance level of osmodehydrated frozen samples with respect to untreated frozen samples. Among osmotic pre-treatments, the osmotic process conducted at 5°C (a temperature not usually applied in the classical range of osmotic dehydration treatment) was very interesting in terms of both sensory and chemical characteristics. In Table 5, the acceptance test data are reported.

The interesting results obtained in OD5 samples drive a deepen investigation in strawberry osmotic dehydration under refrigerated condition (**paper 5**). To provide insight into low temperature OD, whole strawberries were osmo-dehydrated at low temperature (5 °C) in a sucrose syrup (500 g/kg of solution) for different processing times (24, 48, 72, 96, and 120 h); after the treatment, water loss, solid gain, and weight reduction were recorded. Next, the osmo-dehydrated samples were frozen, and after a 5-month storage at 718 8C they were analyzed for drip loss, titrimetric acidity, refractometric index, pH, and sensory acceptance. From a technological point of view, it is important to underline the high quality of samples treated for 96 and 120 h, and that they may potentially be employed as ingredients in foods (such as frozen desserts and cakes) due to the very low drip loss values and high acceptance level. At the same time, the phenolic content of treated samples was very high and comparable to fresh strawberries, and thus the product could be considered as healthy as fresh strawberries (with obvious marketing implications). The low temperature process probably induces a protective mechanism in strawberries that permits improvements in their freezing performance. This hypothesis needs to be confirmed by more in-depth studies on the textural, sensory, and chemical characteristics of fruits. In the present study, we have provided an initial insight in the low temperature osmo-dehydrofreezing of whole strawberries. At the temperature used (5 °C), a few hours of treatment are not sufficient to obtain a substantial WL extent, thus making the technique relatively slow with respect to osmo-dehydration at higher temperatures. On the other hand, at low temperature the process has two major advantages: it permits a higher sensory acceptance level and a higher polyphenolic content with respect to osmo-dehydration at higher temperatures (as demonstrated in previous works) also with respect to the same fresh raw material. This last supposition is supported by the neo-synthesis of low molecular weight phenolic

compounds, probably induced by the combination of osmotic stress and low temperatures. The increase in the phenolic content of strawberries occurs in the first day of treatment, and

decreases slightly in subsequent days, when leaching in the osmotic solution prevailed. In any case, strawberries treated for 1–2 days at 5 °C presented a high acceptance level for consumers and a very high polyphenolic content (in particular anthocyanins), while strawberries treated for 3–5 days presented a reasonable acceptance level, very low drip loss and high phenolic content. Thus, the processing time influences the final quality of strawberries. The strawberries treated for 1–2 days are very suitable for direct consumption after thawing in substitution of fresh fruits, and samples treated for 2–5 days may have interesting applications as ingredients in the food industry.

Table 3 from paper 4. HPLC-DAD/MSD phenolic contents of fresh and processed strawberries. *a-e* different letters in the same row indicate statistically significantly different values (Honestly Significant Differences or HSD by Tukey $p<0.05$).

compound (mg/100 g)	FR3	FR4	TQ	ICF	OD30	OD5	VOD
1.Cyanidin 3-glucoside	0.089 (ab)	0.103 (ab)	0.076 (ab)	0.093 (ab)	0.056 (b)	0.118 (a)	0.063 (b)
2.Pelargonidin 3-glucoside	3.764 (a)	4.455 (a)	3.062 (a)	3.540 (a)	2.927 (a)	4.040 (a)	2.771 (a)
3.Pelargonidin 3-rutinoside	0.209 (ab)	0.250 (ab)	0.172 (ab)	0.211 (ab)	0.147 (b)	0.258 (a)	0.143 (b)
4.Unknown anthocyanin	0.015 (abc)	0.016 (ab)	0.009 (cd)	0.012 (abcd)	0.007 (d)	0.016 (a)	0.010 (bcd)
5.Unknown anthocyanin	0.528 (ab)	0.631 (a)	0.355 (b)	0.411 (ab)	0.347 (b)	0.438 (ab)	0.333 (b)
6.Pelargonidin 3-acetilglucoside	0.031 (a)	0.046 (a)	0.027 (a)	0.038 (a)	0.034 (a)	0.035 (a)	0.028 (a)
7.galloyl derivative	2.322 (b)	2.502 (b)	2.739 (b)	3.572 (b)	2.985 (b)	5.895 (a)	2.322 (b)
8.Caffeoyl glucose	0.062 (a)	0.066 (a)	0.090 (a)	0.079 (a)	0.081 (a)	0.103 (a)	0.072 (a)
9.p-coumaryl glucoside	0.403 (b)	0.533 (b)	0.564 (b)	0.595 (b)	0.369 (b)	1.005 (a)	0.370 (b)
10.Unknown compound	0.025 (a)	0.030 (a)	0.020 (a)	0.022 (a)	0.021 (a)	0.029 (a)	0.017 (a)
11.Unknown compound	0.067 (ab)	0.077 (ab)	0.057 (ab)	0.063 (ab)	0.048 (b)	0.086 (a)	0.044 (b)
12.Unknown compound	0.052 (ab)	0.055 (ab)	0.047 (b)	0.048 (b)	0.045 (b)	0.079 (a)	0.043 (b)
13.ellagic derivative	0.129 (a)	0.117 (a)	0.092 (a)	0.094 (a)	0.097 (a)	0.106 (a)	0.086 (a)
14.Quercetin 3-glucuronide + glucoside	0.654 (a)	0.523 (ab)	0.235 (de)	0.169 (e)	0.277 (de)	0.451 (bc)	0.334 (cd)
15.Ellagic acid	0.257 (a)	0.195 (a)	0.118 (a)	0.180 (a)	0.147 (a)	0.228 (a)	0.169 (a)
16.Unknown compound	0.035 (a)	0.039 (a)	0.022 (a)	0.026 (a)	0.019 (a)	0.029 (a)	0.022 (a)
17.Kaempferol 3-glucuronide	0.179 (a)	0.157 (a)	0.096 (b)	0.097 (b)	0.095 (b)	0.156 (a)	0.103 (b)
18.Kaempferol derivative	0.080 (ab)	0.083 (a)	0.051 (c)	0.059 (abc)	0.047 (c)	0.076 (ab)	0.053 (bc)
19.Kaempferol derivative	0.019 (a)	0.023 (a)	0.014 (a)	0.019 (a)	0.016 (a)	0.019 (a)	0.015 (a)
Total polyphenols	**8.920** (ab)	**9.898** (ab)	**7.846** (b)	**9.330** (ab)	**7.766** (b)	**13.167** (a)	**6.995** (b)
Total polyphenols (WR corrected)	**8.920** (ab)	**9.898** (ab)	**7.846** (b)	**9.330** (ab)	**7.029** (b)	**11.739** (a)	**6.432** (c)

Table 4 from paper 4. SPME-GC-MSD analysis of volatile compounds contents of fresh and processed strawberries. *a-c* different letters in the same row indicate statistically significantly different values (Honestly Significant Differences or HSD by Tukey $p<0.05$). Abbreviations used: ND – not detectable; NQ – not quantifiable. Values are expressed as peak area integration value (ion abundance x time)

N°	compound	FR	TQ	ICF	OD30	OD5	VOD
1	acetaldehyde	8.4 (b)	3.9 (b)	7.5 (b)	30.3 (a)	29.6 (a)	34.6 (a)
2	methyl acetate	52.0 (a)	43.3 (a)	29.8 (a)	30.1 (a)	14.1 (a)	43.9 (a)
3	ethyl acetate	5.3 (bc)	ND	NQ	148.7 (b)	352.3 (a)	99.2 (bc)
4	ethanol	ND	ND	ND	229.6 (a)	289.8 (a)	229.4 (a)
5	methyl butanoate	196.7 (b)	288.2 (ab)	408.1 (a)	180.9 (b)	195.6 (b)	154.2 (b)
6	ethyl butanoate	29.9 (b)	25.3 (b)	28.8 (b)	256.2 (ab)	342.5 (a)	132.1 (ab)
7	3-methylbuthyl acetate	ND	ND	ND	8.8 (a)	9.7 (a)	14.3 (a)
8	2-butenoic acid ethyl ester	ND	ND	ND	10.2 (ab)	19.0 (a)	5.4 (ab)
9	methyl hexanoate	50.1 (a)	26.5 (a)	52.0 (a)	27.1 (a)	19.7 (a)	22.9 (a)
10	buthyl butanoate	24.5 (a)	11.5 (b)	23.5 (a)	11.7 (b)	11.7 (b)	NQ
11	2-hexen-1-al	5.9 (c)	47.2 (ab)	13.2 (bc)	44.8 (abc)	42.5 (abc)	63.6 (a)
12	ethyl hexanoate	36.5 (c)	5.2 (c)	13.4 (c)	555.8 (a)	696.9 (a)	277.5 (b)
13	hexyl acetate	437.3 (a)	28.4 (b)	43.1 (b)	34.6 (b)	58.2 (b)	28.2 (b)
14	2-hexen-1-yl acetate	577.0 (a)	33.1 (bc)	52.2 (b)	12.1 (c)	27.8 (bc)	20.9 (bc)
15	6-methyl-5-hepten-2-one	ND	ND	10.6 (a)	4.5 (b)	5.6 (b)	NQ
16	ethyl-3-hexenoate	ND	ND	ND	3.1 (a)	3.7 (a)	ND
17	nonanal	7.0 (b)	13.7 (a)	10.3 (ab)	7.8 (ab)	7.6 (ab)	9.5 (ab)
18	2-hexen-1-ol	4.4 (a)	ND	4.5 (a)	ND	ND	ND
19	hexyl butanoate	57.8 (a)	4.4 (b)	3.8 (b)	ND	ND	ND
20	ethyl octanoate	ND	ND	3.1 (c)	22.5 (a)	27.7 (a)	12.0 (b)
21	octyl acetate	ND	ND	ND	9.9 (b)	35.8 (a)	5.9 (c)
22	2-hexen-1-yl butanoate	53.8 (a)	6.8 (b)	9.7 (b)	ND	ND	ND
23	2-ethyl-1-hexanol	2.5 (a)	ND	1.9 (a)	2.8 (a)	2.7 (a)	5.3 (a)
24	linalol	16.6 (a)	17.4 (a)	26.5 (a)	21.1 (a)	29.4 (a)	18.1 (a)
25	octanol	ND	ND	3.7 (b)	3.4 (b)	12.9 (a)	ND
26	octyl butanoate	11.2 (a)	3.1 (a)	10.7 (a)	8.7 (a)	8.7 (a)	ND
27	ethyl benzoate	ND	ND	ND	6.3 (a)	4.4 (a)	5.7 (a)
28	phenyl methyl acetate	5.7 (a)	3.5 (a)	6.2 (a)	5.7 (a)	11.8 (a)	4.6 (a)
29	alpha-farnesene	16.5 (a)	ND	ND	ND	ND	ND
30	hexanoic acid	9.9 (b)	15.4 (a)	21.5 (a)	2.6 (b)	19.2 (a)	NQ
31	ethyl 3-phenyl propenoate	ND	ND	ND	2.8 (a)	2.8 (a)	ND
32	gamma-decalactone	13.9 (b)	18.1 (b)	27.9 (ab)	15.9 (b)	57.5 (a)	5.9 (b)

6.3. Further (Vacuum Impregnation) investigation

Aside investigation in the use of VI in cryo-stabilization of frozen fruits, during the PhD project, some deepen studies on VI process were carried out. Some scientific poster were produced (publication IV, V and VI) that are not discussed in the present Thesis. The following two publication (paper 5 and 6), regards studies of VI effects on whole prickly pear texture and the use of VI in adding aroma compounds to apple stick.

An interesting application of Vacuum Impregnation is the treatment of whole fruits and vegetables, as pre-treatment for different processes. The group of Professors H. Mujica-Paz and A. Valdez-Fragoso published some original papers reporting condition of impregnation and osmotic dehydration of whole fruit and vegetables [44-45] and reached interesting results in accelerating the pickling of whole jalapeno peppers [46].

As fruit and vegetables are immersed in aqueous solutions, VI obviously cause changes in fruit and vegetables, due to the inter-changes of chemical compounds between vegetable cell and the external solution. About chemical composition we gave a first insight in paper 1-5 as regards nectarines, apple and strawberry. But some fruits undergo deepen changes in their texture profile, after a VI treatment. A limited number of studies have been carried out to evaluate the effect of vacuum pulse application on firmness of fruits and vegetables subjected to osmotic dehydration treatments [47-48].

Other works have been designed with the specific purpose of increasing tissue strength through immersion of samples in hypertonic or ISs containing calcium salts and a vacuum pulse application [49-51]. Among the few existing reports, there are studies regarding the effect of impregnation treatment with IS on the firmness of kiwi halves [52], mushrooms [53], whole jalapeño peppers [54] and apple slices [55]. With the increasing applications of vacuum pulse for minimal processing of fruits and vegetables [13], the assessment of firmness should be considered for evaluating the effect of these treatments on the quality of fruits and vegetables. Thus, the aim of the research of **paper 6** was to study the effect of impregnation parameters on firmness of green-skinned, whole peeled prickly pear (Opuntia ficus-indica) using a sucrose IS. Prickly pear is an oval elongated berry produced by the cactus Opuntia, which is native of Mexico. This fruit consists of a thick peel, covered with small thin spines, enclosing a sweet juicy pulp intermixed with many hard seeds. The

prickly pear contains about 84–87% water and 11–16% soluble solids. It has a titratable acidity of 0.015–0.049 g citric acid/100 g pulp and a pH of 6.5–7.5.

The firmness of fresh and impregnated peeled prickly pears was determined by puncture test using a texture analyzer TA-XT2 (Texture Technologies Corp., Scardale, NY). A2-mm-diameter stainless steel probe with a flat tip was driven radially into the whole peeled fruits at a speed of 10 mm/s at 11 different positions. The TAXT2 was set to automatically reverse the plunger travel direction when it reached the pre-established depth (30 mm). At the end of each test, the force–displacement curves, the maximum force (Fmax, N), the average force (Fav, N) and the work required to penetrate the samples (Wp, Nm*m) were recorded and used for the evaluation of the firmness. Two peeled whole prickly pears were tested for each impregnation condition, performing seven punctures at different locations on each fruit. The same procedure was used with fresh fruits.

The predicting models of Table 2 of paper 6 allowed plotting of the response surfaces for F_{av} (Fig. 3 of paper 6) and W_p (Fig. 4 of paper 6), at short (3 min) and long (60 min) vacuum application times. Under these conditions, similar graphs were obtained for F_{av} and W_p, but for each firmness parameter, significant differences can be noticed at the two t_v levels.

Whole prickly pear resulted as very sensitive to vacuum impregnation treatments. The firmness of impregnated prickly pear, mainly evaluated as mean average force and work of puncture, decreased significantly with relaxation time and vacuum pressure. The major change in firmness was observed after long vacuum application times. Favorable impregnation conditions cause firmness reduction of around 35%. The softening of impregnated prickly pear tissue was explained in terms of deformation-relaxation, pectin solubilization and calcium leaching phenomena. Further studies should be carried out to evaluate the effect of calcium salts in retaining the firmness of impregnated prickly pear.

Another application of VI have been reported in **paper 7**. In that research work, was evaluated the possibility to impregnate industrial aroma compounds into apple stick to enrich flavor perception. Modifying the flavor 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. The production of convenient, fresh and healthy foods has been widely investigated in the last few years and has included fortified foods with physiologically active compounds (PAC),

like probiotics [56], calcium [57; 58] or zinc salts [13]; osmo-dehydrofreezed fruits enriched with cryo-protectants [12; 59]; or also minimally processed foods incorporated with edible coatings to improve texture [60]. Such impregnation processes are usually performed under vacuum conditions or at atmospheric pressure.

Another technology which has gained a considerable interest in minimally processed food manufacturing is high-intensity ultrasound [61] which has been applied in several food processes [62]. Recently high power ultrasounds have been used to accelerate the mass transfer kinetics involved in osmotic dehydration [63]. The high intensity of the 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 transfers [64].

In this study the methodologies used for the manufacturing of functional food enriched with PAC, as vacuum and atmospheric impregnation, and the innovative technology of ultrasounds will be employed to promote the flavorings enrichment of apple sticks. This investigation will compare different impregnation techniques, such as impregnation at atmospheric pressure (AI), vacuum impregnation (VI), impregnation assisted by ultrasounds (USI) and the combination of vacuum plus ultrasound technologies (VUSI), with the aim of selecting the best procedure. Significant differences ($p<0.05$) were detected between treatments and times for green apple flavouring impregnation. As reported in Table 2 of paper 7, VI and VUSI gave the highest impregnation, compared to AI and USI, mainly at 5.0 min. USI treatments, instead, were not different from AI, for this reason it was supposed that the higher RFs obtained for VUSI were closely related to the vacuum effect. The higher volatiles impregnation obtained in VI and VUSI was due to the fraction of isotonic solution penetrated inside the apple sticks by hydrodynamic mechanism (HDM), that is the pressure gradient developed when the atmospheric pressure is restored. In fact, VI and VUSI samples after treatment had a weight variation of about 14%; on the other hand, no significant weight variations were detected for AI and USI samples. The RFs of the major components of green apple flavorings (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 of paper 7. The findings of the present investigation were 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, instead, was not significantly different from AI. Esters and alcohols had different impregnation behaviors: further studies are necessary to explain

such results, as well as the reduction of ethyl 2-methylbutanoate, 3-methylbutyl acetate and hexyl acetate after some min of treatment.

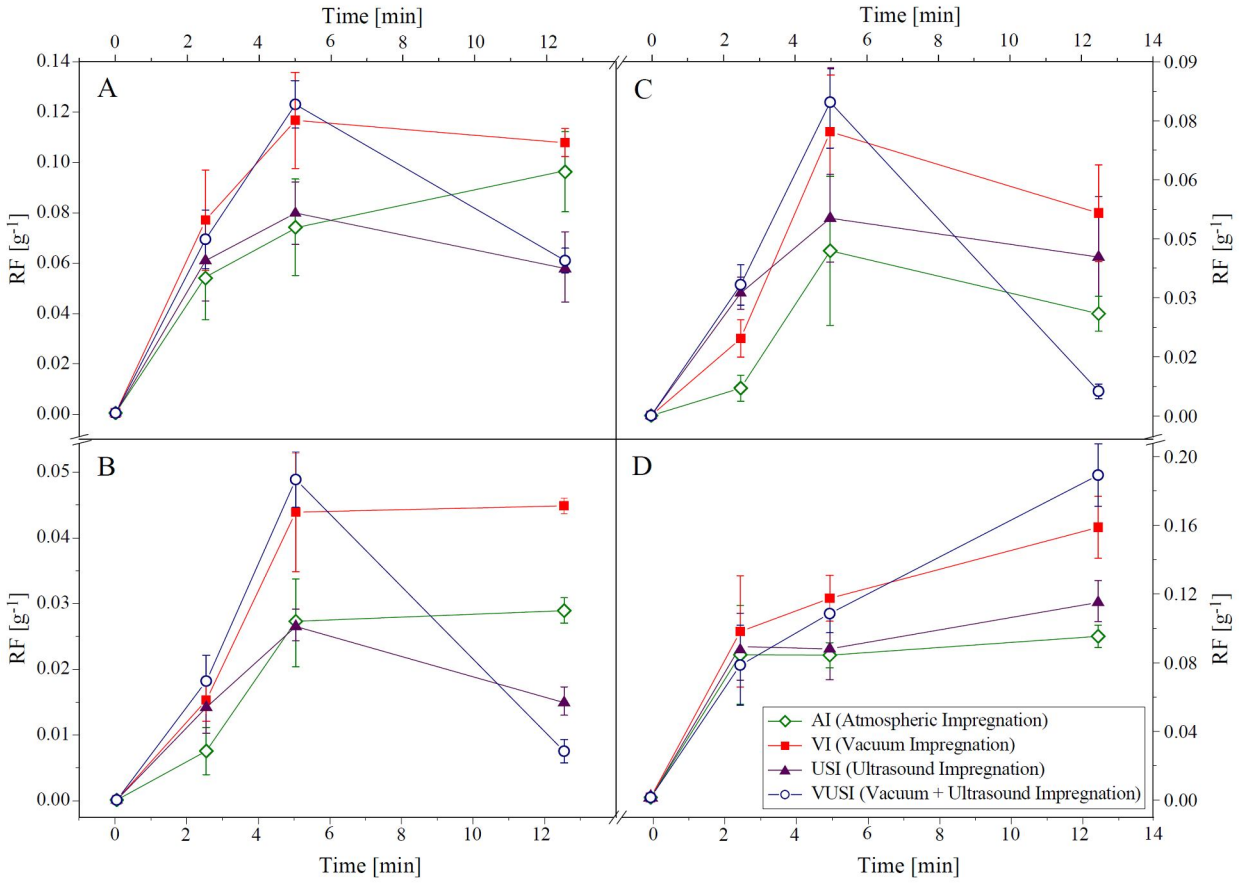

Fig. 2 Impregnation of ethyl 2-methylbutanoate (A), 3-methylbutyl acetate (B), hexyl acetate (C) and hexan-1-ol (D) at different relaxation times (2.5, 5.0 and 12.5 min).

6.4. Analytical and sensory tools development

To bring off the different research of the PhD project, different "tools" were developed:

- Different technological equipment to carry out VI, OD and ICF experiments
- Analytical methods (HPLC and CZE) for phenolic analysis in fruits adapting methods from literature;
- Analytical methods for aroma determination (SPME)
- Sensory evaluation protocols

These tools were pointed out with the valuable assistance of the high skilled investigation team of prof. Lercker and his assistants. In some cases, the researches produced were published as original papers.

In particular, in paper 8 we reported a study of HPLC *vs* CZE comparison of strawberry anthocyanins analyses. In paper 9 we use the SPME-MSD technique and we pointed out a sensory evaluation system for the study of boiled potato off-flavor development.

In **paper 8**, building upon the research of Da Costa et al. [65], who published a new method for the separation of anthocyanins of blackcurrants under acidic conditions, we have optimized a similar method using CZE for the separation of colored pigments in strawberry extracts. With this study it was our intention to reduce the retention time of anthocyanins and improve the efficiency by lowering electro migration dispersion (EMD) due to the strongly acidic conditions employed. The results of CZE analysis (migration time, efficiency, LOD, LOQ and solvent consumption) were compared with those obtained in HPLC adapting a method previously pointed out by our research group. In figure 5 and 6 of paper 8 electropherogram and chromatogram of the two method compared are reported. In table 3 of paper 8 results of comparison between the HPLC and HPCE method are reported.

In this investigation, a new electrophoretic separation technique was developed that allows the separation of the main anthocyanins from a matrix not previously analysed in acidic conditions, such as methanolic strawberry extracts. The optimised method presents the traditional benefits of CE analysis, such as high separation efficiency with a low consumption of solvents and samples; it also results in considerable reduction in analysis time of anthocyanins, with respect to that reported by Da Costa et al. [65]. However, the major volumes injected in HPLC and the different detector employed (diode array detector vs. single wavelength UV–Vis detector in HPCE) enabled to reach lower LOD and LOQ in HPLC, than HPCE. The results obtained denote the high potential of electrophoretic applications, although further studies are required in order to improve its quantification repeatability, and make CZE an effective alternative to HPLC, the technique traditionally employed in the separation of anthocyanins.

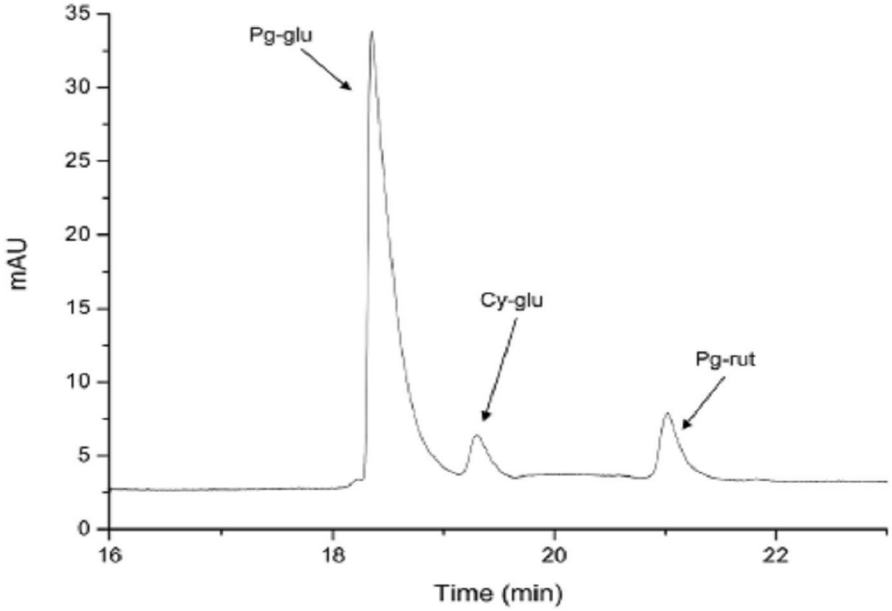

Figure 5. Electropherogram of a strawberry extract recorded with the optimised method. Electrophoretic conditions: fused-silica capillary 45 cm (id 50 μm); applied voltage 23 kV; capillary temperature: 25°C; injection time: 2 s; BGE: 250 mM monobasic sodium phosphate containing 30% v/v ACN and adjusted to pH 1.4 with *ortho*-phosphoric acid; detection at 280 nm with a UV–Vis detector. The peak at 12.5 min was not identified.

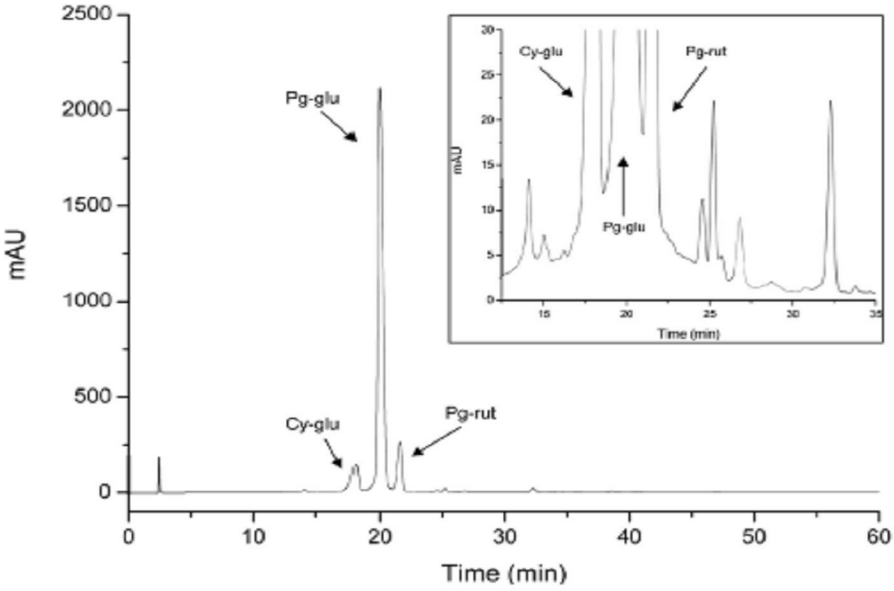

Figure 6. Chromatogram of a strawberry extract. The square on the right shows enlargement of the chromatographic trace between 10 and 35 min. Separation conditions: column C_{18} Luna 250 × 3 mm id, 5 μm particle size; mobile phase (A) 2.5% formic acid in water, (B) 2.5% formic acid in methanol; flow rate 0.5 mL/min; injection volume: 20 μL; detection performed at 510 nm.

Table 3. Sensitivity, analysis time and solvent consumption of HPCE and HPLC

	HPCE	HPLC
LOD (mg/L)	2.06	0.04
LOQ (mg/L)	6.87	0.13
Strawberry extracts A_t (min)[a)]	30	70
Sample volume analysed (nL)	1–50	20×10^3
Running buffer/mobile phase employed for 100 analysis (mL)	50	3500

a) Strawberry extracts A_t: analysis time of strawberry extracts plus system re-equilibration time.

The study presented in **paper 9**, 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. As reported by Petersen et al. [66], 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-flavors 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.

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-odors and off-flavors in boiled potatoes (POF), the effects of the use of food additives after cooking were examined. POF formation, analyzed 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-flavors, in contrast to sodium pyrophosphate. Potassium meta-bisulphite

prevented POF formation, but caused the creation of other off-flavors detected by a trained panel.

7. Conclusions and future broadening

The use of VI or OD as pre-treatment for frozen fruits of high sensory and nutritional quality demonstrate to be very useful. During these studies a very interesting field of application derived from paper 4 and 5, in which OD under refrigerated condition was used, resulting in the production of very high quality frozen strawberries. The matter is that at 5°C the economic cost derived from the long treating time begin to be very important. Other studies have been carried out but are not published yet, regarding the kinetics of OD at low temperature, eventually assisting the process with high agitation system, as ultrasounds waves. Very interesting in this field could be the use of OD at supercooling temperatures. In fact, as reported in some works [67]. It is possible to store some vegetables under their natural freezing point. The capacity of fruits to be impregnated by pressure changes in the VI process was strongly dependent on the fruit porosity. Apple and nectarines were very affected by the VI process, and it was possible to introduce inside their matrix, active compounds, as calcium chloride to enhance fruit texture, or ascorbic acid to avoid phenolic oxidation. What appear evident during the various experiments conducted was the dependence of volume of solution impregnated and fruit porosity, but the same fruit, for example apple, were not impregnated in the same way if their maturation stage was different. An investigation to better understand this observation is being conducted.

Freezing improvement is also be studied, using the ultrasound to assist the freezing process. Some preliminary result in this field seems to be interesting, and a first insight in ultrasound assisted freezing of potato is being to be published, thanks to the work carried out by Dr. Patrizia Comandini and Dr. Mayra Soto-Caballero.

As regard analytical determination, the aim of our work was to find rapid analysis for phenolic determination of fruits. In this direction, we pointed out an aqueous extraction process for the HPLC analysis, that permit to shorten the extraction time, avoidthe use of organic solvent and eliminate the drying step. This research will be soon submitted.

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ORIGINAL PAPER

Phenolic content and antioxidant capacity versus consumer acceptance of soaked and vacuum impregnated frozen nectarines

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Abstract Nectarines (*Prunus persica* L. cv. *Maria Laura*) were manually selected, cut in slices and divided into four groups: fresh, untreated frozen, soaked in osmotic solution and subsequently frozen, and vacuum impregnated (VI) and subsequently frozen. This investigation was focused on evaluation of consumer acceptance with respect to treated versus untreated frozen nectarine slices. In a preliminary acceptance test of untreated frozen nectarine slices, fruits were generally rejected on the basis of a darkened appearance and "oxidized" taste. These negative attributes were probably linked to the activity of polyphenol oxidase (PPO) and depletion of phenols due to cell rupture during freeze–thaw procedures. For these reasons, in order to evaluate the tendency of fruit to oxidation, several analyses were performed: the antioxidant capacity of phenolic fraction and the *o*-diphenol content were estimated by spectrophotometric assays, whereas the hydroxycinnamic acid (chlorogenic and neochlorogenic acids) composition was evaluated by high performance liquid chromatography (HPLC). Phenolic content and antioxidant capacity were found to correlate well with the acceptance level of frozen nectarine slices. In this regard a higher phenolic content associated with a higher acceptance level of nectarine samples.

Keywords Nectarine · Phenols · Vacuum impregnation · Freezing · Sensory evaluation

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Introduction

The main advantage of preserving fruits by freezing is the extended usage of frozen fruits during the off-season. Additionally, frozen fruits can be transported to remote markets that cannot be accessed with fresh fruit. The preservation of fruits by freezing has clearly become one of the most important preservation methods, especially for the processing industry (juice, jam, canned, etc.), and the utilization of frozen fruit for direct consumption by consumers is less frequently employed. The primary reason for the lack of more widespread use of frozen fruits is that there is a severe decline in quality related to the freeze–thaw process of various fruits (e.g., apples, peaches, apricots, melon, plum, etc.). In particular, the freeze–thaw process causes a noticeable drip loss, accompanied by softening and enzymatic browning. These phenomena are due to cell disruption promoted by ice crystal formation during freezing, thereby leading to undesirable physico-chemical changes [1, 2].

Osmotic processes prior to freezing are used to produce several kinds of foods that can be stored for lengthy periods, and after thawing, retain favorable texture, color and flavor [3–6]. Due to the mild processing conditions used, osmotic processes have minimal impact on the nutritional and sensory quality of foods [7–9].

Among the developments in osmotic treatments of foods, vacuum impregnation (VI) is a recent application in fruit processing. In fact, VI is a recent technology applied in the food industry and it is possible to rapidly introduce several types of solutions in the porous structure of foods using this technique. As a consequence of this mass transfer, improvements in the physical–chemical, nutritional and sensory properties of foods take place leading to significant advantages such as longer shelf-life, optimization of further

processes, and the introduction of innovative products in the food market [10].

VI consists in the exchange of the gases occluded in open pores of the food matrix with treating solutions [11]. The impregnation of solutions in pores is dependent on the hydrodynamic mechanism (HDM) and the deformation and relaxation phenomena (DRP). In the former, the solution is drawn into the pores by capillarity, after gas expulsion during the vacuum step; in the latter, which is less effective than the former, gas expansion due to the pressure changes causes pore deformation and subsequent variation in volume of impregnated solution [12, 13].

Generally, VI is carried out in two steps after immersion of the food matrix in treating solution. In the first step, vacuum pressure (30–120 Mbar) is imposed on the system for a short time (from a few minutes to 45 min), thus promoting the expansion and outflow of the internal gas in the product. The releasing of the gas fills the product pores with the native liquid. In the second step atmospheric pressure is restored and compression leads to a large reduction in volume of the remaining gas in the pores filling the porous structure with liquid [14].

VI permits the direct introduction of specific substances and/or ingredients into food pores, thus maintaining the raw food structure; this effect can be exploited as a pre-treatment step in several food technologies such as osmotic dehydration (OD), freezing, canning, frying, etc. [15]. Due to the low temperature applied during VI, the heat damage to tissues is avoided and the color, aroma, and taste of raw materials are highly preserved, especially in vegetables characterized by high porosity. As observed by several researchers [8, 16, 17], after VI the polyphenol oxidase (PPO) activity in fruits decreases due to removal of oxygen from the inner portions of the vegetable matrix.

Moreover, as already mentioned, one advantage of VI consists in the possibility to introduce functional substances endowed with specific purposes into the food matrix, i.e., stabilizing agents, antioxidants, antibacterial, etc. In frozen fruits, impregnation with calcium salts can increase fruit texture through interaction with pectins, in order to avoid the tissue breakdown after thawing out [18, 19]. Moreover, compounds with cryoprotective effects such as glycerol, fructose, sucrose and glucose can be introduced [10].

The viability of fruit and vegetables treated by VI depends on the volume of impregnated solution, which is also linked to fruit porosity. The effective porosity depends on many factors but in general apple has the highest porosity, followed by peach, melon and strawberry at similar levels; pear, prune and apricot have a lower effective porosity level [20]. In general, larger fruit porosity permits more effective vacuum treatment.

In peaches and nectarines a significant amount of phenolic substances are present [21–23]. Polyphenols are important minor compounds with well-known anti-tumoral and anti-inflammatory properties, and their radical scavenger activity may also prevent cardiovascular disease [24, 25]. In vegetables since the PPO activity causes rapid depletion of the phenolic fraction, changes in polyphenols may, therefore, be used as a marker of the oxidative status of foods.

The aim of the present investigation is to evaluate the consumer acceptance of VI, soaked and untreated frozen nectarine slices. In particular, a correlation between negative sensory attributes such as darkening, an "oxidized" taste and phenol oxidation and depletion during thawing was previously found. For these reasons, in addition to consumer acceptance, the phenolic fraction has been assessed by both spectrophotometric (*o*-diphenols content) and chromatographic (HPLC) analyses and the antiradical capacity ($ABTS^{\bullet+}$ assay) was also tested.

Materials and methods

Solvents, reagents and standards

Solvents and reagents were purchased from Merck & Co. Inc. (Darmstadt, Germany). Chlorogenic acid (CA) standard for chromatographic analysis was from Fluka (Buchs, Switzerland).

Raw materials

Nectarines (var. *Maria Laura)* were obtained in local market (Cesena, Italy) (Fig. 1a). Ten fruits were selected according to size (about 85 mm diameter), uniformity of color and degree of ripeness. Each unpeeled fruit was first divided longitudinally (parallel to the apex-base direction) in two halves with a knife and subsequently de-stoned (Fig. 1b); from each half, five spherical wedges were obtained with a manual device (Fig. 1c) and then each wedge was longitudinally divided into two halves (Fig. 1d). Only wedges that were uniform in size (about 20 mm thick, 20 mm wide and 40 mm long) were used.

Sample treatments

The wedges (subsequently referred to as "slices") were divided into four groups (50 in each group):

1. Fresh nectarines (control sample, FR): slices were immediately analyzed on the day of purchase.
2. Untreated frozen nectarines (NT): slices were frozen in a polystyrene box by placing them on a grid which was

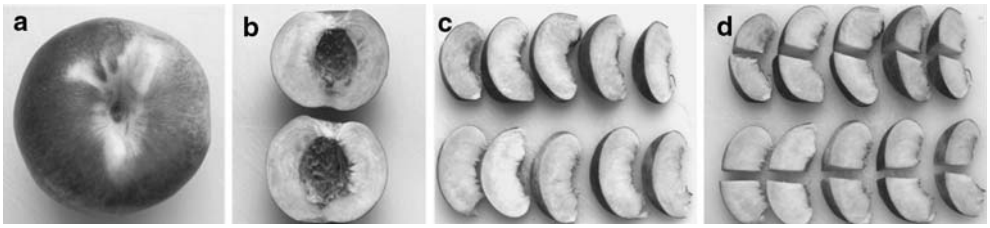

Fig. 1 Sampling of peach slices. **a** whole peach, **b** longitudinal cut and de-stoning, **c** spherical wedge, and **d** wedge division (slices)

then placed above a layer of dry ice. After this more dry ice was distributed over the slices and the chamber was closed. The dry ice was composed of pellets 2 mm in length and the ratio slices/dry ice was exactly 1/7 (w/w); the chamber temperature –79 °C. After 15 min the core product reached the temperature chamber and slices were stored at –18 °C for 1 month. Prior to analyses samples were distributed in plastic trays and thawed at controlled temperature (+18 °C) for 4 h.

3. Soaked nectarines (SK): slices were dipped in a aqueous treating solution containing 40% fructose, 4% calcium chloride dihydrate, 2% ascorbic acid (AA) and 0.4% sodium chloride. Samples were placed under the liquid surface using a perforated grill for 15 min at 18 °C (constant during the process). At the end of the procedure slices were frozen in a polystyrene box as described above and stored at –18 °C for 1 month. Prior to analyses samples were thawed at controlled temperature (+18 °C) for 4 h.
4. Vacuum impregnated nectarines (VI): slices were dipped in a treating solution (as SK samples) contained in a glass chamber connected to a vacuum pump (Vac V-500, Buchi, Switzerland). The vacuum pressure was 30 Mbar. Samples were placed under the liquid surface using a perforated grill for 15 min at 18 °C (constant during the process). Slices were frozen and analyzed as previously described.

Analytical determination

Dry matter (DM), pH, soluble solids (SS) and titratable acidity (TA) of fresh and frozen slices were calculated according to AOAC [26]; the pH meter was a Basic 20 (Crison Instrument, Barcelona, Spain). For fresh samples, analyses were carried out on the day of purchase. The other samples were analyzed after thawing was complete.

Extraction of phenolic compounds

Samples FR, NT, SK and VI were freeze-dried (CINQUEPASCAL LIO2000P, Milan, Italy). For the frozen samples this procedure was performed at the end of the 4-h thawing process. Phenolic extract was prepared from lyophilized sample. About 3 g of powder was extracted with 10 ml of aqueous methanol (20%) (Merck, Darmstadt, Germany) in a centrifuge tube with an Ultraturrax (IKA-Werke mod. T 25 basic, Staufen, Germany) at 15,000 rpm for 3 min. The tube was placed in a sonicated bath at 30 °C for 44 min and then centrifuged at 22,000 rpm (39,600*g*) for 10 min at 30 °C (Avanti J25, Beckman Coulter, Nyon Switzerland). The supernatant was recovered, filtered with a 45 μm cellulose acetate filter (Whatman, Clifton, NJ, USA) and placed in a vial for HPLC analysis.

Determination *o*-diphenol content (*o*-DPH)

According to Bendini et al. [27], 0.5 ml of phenolic extract was diluted tenfold with a 50% aqueous MeOH mixture in a flask; 4 ml of this solution was added together with 1 ml of sodium molybdate dihydrate reagent (Sigma, St Louis, MO, USA) in 50% aqueous EtOH, vortexed for 1 min and allowed to stand at room temperature for 10 min. After this step, the solution was centrifuged (3 min, 1,490*g*) and the absorbance of the supernatant was measured at 370 nm against a reference prepared without sodium molybdate. The *o*-DPH concentration was calculated from a calibration curve (r^2 = 0.9850) using gallic acid (Sigma) as a standard (10–500 mg/l). Results were expressed as gallic acid equivalents. The spectrophotometric analysis was repeated three times for each type of extract.

$ABTS^{\bullet+}$ assay

According to Bendini et al. [27] ABTS (Sigma) was dissolved in H_2O to a concentration of 7 mM. The radical cation of ABTS was obtained by reaction with 2.45 mM potassium persulfate (Sigma) (final concentration) and allowing the stock solution to stand in the dark at room temperature for at least 12 h. Before use, the $ABTS^{\bullet+}$ solution was diluted with EtOH to an absorbance of 0.70 ± 0.02 at 734 nm at 30 °C. Next, 1 ml of this $ABTS^{\bullet+}$ solution was added to 0.01 ml of extract and the decrease in absorbance was recorded for 10 min. Absorbance values were corrected for radical decay using blank solution (0.01 ml of 50% aqueous MeOH). Measurements were made in four replicates and the antioxidant activity was calculated as the Trolox equivalent antioxidant capacity

(TEAC) and expressed as milligram Trolox per kilogram of fresh weight (r^2 = 0.9811).

Chlorogenic and neochlorogenic determination by HPLC-DAD

HPLC analysis was carried out using a HP 1100 Series Instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with a high pressure binary pump, an autosampler ASL (Automatic Liquid Sampler G1313A), a membrane degasser, and a UV–Vis detector (DAD). A X-Terra column (5 μm particle size, 250 × 3.00 mm ID) C18 was used (Waters, Milford, MA, USA). The injection volume was 10 μl. All solvents were filtered through a 0.45 μm nylon filter disk (Lida Manufacturing, Kenosha, WI, USA).

Mobile phases were: A, 2% acetic acid in water; and B, methanol/acetonitrile 50/50. The elution gradient was linear: at 0 min 95% solvent A, at 5 min 90%, at 13 min 86% and at 20 min 80. The acquisition wavelength was set at 320 nm. Identification of CA was performed on the basis of retention time of a relative standard purchased from Sigma-Aldrich Inc. (St Louis, MO, USA). Identification of neochlorogenic acid (NCA) was performed on the basis of spectral characteristics and relative retention time according to literature data [21]. Quantification of both individual compounds was performed on the basis of an external calibration curve of CA (r^2 = 0.9958).

Sensory evaluation analysis: acceptance test

The acceptance tests were carried out on a laboratory scale [28] in the Laboratory of Sensory Analysis of the "Campus di Scienze degli Alimenti, University of Bologna", using individual booths with white neon light. Fresh samples were analyzed on the day of purchase, while frozen samples NT, SK and VI were analyzed after 1 month of storage and at the end of the 4-h thawing process.

An untrained panel of 22 consumers was used (11 males and 11 females between 30- and 50-year old, office-workers). No information about the normal fruit consumption habits of the judges was available. For each descriptor a hedonic scale was represented on a continuous line. Above the line, five sectors were indicated corresponding to five hedonic classes: *extremely unpleasant, unpleasant, neither unpleasant nor pleasant, pleasant, extremely pleasant*. Consumers indicated with a sign on the continuous line the sector corresponding to level of product satisfaction, relative to each descriptor, with the possibility to match the tendency through the upper level or the lower level. The line length is 17 cm and each descriptor "level of acceptance" was measured in centimeters.

Descriptors were: *visual appearance*, *aroma* (defined as peach typical aroma evaluated with direct inspiration), *firmness*, *juiciness*, *sweetness*, *sourness*, *retronasal aroma* (aroma evaluation during chewing), *and overall acceptability*.

Statistical analysis

Data were analyzed using Statistica 6.1 (Statsoft Inc., Tulsa, OK, USA) statistical software. The significance of differences at 5% level between averages was determined by one-way ANOVA using Tukey's test.

Results and discussion

In Table 1, data from different nectarine samples relative to DM, SS, titratable acidity and pH are reported. As is evident, the effects of such processes on nectarine slices led to significant differences, reflecting the relevant impact of vacuum applications in fruit and vegetables processes. In fact, the DM of fresh fruit was 14.11% and this value was unvaried in untreated (NT) samples, while it increased significantly in soaked (SK) nectarines (DM 15.09%) and in VI samples (DM 18.07%). A similar behavior was observed for SS, where VI samples showed the highest value with a 15.20° Brix. On the other hand, the TA was decreased in SK and VI samples with respect to fresh samples, while no significant changes in pH were observed in any samples. As mentioned previously, VI allows the replacement of gas occluded in open pores of the food matrix with the treating solution, thus accelerating mass transfers and leading to higher sugar uptake. Immersion in treating solution (both in soaked and VI samples) probably produces solute leaching (sugars, phenols, organic acid, etc.) as confirmed by the decrease in titratable acidity in both SK and VI samples.

In Table 2 the values relative to the $ABTS^{\bullet+}$ test and *o*-diphenol content for different processes are shown. The antioxidant capacity, measured as TEAC, was higher in the VI sample than in the fresh nectarine slices. This effect could be explained by the presence of AA in the treating solution. AA gave a positive response in the $ABTS^{\bullet+}$ test due to its capacity to act as a radical scavenger, reacting with $ABTS^{\bullet+}$ as was the case for Trolox [29]. This behavior was less evident in SK nectarines, probably because of the lack of vacuum during the process. Thus it may be possible to hypothesize that VI caused greater AA mass transfer, and thus a greater amount of AA was present in the VI nectarines.

With regards to the *o*-DPH content, a significant decrease in NT and SK samples was seen with respect to fresh samples, while in VI samples the initial content of fresh nectarines was not affected. This could also be explained considering AA impregnation of VI slices. In this case, AA did not affect the analytical method used, but

Table 1

Samples	FR	NT	SK	VI
DM	14.11^{c}	14.14^{c}	15.09^{b}	18.07^{a}
SS	11.10^{c}	11.10^{c}	12.05^{b}	15.20^{a}
pH	3.36^{a}	3.30^{a}	3.34^{a}	3.35^{a}
TA	1.11^{a}	1.07^{ab}	1.04^{b}	1.02^{b}

The letters *a–c* in italics in the same row indicate statistically significant values (honestly significantly different (HSD) Tukey, $p < 0.05$)

DM dry matter (%), *SS* soluble solids (Brix in juice by refractometric index), *pH* (in juice), *TA* titratable acidity (% of citric acid in juice), in nectarine slices. *FR* fresh nectarines slices, *NT* frozen nectarine slices, *SK* soaked nectarine slices, *VI* vacuum impregnated nectarine slices

Table 2

Samples	FR	NT	SK	VI
TEAC	3854^{b}	3268^{c}	3479^{c}	6210^{a}
o-DPH	647.48^{a}	347.57^{b}	422.98^{b}	581.61^{a}

The letters *a–c* in italics in the same row indicate statistically significant values (HSD Tukey $p < 0.05$)

TEAC trolox equivalent antioxidant capacity (mg trolox per kg of fw) and *o-DPH o*-diphenols content valued by spectrophotometric assays of nectarine slices. *FR* fresh nectarines slices, *NT* frozen nectarine slices, *SK* soaked nectarine slices, *VI* vacuum impregnated nectarine slices

as widely known AA has a protective effect on phenolic compounds, avoiding oxidation and causing a possible reduction from quinones to phenols. This effect was not observed in SK samples, due to the relatively small AA quantity that impregnated the matrix.

In Fig. 2 the results of HPLC determination of CA and NCA are shown and the CA + NCA content is reported. Recoveries of the extraction method previously calculated in our laboratory for CA and NCA ranged from 80 to 105%. The two phenolic compounds belong to the hydroxycinnamic acid class and are also *o*-diphenols. However, the content of the two phenolic compounds (CA + NCA) in the VI sample was comparable to that in FR, while it resulted decreased in SK and NT with respect to the initial raw material. Although no statistically significant differences were observed in the phenolic content of differently treated samples, a decreasing content of hydroxycinnamic acids in NT samples was more evident with respect to SK samples, and even more than VI samples. To some extent the phenolic content in VI samples could be considered similar to FR samples, while SK peach slices were more similar to NT slices considering the *o*-diphenol content.

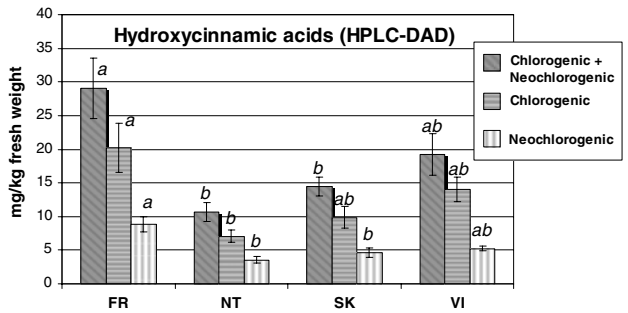

Fig. 2 Results of HPLC-DAD analyses of CA and NCA in fresh and frozen nectarine slices. Letters *a–b* in italics indicate significant differences (HSD Tukey, $p < 0.05$). *FR*, fresh nectarine slices; *NT*, frozen nectarine slices; *SK*, soaked nectarine slices; *VI*, vacuum impregnated nectarine slices

These results can be summarized as follows: the VI process introduced a significant quantity of solutes into nectarine slices, while this effect was less evident in SK samples. VI nectarine slices showed a phenolic content that was unchanged or only slightly decreased. The depletion might be explained considering solute leaching from the fruit into the solution, and the extent of this phenomenon has been demonstrated in our previous work in VI apple slices [30]. Nonetheless, the protective effect of AA should be present only in the outer zones (in the proximity of pores), so the phenols in the inner region of the slices would be subjected to oxidation more than the compounds in contact with the impregnated AA. This could explain differences in phenolic depletion in the fruit, although more studies are needed to confirm this hypothesis.

Finally, in Table 3, the results of the quantitative acceptance test are shown. When rating the overall acceptability attribute, it was observed that VI and SK samples had, respectively, 13.5 and 12.5 points, which was highly similar to that seen for fresh raw material (14.1 points). NT samples had a very low score, thus confirming what found in preliminary tests (see above). In fact, by analyzing each single attribute, it can be noted that NT had very low acceptance level of visual appearance, aroma, sweetness, and retronasal aroma, while the firmness and acidity remained high and comparable with those of other samples. It is likely that the phenolic oxidation occurring during thawing contributed to the decreases in visual aspect, aroma modification and flavor aggravation. Considering visual appearance attribute, the SK sample was similar to VI and FR samples and different from NT samples, in contrast with data from *o*-diphenol and CA and NCA HPLC determination. This can be explained considering that phenolic oxidation in SK samples occurs exclusively in the inner part of nectarine slices, while the protective effect of AA and sugars takes place at the surface of slices.

Regarding aroma punctuation (perceived with direct nasal inhalation above the sample) of SK, it was even higher than VI and FR samples. In particular, the FR

Table 3 Results of acceptance tests carried out on fresh and frozen nectarines slices

Attribute	FR		NT		SK		VI	
Visual appearance	13.5 (±2.7)	*a*	3.0 (±1.3)	*b*	13.6 (±3.1)	*a*	12.3 (±4.0)	*a*
Aroma	4.2 (±4.3)	*cd*	6.6 (±2.3)	*c*	12.7 (±2.3)	*a*	10.3 (±2.5)	*b*
Firmness	12.5 (±1.4)	*a*	12.5 (±1.6)	*a*	12.8 (±2.3)	*a*	12.5 (±0.9)	*a*
Juiciness	9.3 (±5.6)	*a*	6.6 (±4.7)	*a*	10.0 (±3.2)	*a*	10.5 (±6.4)	*a*
Sweetness	6.8 (±3.3)	*b*	4.6 (±2.0)	*c*	14.0 (±2.7)	*a*	13.7 (±2.1)	*a*
Sourness	10.5 (±2.1)	*ab*	13.4 (±3.5)	*a*	8.7 (±0.5)	*b*	8.7 (±0.5)	*b*
Retronasal aroma	14.6 (±1.7)	*a*	4.5 (±1.4)	*d*	6.1 (±2.0)	*c*	12.4 (±2.1)	*b*
Overall acceptability	14.1 (±1.5)	*a*	4.2 (±2.5)	*c*	12.5 (±2.1)	*b*	13.5 (±0.8)	*b*

FR fresh nectarines slices, *NT* frozen nectarine slices, *SK* soaked nectarine slices, *VI* vacuum impregnated nectarine slices. The letters *a–d* in italics in the same row indicate statistically significant values (HSD Tukey, $p < 0.05$)

sample had the poorest values of aroma together with the NT sample, which is difficult to rationalize. It is possible that the cutting zone in the FR slices underwent rapid oxidation and was strongly altered at the moment of tasting or that the VI and SK processes improved aroma in some manner that requires further investigation. The VI sample had an aroma value of 10.3, slightly less than SK, which was probably due to modification of the primary aroma characteristics of peaches. In fact, the VI process affected the chemical composition more than SK, as demonstrated by the DM and refractometric index values. On the other hand, the retronasal aroma of SK samples (perceived during chewing) was very low compared to VI and FR samples, but comparable with NT samples. This descriptor seemed to be more important in determining the final acceptance level of samples. It must be noted that oxidation occurred, at any level, including in the inner part of slices where cells disruption due to ice crystals formation took place. Thus, in the VI slices, AA impregnation even occurred in the inner part of slices in the network formed by pores, thus avoiding phenolic oxidation at any level.

It must be noted that the low value of retronasal aroma was the most important factor in penalizing the NT sample, but it was not sufficient for the consumer to discard the SK sample as "not pleasant". Perhaps the high relevancy on the overall acceptability attribute was due to visual aspect and aroma, or the visual aspect may have influenced the evaluation of other attributes; in fact as previously mentioned, the overall acceptability of SK samples was similar to VI samples, in spite of a lower retronasal aroma. It should be stressed that the consumer judges did not know that the nectarine slices had been frozen and thus probably considered NT slices as aged, while they considered SK and VI slices as "fresh" on the basis of the appearance.

In conclusion, phenolic content and antioxidant capacity appear to be good parameters for determining the acceptance level of frozen nectarine slices. In fact, VI samples, which had comparable amounts of *o*-DPH, CA and NCA to fresh slices, were considered pleasant (13.5) and very near the extremely pleasant level of fresh samples (14.1). In general, the higher the phenolic content, the higher the acceptance level of samples.

In untreated frozen slices, the higher extent of phenolic oxidation caused consumer rejection (overall acceptability = 4.2). SK samples had similar extents of oxidation, but the acceptance level was very high (12.5). In any case, while no significant differences were observed in the ABTS, *o*-DPH, CA and NCA content between SK and NT nectarines, SK acceptability values tended to be slightly higher.

Sourness and sweetness descriptors did not seem to greatly affect the overall acceptability judgment. In any case sweetness was higher in the SK and VI samples, while sourness was higher in NT samples. It should also be underlined that the fresh nectarines were bought at an early stage of ripeness. As described previously, titratable acidity in SK and VI samples was reduced, thus confirming what seen with the consumer test.

Finally there were no significant differences between the firmness acceptance of samples, although it is likely that a wide range of firmness acceptance in consumer evaluation exists. Softer or harder slices were probably associated with riper or greener fruits, respectively.

Conclusion

Freezing of fruit can be considered an optimal technology to preserve the nutritional and chemical characteristics of fruit for extended periods. Unfortunately, formation of ice crystals during freezing causes cell disruption. This phenomenon becomes evident during thawing, prior to consumption, with acceleration of enzymatic reactions and loss of quality. In particular, phenolic oxidation takes place, leading to formation of browning and off-flavor. Using osmotic processes like soaking or vacuum impregnating

fruits in solutions containing antioxidant (AA) for a short time (e.g., 15 min), it is possible to maintain fruit quality after thawing. In this investigation, this effect was more evident in VI samples than SK samples, and VI was an effective technology for pre-treatment of fruit prior to freezing. Moreover, consumer acceptance of frozen fruit is strongly conditioned by oxidative status. In conclusion, phenolic content and antioxidant capacity appear to be good parameters for determining the acceptance level of frozen nectarine slices. In general, the higher the phenolic content, the higher the acceptance level of samples.

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ORIGINAL PAPER

Effect of vacuum impregnation on the phenolic content of Granny Smith and Stark Delicious frozen apple *cvv*

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Abstract ABTS•+ test, *o*-diphenols (spectrophotometric) and HPLC-DAD phenolic content in vacuum impregnated apple slices from Stark Delicious and Granny Smith *cvv.* were studied. Vacuum impregnation (VI) was carried out in a pilot plant using an aqueous solution containing 37.9% dextrose, 15.2% sucrose, 1.0% ascorbic acid, 0.25% calcium chloride and 0.25% sodium chloride. The impregnation time was 30 min and the vacuum pressure was 100 mbar; the temperature was 25 °C during all processing steps and the solution/fruit ratio was 11:1. At the end of VI, samples were left 5 min in syrup. They were then rinsed with water, drained in a vibrating screen and quickly frozen. A significant decrease ($p < 0.05$) of the *o*-diphenol content was seen (17.84 and 12.32% of the initial content in Stark and Granny varieties, respectively). The same trend was confirmed by HPLC-DAD where reductions in total phenols were 21.57 and 26.86% in Stark and Granny, respectively. Individual phenolic compounds showed different rates of depletion, although in some cases there was no reduction. The ABTS•+ test was strongly affected by the presence of ascorbic acid (AA), which was much higher in treated samples. Sensory evaluation showed higher values of hardness, crispness, juiciness and sourness in VI Granny Smith than VI Stark delicious, with a higher retention of texture parameters in the former. Finally, sweetness was slightly higher in VI Stark.

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Introduction

Fresh fruits contain nutritional and healthful constituents such as minerals, vitamins such as C, E and A, phytochemicals such as folates, glucosinolates, carotenoids, flavonoids and phenolic acids, in addition to lycopene, selenium, and dietary fibers. Recently, polyphenols have received increasing interest as they possess anti-inflammatory, anti-histaminic and anti-tumor activities, and also act as free radical scavengers by protecting against cardiovascular disease [1–5]. Apples, in particular, are rich in phenolic compounds, which are of unquestionable importance due to their contribution to the color, taste and flavor characteristics of both apples and their derived products. In some cases, the major food sources of antioxidants are derived from tea, onions and apples [2]. The most important polyphenolic compounds present in apple are phenolic acids and flavonoids such as flavanols or catechins, flavonols, dihydrocalchones and anthocyanidins, while their relative content depends on several variables [6–8].

The newest trend in the food industry is the development of ready-to-eat, healthy products with a high sensory content. Fresh fruit is an excellent raw material, but is difficult to conserve for long periods of time without modifying its nutritional distinctiveness. Osmotic treatments prior to freezing are used to produce several kinds of food products that can be stored for lengthy periods and after thawing retain good texture, color, and flavor [9–12]. Due to the mild processing conditions used, osmotic treatments have minimal impact on the nutritional and sensory quality of foods [13–15].

Vacuum impregnation (VI) is a recent development in the osmotic treatment of foods. VI of a porous product consists in exchanging the internal gas or liquid occluded in open pores with an external liquid phase due to the action of hydrodynamic mechanisms (HDM) promoted by pressure changes and deformation–relaxation phenomena (DRP) [16, 17]. As a consequence, some mass transfer processes, such as air-drying and osmotic dehydration (OD), are improved with a consequent change in food composition [18]. During VI processes other physical–chemical processes such as osmosis and diffusion occur in addition to impregnation.

During osmotic treatment, two important mass transfers are driven from concentration gradients: a flow of water from the food matrix to the liquid and a flow of solute from the liquid to the food matrix. A third mass transfer involves food solutes leaching in the solution. Although leaching is generally considered to be quantitatively negligible [19], it could result in deterioration of the food matrix. Using pulsed vacuum osmotic dehydration (PVOD), Peiró et al. [20, 21] found a detectable loss of citric acid, ascorbic acid, galacturonic acid and other components from grapefruit and pineapple in an osmotic solution that had been reutilized.

As reported by Andrès et al. [22], in addition to solute leaching, at the end of the vacuum step during VI processes, there is a loss of native liquid contained in the intercellular spaces (pores) due to internal gas expansion in many types of fruits. Moreover, in addition to mass fluxes, structural changes in tissue such as cell alteration due to deformation and breakage of cellular elements associated with dehydration and gas–liquid exchanges also occur. All these phenomena provoke changes at many levels (reviewed in Chiralt and Talens [23]).

With regards to chemical changes of osmotically treated foods, several studies have been performed that have focused mainly on the profile of volatile compounds. Modifications in the aromatic profile depend on osmotic process variables, such as temperature, solute type and concentration, pressure, and the solution/fruit ratio [24]. For example, osmotic treatment results in a greater retention of vitamin C and chlorophyll of frozen kiwifruit slices during storage at –10 °C and strawberries treated with sugar solutions can also stabilize the antocyanin content [25].

It must be considered that in industrial osmotic processes solutions are reused, so that their content in food components reaches equilibrium with the raw material, reducing the lixiviation process [26]. In reality, industrial solution (syrups) management requires frequent supplements and/or thermal treatment in order to maintain a gradient between the two phases. Thus, a more complex theory is necessary to accurately describe food modification during industrial osmotic processes [27].

All these modifications must be studied in order to minimize nutritional depletion of the raw material. To the best of our knowledge, few studies have been performed on modifications of the phenolic profile during osmotic treatment of apple. The primary aims of the present investigation was to evaluate changes in the amount of biophenolics in frozen apple slices from two varieties (Granny Smith and Stark Delicious) following VI and to assess the sensory characteristics of slices from the two varieties after VI, cryofreezing and thawing.

Experimental

Samples

Two varieties of apples (300 Granny Smith and 300 Stark Delicious) were bought at a local market. Each variety was randomly divided in two subgroups of 150 apples: control samples and VI samples. Fifty apples from each of the control samples were used for sensory analysis of fresh material (described below). The remaining 100 apples were mechanically peeled, cored and sliced (16 slices per apple) in a few minutes at a pilot plant at a local factory. Next, slices corresponding to about 50 apples were ground with a chopper and the dry matter was calculated. Juice was extracted by centrifugation to quantify soluble solids and measure pH and titratable acidity. The remaining slices were immediately frozen in a Cryo CM 85 liquid nitrogen freezer (Linde Gas Italia, Milan, Italy) at –73°C, in 7 min and stored for 7 days at –18°C. Following this, slices were freeze-dried (CINQUEPASCAL LIO2000P, Milan, Italy) and then crushed to obtain a powder for extraction of phenolics. In our experience neither cryofreezing nor frozen storage at –18°C for 7 days significantly affects the phenolic content of either peaches or apples (data not published). Similar findings have also been reported by other authors for other fruits [28, 29]. For this reason, the data for control samples was considered to be the same as fresh material.

The second subgroup of apples was mechanically peeled, cored and sliced (16 slices per apple) in a few minuts at the same pilot plant and were transported to a local pilot vacuum candying plant and vacuum impregnated using an aqueous solution containing 37.9% dextrose, 15.2% sucrose, 1.0% ascorbic acid, 0.25% calcium chloride, and 0.25% sodium chloride. Impregnation was carried out for 30 min at a vacuum pressure of 100 mbar. The temperature was 25 °C during all processing and the solution/fruit ratio was 11:1. At the end of the VI step, samples were left for 5 min in the impregnating solution and then rinsed with water, drained in a vibrating screen, and finally frozen in a Cryo CM 85 liquid nitrogen freezer (Linde Gas Italia, Milan, Italy) at –73°C in 7 min. Samples

were stored for 7 days at –18°C, and slices corresponding to about 50 apples were used for sensory analysis of treated apples (see below). Another 50 apples were used for determination of dry matter, soluble solids, pH and titratable acidity, while the remaining slices (about 50 apples) were freeze-dried (CINQUEPASCAL LIO2000P) and then crushed to obtain a powder for extraction of phenolics.

Analyses were carried out in triplicate.

Analyses of dry matter, soluble solids, titratable acidity

Dry matter, pH, soluble solids and titratable acidity of fresh and frozen slices were calculated according to AOAC [30].

Mass transfer determination

Solid gain (SG), water loss (WL) and weight reduction (WR) were calculated as described by Giangiacomo et al. [31].

Extraction of phenolic compounds

A phenolic extract was prepared from lyophilized sample. About 3 g of powder were extracted with 10 ml of aqueous methanol (20%) (Merck, Darmstadt, Germany) in a centrifuge tube with an Ultra Turrax (IKA-Werke mod. T 25 basic, Staufen, Germany) at 15,000 rpm for 3 min. The tube was placed in a sonicated bath at 30 °C for 44 min and then centrifuged at 22,000 rpm (39,600 g) for 10 min at 30 °C (Avanti J25, Beckman Coulter, Fullerton, CA, USA). The supernatant was recovered and filtered through a 45 µm cellulose acetate filter (Whatman, Clifton NJ, USA) and used for HPLC analysis. Recoveries of the extraction method were previously calculated for each compound. The medium recoveries in subsequent extraction were 80% in the first extraction, 14% in the second, 5% in the third and less then 1% in the fourth. Only one extraction was carried out in the analysis and the final amount of phenolics were estimated on the basis of calculated recoveries.

Determination of total *o*-diphenol

According to Bendini et al. [32], 0.5 ml of phenolic extract was diluted tenfold with a 50% aq. MeOH mixture in a flask; 4 ml of this solution was added together with 1 ml of sodium molybdate dihydrate (Sigma, St Louis MO, USA) in 50% aq EtOH, vortexed for 1 min and allowed to stand at room temperature for 10 min. After this step, the solution was centrifuged (3 min, 1,490 g) and the absorbance of the supernatant was measured at 370 nm against a reference prepared without sodium molybdate reagent. The *o*-diphenol concentration was calculated from a calibration curve ($r^2 = 0.9850$) using gallic acid (Sigma, St Louis MO, USA) as a standard (10–500 mg l^{-1}). Results were expressed as gallic acid equivalents. The spectrophotometric analysis was repeated three times for each type of extract.

$ABTS^{\bullet+}$ assay

According to Bendini et al. [32] ABTS (Sigma, St Louis, MO, USA) was dissolved in H_2O to a concentration of 7 mM. The radical cation of ABTS was obtained by reaction with 2.45 mM potassium persulfate (Sigma, St Louis MO, USA) (final concentration) and allowing the stock solution to stand in the dark at room temperature for at least 12 h. Before use, the ABTS•+ solution was diluted with EtOH to an absorbance of 0.70 ± 0.02 at 734 nm at 30 °C. Next, 1 ml of this $ABTS^{\bullet+}$ solution was added to 0.01 ml of extract and the decrease in absorbance was recorded for 10 min. Absorbance values were corrected for radical decay using a blank solution (0.01 ml of 50% aq. MeOH). Measurements were made in four replicates and the antioxidant activity was calculated as the Trolox equivalent antioxidant capacity (TEAC) ($r^2 = 0.9811$).

HPLC-DAD phenolic determination

HPLC analysis was carried out on a Jasco-HPLC (Jasco corporation, Tokyo, Japan), equipped with a binary pump (Jasco PU 1580 intelligent HPLC pump), an autosampler (Jasco AS 2055 plus intelligent sampler), a HP Diode-Array UV-VIS Detector (Jasco MD 1510 multiwavelength detector). A Luna C18 (Phenomenex, St Torrance CA, USA) column (5 µm particle size, 250 mm × 3.00 mm ID) was used and 20 µl of phenolic extract were injected. All solvents were filtered through a 0.45 µm nylon filter disk (Lida Manufacturing, Kenosha WI, USA).

Solvents for the mobile phase were: A, 1% formic acid in water and B, 1% formic acid in methanol. The elution conditions were those described by Alonso-Salces et al. [33] and identification of phenolic compounds was performed on the basis of retention time of a relative standard when available or tentatively performed on the basis of spectral characteristics and relative retention time according to literature data. Quantification of individual compounds was performed on the basis of a external calibration curve of the relative standard (when available) or on the basis of a standard of the same phenolic class: hydroxycinnamic acids were quantified with chlorogenic acid, procyanidins by procyanidin B2, (+)-catechin, phloretin derivatives and phloridzin by (-)-epicatechin, and flavonols by quercetin.

Sensory evaluation analysis

For sensory tests a trained panel was used. Ten judges were trained and calibrated on three tactile descriptors (hardness, crispness, juiciness) and two gustatory descriptors (sourness, sweetness). Descriptors were those described by Daillant-Spinnler et al. [34]. Sensory tests were performed in three different sessions on three consecutive days. During the 3 days apples were stored at controlled temperature (+2 °C). Each sample was analyzed in triplicate. The presentation pathway of samples was randomized. Only five descriptors were quantified by judges. The range of descriptors was from 0 (absent) to 5 (extreme). It was possible to match middle values. As it was not our intent to evaluate the aroma profile no measurements were made in this regard.

Statistical analysis

Data were analyzed using Statistica 6.0 (Statsoft, Tulsa OK, USA) statistical software. Differences were considered significant when $p < 0.05$; a one-way ANOVA using Tukey's test was used.

Results and discussion

The amount of solution impregnated at the end of the VI process depends on many factors, but is greater in food matrixes with a higher porosity. It has been reported that apples have a high porosity value and about 11–19% of fruit volume can be filled with external solutions [22]. In our experiments, vacuum impregnated apples did not increase in weight: as reported in Table 1, VI resulted in a weight reduction (WR) of apple slices due to the dewatering promoted by immersion of fruit in a hypertonic solution. Thus, it was possible to hypothesize that dewatering was quantitatively more important than impregnation.

In Tables 1, 2, the results of analyses carried out on the two cultivars are shown. In the ''control sample'' column, the results relative to fresh apples are detailed and values refer to 100 g of fresh sample. In the ''VI sample'' column, results are relative to the treated samples and values refer to the weight of vacuum impregnated sample corresponding to 100 g of an initial ''control sample''. As already mentioned, this corresponded to a weight reduction of 100-WR g. Finally, in the third column analytical changes between the initial sample and treated samples are expressed as:

$$\text{RED}\% = \frac{C_{\text{bf}} - C_{\text{at}}}{C_{\text{bf}}} \times 100$$

where

C_{bf} = Content of analytes in 100 g of fresh sample

C_{at} = Content of analytes in 100 g of fresh sample submitted to VI treatment

Positive values of RED% indicate a percentage reduction of the initial value, while negative numbers indicate a percentage increase.

As reported in Table 1, the results of WR were important for both cultivars after VI. In particular, the Granny Smith variety showed a significantly greater reduction of initial weight compared to the Stark variety. The water loss (WL) of the two varieties was significantly different, and was greater in Granny Smith apple slices. In contrast the solid gain (SG) was significantly higher in Stark. For sample preparation, we submitted apple slices to a vacuum step of 30 min at a vacuum pressure of 100 mbar. Following this, the atmospheric pressure was restored and apple slices were allowed to remain in the solution for 5 min. It seems apparent that this process consists not only a impregnation of the product with the solution, but also a flow of water (and solutes) from fruit to the solution, and a flow of solutes from the solution to fruit slices. This type of process might be more accurately referred to as dewatering–impregnation–soaking, although we continue to refer to it as VI.

Excluding values of dry matter and of soluble solids, which were the same, Granny Smith variety had a lower juice pH and a higher juice titratable acidity thus confirming it as a sour variety. To explain the differences in mass transfer, large differences in the initial structure of the two varieties can be hypothesized. With regards to juice pH and juice acidity measured at the end of VI, the juices extracted from Stark slices were enriched in soluble solids more than Granny slices, as determined by the SG values.

Titratable acidity in juice showed less distinct behavior. In fact, while in the Granny Smith variety it decreased by 19.1%, in the Stark variety it increased by about 50%. This trend can be partially explained considering that 1.0% of ascorbic acid was present in the treatment solution. Thus, ascorbic acid should be impregnated in both Stark and Granny apples slices, affecting titratable acidity (and pH) more in Stark than Granny Smith, where the initial acidity was higher and leaching of organic acids should be more prominent, leading to a greater concentration gradient. However, this impregnation cannot completely explain the increase in acidity in Stark. Moreover, we performed acidity titration of fresh material by directly homogenizing apple slices in a chopper, while titrations on treated samples were performed by grinding frozen slices. As observed by other authors, freezing causes cellular disruption, thus improving extraction procedures of analytes [35]. Finally, the freezing process itself could have caused acidity

Table 1 Results of mass transfers (WR, SG, WL), dry matter, soluble solids, pH, tritratable acidity, *o*-diphenols and ABTS$^{\bullet+}$ test in Granny Smith and Stark Delicious apple varieties before and after VI

Mass transfers	Stark Delicious		Granny Smith	
	Mean	SD	Mean	SD
Weight reduction, WR (%)	10.88^{b}	0.57	15.57^{a}	0.43
Water loss, WL (%)	17.53^{b}	0.42	20.08^{a}	0.43
Solid gain, SG (%)	6.65^{b}	0.06	4.51^{a}	0.30

Analyses	VI sample (100-WR g)		Control sample (100 g)			VI sample (100-WR g)		Control sample (100 g)		
	Mean	SD	Mean	SD	RED (%)	Mean	SD	Mean	SD	RED (%)
Dry matter (g)	21.39^{a}	0.14	14.74^{c}	0.09	–45.12	19.17^{b}	0.09	14.66^{c}	0.11	–30.76
Soluble solids in juice (°Brix)	21.50^{a}	0.20	12.90^{c}	0.10	–66.67	20.00^{b}	0.20	12.80^{c}	0.10	–56.25
pH in juice	3.90		4.24		–	3.27		3.27		–
Titratable acidity in juice (g/L malic acid)	0.36^{c}	0.01	0.24^{d}	0.01	–50.00	0.72^{b}	0.01	0.89^{a}	0.01	19.10
o-diphenols (mg gallic acid)	703.0^{ab}	35.4	855.6^{a}	88.7	17.84	616.41^{b}	19.6	703.2^{ab}	55.2	12.34
ABTS•+ (mg TEAC)	2800.7^{a}	192	1361.3^{b}	201	–105.75	2921.6^{a}	183	1009.6^{b}	142	–189.38

In the ''control sample'' column the results relative to fresh apples are detailed and values refer to 100 g of fresh sample. In the ''VI sample'' column, results are relative to 100 g of initial fresh sample after VI treatment (corresponding to a sample weight of 100-WR g). The RED% indicates an increase (negative values) or decrease (positive values) of the initial content after VI

Letters a–d in superscript indicate statistically significant differences between columns (HSD Tukey $p < 0.05$)

changes in the food material and more analyses will be necessary to further understand this aspect.

The content of *o*-diphenols after VI was decreased about 18% in Stark and 12% in Granny, thus indicating that VI provoked changes in the *o*-diphenol content, probably by leaching in the solution.

Considering the ABTS$^{\bullet+}$ test, it must be kept in mind that 1% ascorbic acid was present in the treating solution that influenced the results as reflected in the larger values in the ''VI sample'' column. Thus, this type of test provides little information about changes in mass and it can only furnish information about the antioxidant food status. It is important to note that the high values of TEAC in treated samples indicate a large content of ascorbic acid. As is known, ascorbic acid protects phenolic compounds from oxidation to quinones, and even reduces quinone to phenols. Therefore, we can affirm with near certainty that the decrease in the phenolic content due to oxidation was prevented in the VI process. Lastly, it is of interest to notice that the ABTS$^{\bullet+}$ value of fresh Stark apples was greater than that of Granny Smith.

In Table 2 the phenolic profiles of the two varieties before and after the VI process are reported. Considering the fresh (peeled) slices, a greater content of total phenolics was found in Stark Delicious compared to Granny Smith. The value of 520.58 mg in Granny Smith was comparable to data from the literature, while the values of Stark Delicious were slightly higher than the values of phenolic content in red apples [36].

It is important to focus on the changes in the total phenolic content due to VI and in both varieties there was a significant reduction. As already mentioned, this reduction may be primarily due to solute leaching in the treating solution, and thus VI may cause a slight decrease in the nutritional content of apple slices.

By analysis of a single class of phenols, it would appear that this reduction is mainly due to the flavan-*3*-ol class. In fact, it was the most prominent group both in the Stark (80.7%) and Granny (83.4%) variety, accounting for 93.4 and 90.7% of the total phenolic reduction, respectively. Contribution to the total reduction by the other phenolic classes can be considered less important. In particular, the hydroxycinnamic acids were reduced in Stark apples, but not in Granny Smith apples, while the dihydrochalcone content was decreased in Granny, but unchanged in Stark.

Focusing on the flavan-*3*-ol class, the most abundant compounds were procyanidin B2 and the procyanidin trimer (compound 11), which was particularly high in the Stark variety. It was also observed that the VI step affected only procyanidins and their RED% was the higher than any other compound. The average reduction in procyanidins (compounds 8-11) was in any case higher in Stark than Granny Smith apples. In contrast, (+)-catechin remained constant or showed no significant reduction in both *cvv.*, while (-)-epicatechin showed a significant reduction of 8.58% only in Granny Smith apples. The main reason for this may be related to the procyanidin distribution in the whole fruit. In fact, they are usually particularly abundant

Table 2 HPLC-DAD determination of phenolic compounds in Granny Smith and Stark Delicious varieties before and after VI

Compound (mg)	VI sample (100-WR g)		Control sample (100 g)		Stark	VI sample (100-WR g)		Control sample (100 g)		Granny
	Mean	SD	Mean	SD	RED (%)	Mean	SD	Mean	SD	RED (%)
1. Caffeic acid	1.19^{c}	0.07	1.31^{c}	0.06	9.02	3.03^{b}	0.08	3.95^{a}	0.09	23.36
2. Caffeoylquinic acid	38.47^{b}	2.00	43.66^{a}	0.40	11.89	25.02^{c}	2.95	28.70^{c}	0.27	12.85
3. *p*-coumaroylquinic acid	11.53^{a}	0.58	11.81^{a}	0.26	2.40	1.49^{b}	0.11	1.72^{b}	0.03	13.70
4. Unknown compound	1.08^{c}	0.04	1.42^{a}	0.02	24.00	1.26^{b}	0.05	1.46^{a}	0.03	13.91
5. Cynnamic acid	–	–	–	–	–	0.55^{b}	0.04	0.61^{a}	0.02	9.13
6. (+)-Catechin	23.44^{a}	0.95	26.54^{a}	0.49	11.66	12.80^{b}	0.78	14.42^{b}	2.94	11.23
7. (-)-Epicatechin	62.44^{a}	0.37	63.31^{a}	0.60	1.37	47.49^{c}	0.56	51.95^{b}	2.91	8.58
8. Procyanidin-B2	92.82^{d}	2.36	147.59^{c}	10.1	37.11	126.60^{b}	7.98	190.13^{a}	5.05	33.41
9. Procyanidin dimer (*t.i.**)	17.81^{c}	0.71	34.42^{a}	1.57	48.26	16.68^{c}	1.51	24.22^{b}	0.35	31.13
10. Procyanidin dimer (*t.i.**)	28.89^{c}	2.22	58.74^{a}	2.39	50.82	40.57^{b}	3.43	68.46^{a}	6.27	40.74
11. Procyanidin trimer (*t.i.**)	156.03^{b}	4.29	177.72^{a}	2.52	12.20	63.33^{d}	2.24	85.14^{c}	1.83	25.62
12. Unknown flavonol	0.64^{b}	0.05	0.82^{a}	0.11	22.02	0.52^{b}	0.06	0.54^{b}	0.01	3.65
13. Unknown flavonol	0.63^{c}	0.05	0.86^{c}	0.05	26.76	1.31^{b}	0.16	1.68^{a}	0.09	21.74
14. Phloretin-2′-*O*-xyloglucoside (*t.i.**)	11.66^{d}	0.13	16.01^{c}	0.37	27.12	26.54^{b}	1.32	31.59^{a}	1.04	16.01
15. Phloretin-2′-*O*-glucoside (*t.i.**)	47.32^{a}	2.43	45.58^{a}	2.63	–3.82	13.44^{b}	1.09	16.00^{b}	1.96	16.01
Total hydroxycinnamic acids	52.27^{b}	2.66	58.21^{a}	0.61	10.20	31.34^{c}	3.05	36.45^{c}	0.42	14.01
Total flavan-3-ols	381.44^{c}	5.36	508.32^{a}	7.86	24.96	307.48^{d}	16.5	434.32^{b}	5.05	29.21
Total flavonols	1.27^{d}	0.09	1.68^{c}	0.17	24.45	1.83^{bc}	0.22	2.22^{ab}	0.10	17.33
Total dihydrochalcones	58.98^{a}	2.47	61.58^{a}	2.60	4.22	40.10^{c}	2.39	47.59^{b}	2.99	15.74
Total phenols	493.96^{b}	6.70	629.78^{a}	9.93	21.57	380.75^{c}	11.3	520.58^{b}	2.21	26.86

In the ''control sample'' column the results relative to fresh apples are detailed and values refer to 100 g of fresh sample. In the ''VI sample'' column, results are relative to 100 g of initial fresh sample after VI treatment (corresponding to a sample weight of 100-WR g). The RED% indicates an increase (negative values) or decrease (positive values) of the initial content after VI

(*t.i.**) tentatively identified compounds on the basis of spectral data and relative retention times in [33]

Letters a–d in superscript indicate statistically significant differences between columns (HSD Tukey $p < 0.05$)

in peels and in the outer fruit tissue just below the peel. Considering that our sample preparation consisted in fruit peeling and slicing, it is thus obvious that the external tissue of each fruit was in contact with the treatment solution, while only part of the internal tissue was exposed (in the cutting zone). As reported by Mavroudis et al. [37], in the first thin layer of apple tissue in contact with hypertonic solution cell death occurs due to strong osmotic shock. Cell death is responsible for the loss of cell structure and membrane semi-permeability and causes solid uptake and the release of inner cellular components.

Other classes of phenolic compounds present in fresh slices were the dihydrochalcones (compounds 14 and 15) and hydroxycinnamic acids (compounds 1–5). Dihydrocalchones were 9.8 and 9.1% of total phenols, respectively, in Stark and Granny Smith apple slices. While phloretin-2′-*O*-xyloglucoside decreased significantly in treated samples of Stark and Granny, the phloretin-2′-*O*-glucoside (phloridzin) content remained constant in Stark, while it showed a significant reduction of 16.01% in Granny Smith. This can be explained considering that the phloretin derivative (compound 14) could be transformed to phloridzin by the hydrolysis of a xylose residue, which should be more prevalent in Stark than in Granny. In fact, reduction of compound 14 in Stark is higher, which is likely due to the presence of both leaching and xylose hydrolysis, while in Granny Smith only the contribution of leaching should be present, although this needs further investigation. Another reason may be related to the distribution of dihydrocalcones in the different fruit areas in the two *cvv*, but this was not determined in these experiments.

The hydroxycinnamic acid group was 9.8 and 7.0% of total phenols, respectively, in Stark and Granny Smith. Among these, the most abundant compound was caffeoylquinic acid (chlorogenic acid) in both cultivars, while a small amount of cinnamic acid was found in the Granny Smith variety, but was undetectable in Stark. Nonetheless, *p*-coumaroylquinic acid was more abundant in Stark and more caffeic acid was present in Granny. The only statistically significant reductions found concerning the hydroxycinnamic derivatives was caffeic acid, unknown compound 4 and cinnamic acid in Granny slices, and

chlorogenic and compound 4 in Stark slices. The other compounds were not affected by VI.

Finally, flavonol compounds were not detected, except for compounds 12 and 13 that were probably of flavonol structure as deduced by their absorbance at 355 nm and their relative retention times. However, they were found in low abundance, although they showed a significant reduction in both cultivars after VI. This is reasonable considering that flavonols are quite exclusively present in peels and in the more external tissue.

As already seen, the changes in phenolic compounds is not a negligible phenomenon, as confirmed by other authors, for some micronutrients [20, 21], and reached more than 20% of depletion of the initial content. Moreover, it is important to note that VI frozen slices presented a small content of moisture and highly dry matter content. Thus, in Table 3 we compared the phenolic content of 100 g of fresh apples and the phenolic content of 100 g of treated apples in the two varieties.

In this case, in the third column, analytical changes between the initial sample and treated samples are expressed as:

$$\mathrm{RED}\% = \frac{C_{\mathrm{bf}} - C_{\mathrm{at}}}{C_{\mathrm{bf}}} \times 100$$

where

C_{bf} = Content of analytes in 100 g of fresh sample

C_{at} = Content of analytes in 100 g of treated sample

Due to water loss, the compounds were concentrated in the treated sample. In fact, in treated Stark and treated Granny apple slices, a total reduction in phenol content by only 11.99 and 13.37%, respectively, was found compared to fresh apples. This reduction was completely due to the loss of procyanidin. In some cases, the phenol content was higher than the initial fresh apples used. For example the phloridzin, (-)-epicatechin and *p*-coumaroylquinic acid content of VI Stark apples was significantly higher than in fresh samples. In Granny Smith only (-)-epicatechin showed such a behavior, but it can be noticed that, though not significant, there was a slight tendency towards higher values of hydroxycinnamic acids compared to fresh apples. Thus, the treated VI samples in both varieties had a phenolic content similar to fresh apples.

Table 3 HPLC-DAD determination of phenolic compounds in vacuum impregnated apple slices from Granny Smith and Stark Delicious varieties, comparing the phenolic content of 100 g of fresh apples and the phenolic content of 100 g of treated apples in the two varieties

Compound (mg)	VI sample (100 g)		Control Sample (100 g)		Stark	VI sample (100 g)		Control Sample (100 g)		Granny
	Mean	SD	Mean	SD	RED %	Mean	SD	Mean	SD	RED %
1. Caffeic acid	1.34^{c}	0.07	1.31^{c}	0.06	–2.08	3.59^{b}	0.09	3.95^{a}	0.09	9.23
2. Caffeoylquinic acid	43.17^{a}	2.24	43.66^{a}	0.40	1.14	29.63^{b}	3.49	28.70^{b}	0.27	–3.22
3. *P*-coumaroylquinic acid	12.93^{a}	0.65	11.81^{b}	0.26	–9.52	1.76^{c}	0.13	1.72^{c}	0.03	–2.22
4. Unknown compound	1.22^{b}	0.04	1.42^{a}	0.02	14.72	1.49^{c}	0.06	1.46^{c}	0.03	–1.97
5. Cynnamic acid	–	–	–	–	–	0.66^{a}	0.04	0.61^{a}	0.02	–7.63
6. (+)-Catechin	26.31^{a}	1.06	26.54^{a}	0.49	0.88	15.16^{b}	0.93	14.42^{b}	2.94	–5.14
7. (-)-Epicatechin	70.06^{a}	0.41	63.31^{b}	0.60	–10.66	56.25^{c}	0.66	51.95^{d}	2.91	–8.28
8. Procyanidin-B2	104.15^{c}	2.65	147.59^{b}	10.1	29.43	149.95^{b}	9.45	190.13^{a}	5.05	21.13
9. Procyanidin dimer (*t.i.**)	19.98^{c}	0.80	34.42^{a}	1.57	41.94	19.76^{c}	1.78	24.22^{b}	0.35	18.43
10. Procyanidin dimer (*t.i.**)	32.42^{d}	2.49	58.74^{bc}	2.39	44.81	48.05^{c}	4.06	68.46^{ab}	6.27	29.81
11. Procyanidin trimer (*t.i.**)	175.08^{a}	4.82	177.72^{a}	2.52	1.48	75.01^{c}	2.65	85.14^{b}	1.83	11.90
12. Unknown flavonol	0.71^{ab}	0.05	0.82^{a}	0.11	12.50	0.62^{b}	0.07	0.54^{b}	0.01	–14.12
13. Unknown flavonol	0.71^{b}	0.05	0.86^{b}	0.05	17.82	1.55^{a}	0.19	1.68^{a}	0.09	7.31
14. Phloretin-2'-*O*-xyloglucoside (*t.i.**)	13.09^{c}	0.15	16.01^{b}	0.37	18.23	31.43^{a}	1.56	31.59^{a}	1.04	0.52
15. Phloretin-2'-*O*-glucoside (*t.i.**)	53.10^{a}	2.73	45.58^{b}	2.63	–16.49	16.07^{c}	1.29	16.00^{c}	1.96	–0.42
Total hydroxycinnamic acids	58.65^{a}	2.98	58.21^{a}	0.61	–0.76	37.12^{b}	3.61	36.45^{b}	0.42	–1.85
Total flavan-3-ols	428.00^{b}	6.01	508.32^{a}	7.86	15.80	364.18^{c}	19.5	434.32^{b}	5.05	16.15
Total flavonols	1.42^{b}	0.10	1.68^{b}	0.17	15.23	2.17^{a}	0.26	2.22^{a}	0.10	2.09
Total dihydrochalcones	66.18^{a}	2.77	61.58^{a}	2.60	–7.47	47.49^{b}	2.84	47.59^{b}	2.99	0.20
Total phenols	554.25^{b}	7.52	629.78^{a}	9.93	11.99	450.97^{d}	13.3	520.58^{c}	2.21	13.37

The RED% indicates an increase (negative values) or decrease (positive values) of the initial content after VI

(*t.i.**) tentatively identified compounds on the basis of spectral data and relative retention times in [33]

Letters a–d in superscript indicate statistically significant differences (HSD Tukey $p < 0.05$)

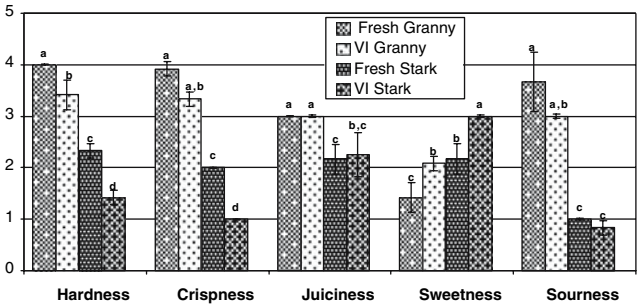

Fig. 1 Results of sensory evaluation of both fresh and VI + cryofreezing Stark Delicious and of fresh and VI and cryofreezing Granny Smith apple slices. Letters a–d in bold indicate statistically significant differences (HSD Tukey $p < 0.05$)

The second aim of the present work was to evaluate the effects of VI and cryofreezing on the sensory properties of the two apple cultivars compared to fresh material. In Fig. 1 the results of sensory evaluation are shown. The lack of a control sample (apples frozen without any previous treatment) is principally due to the very low sensory level of taste acceptance and the unwillingness of judges to test unpleasant slices. Untreated frozen apple slices had no hardness and crispness, and underwent rapid enzymatic browning and extreme loss of internal juice. Such phenomena were not present in the treated apples.

As is evident, fresh Granny Smith apple slices presented higher values of hardness, crispness, juiciness and sourness than Stark Delicious. Sourness was very high in Granny Smith apples, while it was quite low in Stark. Finally, sweetness was slightly higher in Stark.

The VI and cryofreezing process caused a depletion of texture parameters, but treated Granny Smith apples conserved about 85% of initial hardness and crispness, while Stark had only 50% of hardness and 60% of crispness. Juiciness did not seem to be affected by the dehydrofreezing process in either variety. Granny Smith also presented a decrease in sourness, while Stark was not affected. Sweetness increased in both varieties, reaching very high values in Stark.

Conclusion

Vacuum impregnation prior to freezing preserved the sensory characteristics of apples, resulting in retention of texture and flavor. When compared to fresh raw material, Granny Smith presented a higher retention of texture parameters and was less affected by the VI + freezing process.

With regards to the behavior of phenolic compounds after VI, we found a comparable depletion of more than 20% in Stark and 25% in Granny of total phenols. In reality the phenolic decrease was mostly due to procyanidins and only slightly due to hydroxycinnamic and dihydrochalcones. Not withstanding, there were differences in the decrease of individual compounds. Indeed, in addition to leaching of solutes in the treatment solution, other reactions could take place such as hydrolysis of sugar residues or other consumption reactions (among which the most important is oxidation). In these experiments, oxidation of phenolics was prevented by using 1.0% ascorbic acid in the treatment solution.

Nonetheless, it was noticed that the phenolic leaching occurring during the VI treatment is compensated by the concentration effect due to the loss of water at the end of the process. In fact, 100 g of VI frozen samples presents nearly the same content of antioxidants as 100 g of fresh sample.

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ERRATUM

Effect of vacuum impregnation on the phenolic content of Granny Smith and Stark Delicious frozen apple *cvv*

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Erratum to: Eur Food Res Technol
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The original version of this article unfortunately contained errors. In the "Results and discussion" section, page 5 of the paper, line 26, value 520.58 must be substituted with value 52.058.

The captions of Table 1–3 were incorrect. The correct versions are given below.

- **Table 1.** Data of *o*-diphenol and ABTS assay must be considered one order of magnitude less (one tenth)
- **Table 2.** Phenolic content data and standard deviation must be considered one order of magnitude less. RED% remain the same
- **Table 3.** Phenolic content data and standard deviation must be considered one order of magnitude less. RED% remain the same

The online version of the original article can be found under doi:10.1007/s00217-007-0624-x.

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SHORT COMMUNICATION

Effect of frozen storage on the phenolic content of vacuum impregnated Granny Smith and Stark Delicious apple *cvv*.

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Abstract The phenolic fraction modification was evaluated in untreated vacuum impregnated frozen apple slices from two varieties (Granny Smith and Stark Delicious) a few days after freezing and after 12 months of frozen storage. After 12 months the phenolic content of both fresh and vacuum impregnated apple slices varied greatly. The most important change was the increase of many polymeric flavan-3-ols, which may be due to hydrolysis of polymeric procyanidins. Furthermore, there was significant correlation ($r^2 = 0.52$, $P < 0.05$) between the increase in flavan-3-ols and the decrease in pH. In fact, control Stark slices presented the highest pH, and had no statistically significant increase in flavan-3-ols. Other classes of phenolic compounds were decreased in apple slices following frozen storage.

Keywords Apples · Phenols · Vacuum impregnation · Frozen storage

Introduction

Fresh fruits contain nutritional and healthful constituents such as minerals, Vitamins A, C, and E, dietary fibre, folates, carotenoids and selenium in addition to phytochemicals such as, glucosinolates, flavonoids, phenolic acids and lycopene. Recently, polyphenols have received increasing interest due to their anti-inflammatory, antihistaminic and anti-tumour activities, and also act as free radical scavengers and protect against cardiovascular disease [1–3]. The most important polyphenolic compounds present in apple are phenolic acids and flavonoids such as flavanols or catechins, flavonols, dihydrocalchones and anthocyanidins, while their content depends on several variables [4–6]

Osmotic treatments prior to freezing are used to produce several kinds of food products that can be stored for lengthy periods and present a good quality of texture, colour and flavour after thawing [7–10]. Due to the mild processing conditions used, osmotic treatments have minimal impact on the nutritional and sensory quality of foods [11–13].

Vacuum impregnation (VI) is a recent development in the osmotic treatment of foods [14–15]. During VI processes, in addition to impregnation with treating solutions, other physical–chemical processes such as osmosis and diffusion also occur.

Several studies have been performed on the chemical changes of osmotically treated foods, although they have mainly focused on the profile of volatile compounds. Modifications in the aromatic profile depend on osmotic process variables, such as temperature, solute type and concentration, pressure and solution/fruit ratio [16]. For example, osmotic treatment results in a greater retention of Vitamin C and chlorophyll of frozen kiwifruit slices during storage at −10 °C, and treatment of strawberries with different sugar solutions can also stabilise the anthocyanin content [17].

In a previous report [18] we studied the sensory characteristics and phenolic fraction in both untreated and treated (vacuum impregnated) apple slices from two varieties (Granny Smith and Stark Delicious). Therein it was concluded that vacuum impregnation prior to freezing preserved the sensory characteristics of apples, resulting in

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retention of texture and flavour. In fact, when compared to fresh raw material Granny Smith presented a higher retention of texture parameter than Stark Delicious, and was less affected by the VI and freezing processes.

With regards to the behaviour of phenolic compounds after VI, we found a comparable depletion of total phenols of more than 20% in Stark and 25% in Granny. This decrease in the phenolic content was mostly due to procyanidins, and less so to hydroxycinnamic and dihydrochalcones. Not withstanding, there were decreases in individual compounds. Indeed, in addition to leaching of solutes in the treatment solution, other reactions take place such as hydrolysis of sugar residues or other consumption reactions (among which the most important is oxidation). In those experiments, the oxidation of phenolics was prevented by the addition of 1.0% ascorbic acid in the treatment solution.

Finally, looking at the slices obtained by the dehydro-freezing process (VI), on a fresh weight basis the decrease in phenolics was proportionate to their concentration, and the final product presents nearly the same content of antioxidants.

In the present communication we have investigated the behaviour of polyphenolic compounds in both untreated and vacuum impregnated frozen apple slices from two varieties (Granny Smith and Stark Delicious) after 12 months of storage at -18°C.

Experimental

Samples

Sample preparation has been described in detail elsewhere [18]. Briefly two varieties of apples (Granny Smith and Stark Delicious) were bought at a local market. Both fresh slices (control samples) and vacuum impregnated slices (VI samples) from both types of apples were analysed. The VI apples were placed in a pilot vacuum candying plant and vacuum impregnated with an aqueous solution containing 37.9% dextrose, 15.2% sucrose, 1.0% ascorbic acid, 0.25% calcium chloride and 0.25% sodium chloride. The impregnation time was 30 min at a vacuum pressure of 100 mbar. The temperature was maintained at 25 °C during all processing times and the solution/fruit ratio was 11:1. At the end of the VI step, samples were left for 5 min in syrup and then rinsed with water, drained in a vibrating screen and finally frozen in a Cryo CM 85 liquid nitrogen freezer (Linde Gas Italia) at −73 °C in 7 min. Samples were stored at −18 °C.

Extraction of phenolic compounds and HPLC-DAD phenolic determination

Analysis of phenolic compounds was carried out 12 months after VI treatment. During the entire storage period the temperature was maintained at −18 °C. HPLC analysis is detailed in [18]. Briefly a phenolic extract was prepared from lyophilized samples by extracting about 3 g of powder with 10 ml of aqueous methanol (20%) (Merck, Darmstadt, Germany) in a centrifuge tube with Ultraturrax (IKA-Werke mod. T 25 basic, Staufen, Germany) at 15,000 rpm for 3 min. The tube was placed in a sonicated bath at 30 °C for 44 min and then centrifuged at 22,000 rpm (39,600*g*) for 10 min at 30 °C (Avanti J25, Beckman Coulter, USA). The supernatant was recovered and filtered through a 45 µm cellulose acetate filter (Whatman, Clifton NJ, USA) and used for HPLC analysis. HPLC analysis was carried out on a Jasco HPLC (Jasco corporation, Tokyo, Japan), equipped with a binary pump (Jasco PU 1580 intelligent HPLC pump), an autosampler (Jasco AS 2055 plus intelligent sampler) and an HP Diode-Array UV-VIS Detector (Jasco MD 1510 multiwavelength detector). A Luna C18 (Phenomenex, St Torrance CA, USA) column (5 µm particle size, 250 mm × 3.00 mm ID) was used and 20 µl of phenolic extract were injected. All solvents were filtered through a 0.45 µm nylon filter disk (Lida Manufacturing, Kenosha WI, USA).

Solvents for the mobile phase were A: 1% formic acid in water and B: 1% formic acid in methanol. Identification of phenolic compounds was performed on the basis of the retention time of a relative standard when available or tentatively performed on the basis of UV-VIS spectral characteristics and relative retention time according to literature data [18]. Quantification of individual compounds was performed on the basis of a external calibration curve of the relative standard (when available) or on the basis of a standard of the same phenolic class: hydroxycinnamic acids were quantified with chlorogenic acid at 320 nm, procyanidins with procyanidin B2 at 280 nm, (+)-catechin, phloretin derivatives and phloridzin with (−)-epicatechin at 280 nm, and flavonols with quercetin at 360 nm.

Statistical analysis

Data were analysed using Statistica 6.0 (Statsoft, Tulsa OK, USA) software. The significance of differences at a 5% level between averages was determined by one-way ANOVA using Tukey's test.

Results and discussion

After 12 months of storage the phenolic content of both treated and untreated apple slices varied greatly (Table 1). It should be remembered that control samples and VI samples initially differed in terms of acidity, ascorbic acid content and dry matter due to the solid gain and water loss that occurred during the VI treatment. In this paper [18] the initial chemical characteristics are reported. In addition we

Table 1 Phenolic content of fresh and vacuum impregnated apples slices from Stark Delicious and Granny Smith *cvv*. (t0) and (t1) columns indicate, respectively control time (see reference [18]) and after 12 months of storage

Compound (mg/kg fresh weight)	VI sample–Stark					Control sample–Stark					VI sample–Granny					Control sample–Granny				
	Mean (t0)	SD	Mean (t1)	SD	Var%	Mean (t0)	SD	Mean (t1)	SD	Var%	Mean (t0)	SD	Mean (t1)	SD	Var%	Mean (t0)	SD	Mean (t1)	SD	Var%
1. Caffeic acid	1.34^{de}	0.07	1.53^{d}	0.00	14.34	1.31^{e}	0.06	1.24^{e}	0.03	−5.01	3.59^{b}	0.09	2.25^{c}	0.07	−37.44	3.95^{a}	0.09	2.13^{c}	0.08	−45.96
2. Caffeoylquinic acid	43.17^{a}	2.24	42.17^{a}	0.68	−2.32	43.66^{a}	0.40	31.72^{b}	0.25	−27.36	29.63^{b}	3.49	23.90^{c}	1.28	−19.32	28.70^{b}	0.27	20.67^{c}	0.92	−27.97
3. *p*-Coumaroylquinic acid	12.93^{a}	0.65	6.66^{c}	0.10	−48.48	11.81^{b}	0.26	6.37^{c}	0.07	−46.03	1.76^{d}	0.13	0.81^{e}	0.03	−54.16	1.72^{d}	0.03	0.86^{e}	0.06	−49.84
4. Unknown compound	1.22^{b}	0.04	0.82^{d}	0.01	−32.38	1.42^{a}	0.02	0.84^{d}	0.01	−40.66	1.49^{a}	0.06	0.85^{d}	0.03	−42.76	1.46^{a}	0.03	0.97^{c}	0.04	−33.48
5. Cinnamic acid	ND	–	ND	–	–	ND	–	ND	–	–	0.66^{a}	0.04	ND	–	–	0.61^{a}	0.02	ND	–	–
6. (+)-Catechin	26.31^{d}	1.06	65.98^{a}	0.87	150.77	26.54^{d}	0.49	47.28^{b}	1.61	78.16	15.16^{e}	0.93	36.06^{c}	1.98	137.86	14.42^{e}	2.94	39.58^{c}	1.58	174.50
7. (−)-Epicatechin	70.06^{b}	0.41	93.95^{a}	1.72	34.10	63.31^{bc}	0.60	62.82^{bc}	1.35	−0.77	56.25^{cd}	0.66	51.42^{d}	0.72	−8.59	51.95^{d}	2.91	64.19^{b}	5.18	23.55
8. Procyanidin-B2	104.15^{e}	2.65	159.31^{cd}	4.16	52.97	147.59^{d}	10.1	181.60^{bc}	8.41	23.05	149.95^{d}	9.45	179.96^{bc}	14.38	20.01	190.13^{b}	5.05	275.65^{a}	10.68	44.98
9. Procyanidin dimer (t.i.)	19.98^{e}	0.80	45.52^{ab}	1.02	127.82	34.42^{c}	1.57	48.70^{a}	1.02	41.49	19.76^{e}	1.78	27.86^{d}	1.73	40.98	24.22^{d}	0.35	43.62^{b}	1.26	80.12
10. Procyanidin dimer (t.i.)	32.42^{f}	2.49	96.03^{b}	2.01	196.20	58.74^{de}	2.39	96.63^{b}	3.21	64.50	48.05^{e}	4.06	79.81^{c}	6.67	66.09	68.46^{cd}	6.27	127.88^{a}	7.18	86.79
11. Procyanidin trimer (t.i.)	175.08^{a}	4.82	82.78^{c}	3.50	−52.72	177.72^{a}	2.52	87.64^{c}	2.33	−50.69	75.01^{cd}	2.65	65.67^{d}	4.99	−12.45	85.14^{c}	1.83	114.57^{b}	7.83	34.56
12. Unknown flavonol	0.71^{abc}	0.05	0.54^{cd}	0.02	−24.10	0.82^{ab}	0.11	0.60^{cd}	0.02	−27.31	0.62^{bcd}	0.07	0.44^{d}	0.06	−28.98	0.54^{cd}	0.01	0.91^{a}	0.09	69.21
13. Unknown flavonol	0.71^{cd}	0.05	0.62^{d}	0.02	−12.61	0.86^{cd}	0.05	0.69^{cd}	0.01	−19.61	1.55^{ab}	0.19	0.91^{c}	0.06	−41.14	1.68^{a}	0.09	1.33^{b}	0.05	−20.87
14. Phloretin-2′-*O*-xyloglucoside	13.09^{c}	0.15	15.84^{c}	0.42	21.02	16.01^{c}	0.37	16.88^{c}	0.46	5.41	31.43^{b}	1.56	33.72^{b}	1.70	7.27	31.59^{b}	1.04	38.58^{a}	2.06	22.14
15. Phloretin-2′-*O*-glucoside	53.10^{a}	2.73	57.38^{a}	1.57	8.05	45.58^{b}	2.63	53.20^{a}	0.82	16.72	16.07^{cd}	1.29	13.54^{d}	1.01	−15.77	16.00^{cd}	1.96	19.81^{c}	1.24	23.83
Total hydroxycinnamic acids	58.65^{a}	2.98	51.19^{b}	0.79	−12.72	58.21^{a}	0.61	40.18^{c}	0.36	−30.98	37.12^{c}	3.61	27.81^{d}	1.36	−25.08	36.45^{c}	0.42	24.64^{d}	1.06	−32.40
Total flavan-3-ols	428.00^{c}	6.01	543.57^{b}	1.37	27.00	508.32^{b}	7.86	524.68^{b}	10.91	3.22	364.18^{d}	19.50	440.77^{c}	25.23	21.03	434.32^{c}	5.05	665.49^{a}	32.28	53.22
Total flavonols	1.42^{bc}	0.10	1.16^{c}	0.05	−18.36	1.68^{b}	0.17	1.29^{c}	0.03	−23.37	2.17^{a}	0.26	1.35^{bc}	0.10	−37.67	2.22^{a}	0.10	2.24^{a}	0.09	1.04
Total dihydrochalcones	66.18^{abc}	2.77	73.22^{a}	1.91	10.63	61.58^{b}	2.60	70.08^{a}	0.65	13.80	47.49^{c}	2.84	47.25^{c}	2.38	−0.50	47.59^{c}	2.99	58.40^{b}	3.19	22.71
Total phenols	554.25^{c}	7.52	669.13^{b}	1.62	20.73	629.78^{b}	9.93	636.22^{b}	11.90	1.02	450.97^{d}	13.30	517.19^{c}	28.51	14.68	520.58^{c}	2.21	750.76^{a}	35.27	44.22

Var% refers to changes in compounds after (t1) storage with respect to t0: positive values indicate an increase, while negative values indicate a decrease. Superscript letters a–d indicate statistically significant differences between columns (HSD Tukey $P < 0.05$)

ND not detected; *t.i.* tentatively identified

determined dry matter after 12 months storage, which was unchanged (data not shown).

In Table 1 the percentage variation (var %) of individual phenolic compounds is shown. Positive values of var % indicates an increase, whereas negative values indicate a reduction.

It should be noted that a similar increase in total phenols occurred in VI samples in both Granny Smith and Stark Delicious varieties. In control samples there was a different behaviour: in fact, control Granny Smith slices presented the highest increase in phenolics, while control Stark slices showed no such increase. The increase in total phenolics followed the same trend as the increase in flavan-3-ols in all samples. In particular (+)-catechin presented the greatest increase compared to (−)-epicatechin and other procyanidins. This could be explained by hypothesising that hydrolysis of polymeric procyanidins occurred during the frozen storage. This is confirmed by observation that the only flavan-3-ol compound that decreased during frozen storage (in three of four samples) was the procyanidin trimer (compound 11). Other procyanidins might have been formed from this compound. In any case other polymeric procyanidins should be present but were not detected with the analytical methods employed.

Furthermore, there was a correlation ($r^2 = 0.52$, $P < 0.05$) between the increase in flavan-3-ols and the decrease in pH. In fact, control Stark slices presented the highest pH, and had no statistically significant increase in flavan-3-ols.

A comparable behaviour of flavan-3-ols was observed in the dihydrochalcones class (compound 14 and 15), although only in a few samples.

In contrast hydroxycinnamic acids and flavonols generally showed a decrease that may be attributable to degradation pathway via enzymatic or hydrolytic reactions.

It is known that enzyme activity is critically impaired at −18 °C, although residual activity could be present in the unfrozen rubbery-state water fraction. Thus in frozen foods, both enzymatic and hydrolytic reactions could take place in this fraction. In particular lyases could convert polyglycosylated phenols to tri-di-mono glycosylated phenols or could liberate the aglycon moiety. Moreover acidic hydrolysis of polymeric polyphenols in fruit could occur. To the best of our knowledge there are no other reactions that could take place.

In conclusion, during the frozen storage of apple slices there is an apparent increase in many phenolic substances, but this is likely due to hydrolysis of polymeric phenols. This increase seems to be independent of the VI treatment applied, and is better correlated with pH and titratable acidity values.

Other phenolic compounds also decrease significantly in apple slices probably due to PPO activity, although oxidation should be prevented in VI samples due to the high ascorbic acid content derived from the VI treatment and the very low polyphenoloxidase (PPO) activity at −18 °C.

In future investigations we will attempt definitive identification of phenolic compounds, and in particular will study the hydrolysis of procyanidins by identifying the different polymeric procyanidin compounds and monomers that are formed. Moreover a thorough study of enzymatic activities and pathways in frozen fruit during storage is deemed necessary to verify the results obtained in the present analysis.

Finally, the results obtained after 12 months storage provide preliminary insight into the phenolic changes during frozen storage of fresh and vacuum impregnated apples. Lastly, it would be worthwhile to examine the storage effects at intermediate storage times to better understand the changes in phenolic compounds during storage.

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Osmotic dehydrofreezing of strawberries: Polyphenolic content, volatile profile and consumer acceptance

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ABSTRACT

We evaluated dehydrofreezing in strawberries in terms of retention of healthy compounds (i.e. polyphenolics) and sensory qualities for direct consumption in substitution of fresh fruit. Different osmodehydration (OD at 30 °C and 5 °C), vacuum osmodehydration (VOD at 30 °C) and immersion chilling freezing (ICF) processes were applied in sucrose syrup. Samples were analyzed for dry matter, soluble solids, pH, titratable acidity, mass transfers, polyphenolic content by HPLC-DAD/MSD, volatile profile by SPME-GC/MSD and consumer acceptance. The results of sensory evaluation, in particular, confirmed the cryoprotective effects of osmotic processes of fruits with respect to untreated frozen control samples. The OD samples at 5 °C presented a water loss to solid gain ratio comparable to OD at 30 °C and VOD samples. Moreover, while osmo-dehydrofreezing at relatively high temperatures caused a slight depletion of phenolic compounds, the samples osmodehydrated at 5 °C showed higher polyphenolic retention. Data on aromatic compounds showed that some compounds increased (e.g. ethanol and acetaldehyde), while others did not appear to be affected by the pre-treatments.

1. Introduction

Strawberries are very popular fruits available during the spring–summer period, although they are highly perishable and susceptible to bruises and fungal attacks. A good way to preserve strawberries (and fruits in general) is through the use of freezing technologies that combine low temperature and water activity (a_w) reduction associated with the cryoconcentration of the fruit liquid phase during ice crystal formation. However, because of the high freezable water content of strawberries, freezing leads to significant cellular damage, and several chemical–physical and organoleptical deteriorations take place, especially when fruits are thawed, with subsequent loss of product quality (Blanda, Cerretani, Bendini, Cardinali, & Lercker, 2008; Martínez-Navarrete et al., 2001).

In recent years, several studies have highlighted the importance of dehydration pre-treatment before freezing process (dehydrofreezing) in order to reduce the water content and limit ice crystal damage in foods (Chiralt et al., 2001). Osmotic treatments prior to freezing are used to produce several kinds of fruits that can be stored for long periods of time with good retention of texture, colour and flavour after thawing (Dalla Rosa & Spiess, 2000; Maestrelli, Lo Scalzo, Lupi, Bertolo, & Torreggiani, 2001; Sormani, Maffi, Bertolo, & Torreggiani, 1999).

During osmotic dehydration in hypertonic solutions, two important mass transfers are driven from concentration gradients: flow of water from the food matrix to the liquid and flow of solute from the liquid to the food matrix. A third mass transfer involves food solutes leaching in the solution. Although leaching has been generally considered to be quantitatively negligible (Dixon & Jen, 1977), it can result in loss of the nutritional content of foods. This has been demonstrated by Peiró-Mena, Dias, Camacho, and Martínez-Navarrete (2006) and Peiró-Mena, Camacho, and Martínez-Navarrete (2007) who found that the amount of citric acid, ascorbic acid, galacturonic acid and other components from grapefruit and pineapple decreased after immersion in concentrated solutions. We have also demonstrated depletion of the polyphenolic content in vacuum impregnated apple slices, mainly due to solute leaching in the treating solution (Blanda, Cerretani, Bendini, Cardinali, Scarpellini, & Lercker, 2008).

In reality, during osmotic processes, "osmosis" is not the only phenomenon taking place, and other phenomena connected to cell viability, membranes integrity, enzyme activity, hydrolysis of polymeric compounds, fermentative metabolism, etc., occur. All these changes depend on the particular technique used, i.e. osmotic dehydration (OD), vacuum and pulsed vacuum osmotic dehydration (VOD, PVOD), immersion chilling freezing (ICF) and conditions used (time, temperature, type of solutions, pressure, type of foods

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and foods previous handling and processing, etc.) (Fito, Chiralt, Barat, Spiess, & Behsnilian, 2001; Lucas & Raoult-Wack, 1998).

In strawberries, changes may occur at any level and involve the cell structure of fruits and chemical composition (Chiralt & Talens, 2005). Processed fruits undergo changes in sensory attributes such as texture and colour (Chiralt et al., 2001; Moraga, Martínez-Navarrete, Martínez-Monzó, & Chiralt, 2000), as well as changes in the profile of volatile compounds (Escriche, Chiralt, Moreno, & Serra, 2000; Talens, Escriche, Martìnez-Navarrete, & Chiralt, 2002). Other quality attributes, such as taste or flavour related to fruit composition like major sugars and acidity, may also be altered during these processes (Viberg & Sjoholm, 1998).

Recently, we have assessed the use of dehydrofreezing for obtaining frozen fruits (apples and peaches) with a high content of healthy phytochemicals (polyphenolic retention) and favourable sensory qualities for direct consumption in substitution of fresh fruit (Blanda, Cerretani, Bendini, Cardinali, & Lercker, 2008; Blanda, Cerretani, Bendini, Cardinali, Scarpellini, & Lercker, 2008) In the present study, we pre-treated strawberry fruits using different processes, and after freezing we evaluated changes in chemical properties with respect to fresh raw material. Consumer acceptance was also assessed after thawing.

2. Materials and methods

2.1. Instruments

Phenolic extracts HPLC analyses were performed with an HP 1100 series instrument (Agilent Technologies, Palo Alto, CA), equipped with a binary pump delivery system, a degasser, an autosampler, a diode array UV–vis detector (DAD) and a mass spectrometer detector (MSD). The HPLC column used was a C_{18} Luna column, 5 μm, 15 cm × 3.0 mm (Phenomenex, Torrance, CA), with a C_{18} pre-column (Phenomenex) filter.

2.2. Reagents, stock solutions and reference compounds

p-Cumaric acid, pelargonidin chloride, ellagic acid and kaempferol were acquired from Sigma (St. Louis, MO). Stock solutions containing these analytes were prepared in methanol at a concentration of 2000 μg mL^{-1} for *p*-cumaric, 2000 μg mL^{-1} for pelargonidin, 2000 μg mL^{-1} for ellagic acid and 2500 μg mL^{-1} for kaempferol. These compounds were used to construct calibration curves in a range of 1–500 μg mL^{-1}. Methanol and HPLC-grade water were from Merck (Darmstadt, Germany). Distilled water was deionized using a Milli-Q system (Millipore, Bedford, MA).

2.3. Samples

Strawberries (*cv.* Alba) were bought at a local market. From 48 kg of strawberries at the pink stage of ripeness, fruits without damage or fungal attacks (ranging from 23 to 27 g) were selected to obtain a final sample of 24 kg. After stalk removal, the entire sample was randomly divided into six aliquots of 4 kg each.

(1) Fresh strawberries (FR sample): whole fruits were washed in tap water, drained and immediately analyzed as described below.
(2) Fresh frozen strawberries (TQ sample): whole fruits were washed in tap water, drained and immediately frozen in a freezing chamber in direct contact with dry ice pellets (2 mm diameter). After 30 min, fruits reached a temperature of −79.8 °C and were then stored in a conventional freezer at −18 °C for 1 month until analysis as described below.
(3) Immediately chilled-frozen strawberries (ICF sample): washed and drained fruits were submerged in a sucrose solution (69 g of sucrose in 100 g of solution) (which remained liquid at the temperature used) kept at constant temperature of −19 °C for 24 h. The fruit:syrup ratio was 1:5 (w/w). At the end of the process, strawberries were accurately and rapidly washed with cold water and stored at −18 °C in a conventional freezer for 1 month.
(4) Osmodehydrated strawberries (OD30 sample): whole fruits were washed in tap water, drained and placed in an impregnating chamber. Sucrose syrup (50 g of sucrose in 100 g of solution) was then added and fruits were kept submerged using a grid. The conditions were adapted from Escriche et al. (2000); briefly, the fruit:syrup ratio was 1:5 (w/w), and the syrup temperature was kept constant during the processing (30 °C). Osmotic dehydration was continued for 4 h. At the end of the process, fruits were accurately drained from residual syrup and quickly frozen as described previously. They were then stored for 1 month at −18 °C in a conventional freezer.
(5) Osmodehydrated strawberries (OD5 sample): fruits were processed as sample OD30, but the process temperature was 5 °C (kept constant during the processing in a controlled refrigerator) with a processing time of 24 h. At the end of the process, fruits were accurately drained from residual syrup, quickly frozen as described previously and stored for 1 month at −18 °C in a conventional freezer.
(6) Vacuum impregnated + osmodehydrated strawberries (VOD sample): whole fruits were washed in tap water, drained and put in a vacuum chamber connected to a vacuum pump. Sucrose syrup (50 g of sucrose in 100 g of solution) was then added and fruits were kept submerged using a grid. Conditions used were adapted from Escriche et al. (2000); the fruit:syrup ratio was 1:5 (w/w) and the syrup temperature was kept constant during processing (30 °C). A vacuum step at 100 mbar was applied for 5 min. After this, osmotic dehydration was continued for 4 h. At the end of the process, fruits were accurately drained from residual syrup and quickly frozen as described and stored for 1 month at −18 °C in a conventional freezer until analysis.

For each subsample, analyses were carried out in triplicate on aliquots of 19 strawberries (about 475 g). Due to the high variability in strawberry chemical features (sequential ripening of fruit), the representativeness of aliquot size was studied by analyzing eight aliquots of the FR subsample. The results showed that an aliquot size of 19 strawberries was a good compromise between subsample representativeness and laboratory constraints. In the Section 3 we reported the results of two of the eight aliquots analyzed for the FR subsample (FR3 and FR4). These two aliquots presented the greatest differences in the total phenolic content, and thus they represented the extent of variability of raw material.

2.4. Analyses of dry matter, soluble solids, titratable acidity

Dry matter (DM), pH, soluble solids (SS) and titratable acidity (TA) of fresh and frozen slices were calculated according to AOAC (2000).

2.5. Mass transfer determination

Solid gain (SG), water loss (WL) and weight reduction (WR) were calculated as described by Giangiacomo, Torreggiani, & Abbo (1987) using the equation:

$$WL = \frac{w_{wo} - (w_t - w_{st})}{(w_{wo} + w_{so})}$$

$$SG = \frac{(w_{st} - w_{so})}{(w_{wo} + w_{so})}$$

$$WR = WL - SG$$

where w_{wo} = mass of water in fruit before treatment; w_t = mass of fruits after treatment; w_{st} = mass of solids (dry matter) in fruit after treatment; and w_{so} = mass of solids (dry matter) in fruit before treatment.

2.6. Phenolic extract

Phenolic extracts were obtained by adapting the method from Gil, Holcroft, & Kader (1997). Briefly, 19 fruits (fresh or frozen) were ground in a blender and then 5 g of homogenate was extracted with 15 mL of 95% aqueous methanol (v/v) in a centrifuge tube and homogenized with an Ultra Turrax (IKA-Werke T 25 basic, Staufen, Germany) at 15,000 rpm for 3 min. Following this, the tube was centrifuged at 22,000 rpm (39,600 *g*) for 10 min at 10 °C (Avant J25, Beckman Coulter, Fullerton, CA). The supernatant was recovered and a second extraction was performed on the sample residue. The two extractions were then combined and evaporated in a vacuum centrifuge to complete dryness (MIVAC DUO, Genevac, Ipswich, England). The concentrated sample was dissolved in 5 mL of acidified water (3% v/v formic acid) and then passed through a Strata C_{18}-E 55 μm 70 A cartridge (Phenomenex) that had been previously activated with methanol followed by water and then acidified water (3% v/v formic acid). Anthocyanins and other phenolics were adsorbed onto the column while sugars, organic acids and other highly water soluble components were eluted with 10 mL of acidified water (3% v/v formic acid). Anthocyanins and other phenolic compounds were then recovered with 1.8 mL of methanol containing formic acid (3% v/v). Both the water and methanolic extracts were filtered through a 0.45 μm filter (Whatman, Clifton, NJ) and injected in HPLC for analysis of phenolics as described below.

2.7. HPLC-DAD/MS phenolic analysis

Detector wavelengths were set at 280, 320, 350 and 520 nm. Identification was also made using MSD using an electrospray (ESI) interface operating in positive and negative mode using the following conditions: drying gas flow, 9.0 L min^{-1}; nebulizer pressure, 50 psi; gas drying temperature, 350 °C; capillary voltage, 3000 V; fragmentor voltage, 60 V. Phenolic compounds were tentatively identified based on their UV–vis and mass spectra obtained by HPLC-DAD/MSD (Table 1). Mobile phases were: A, acidified water (2.5% v/v formic acid); and B, methanol containing formic acid (3% v/v). The elution gradient was linear: at 0 min 85% solvent A held for 5 min, from 5 to 20 min 65% A was reached and held constant until 25 min, from 25 to 35 min 50% held constant until 45 min, from 45 to 50 min 34% and finally at 59 min 85% solvent A was restored. A 10 min post run equilibration was performed.

To carry out quantification in HPLC-DAD, five standard calibration curves were constructed using four commercial reference compounds, *p*-cumaric acid, pelargonidin, ellagic acid and kaempferol. Anthocyanins (compounds **1**–**6** in Table 1) were quantified using the calibration curve of pelargonidin at 520 nm ($r^2 = 0.9952$); compound **7** was quantified using the ellagic acid curve at 280 nm ($r^2 = 0.9995$); phenolic acids (compounds **8**–**12**) were quantified on the basis of a *p*-cumaric standard calibration curve at 320 nm ($r^2 = 0.9926$); ellagic acid (compound **15**) and ellagic derivatives (compound **13**) were quantified using the ellagic acid curve at 350 nm, respectively ($r^2 = 0.9998$); finally, flavonols (compounds **14** and **16**–**19**) were quantified using a kaempferol calibration curve at 350 nm ($r^2 = 0.9890$). The total phenolic content (TP) was determined as the sum of phenolic compounds identified and quantified.

2.8. SPME-GC/MSD analysis

Nineteen frozen strawberries (about 475 g) were thawed at room temperature until the temperature reached −3 °C. Strawberries were then cut in small pieces, uniform in size (cubes of about 3 mm edge), and the pieces were mixed. About 37.8 g of cubes were placed in a 135 mL glass bottle and closed with a screw cap equipped with a silicon disk. The bottle was then introduced in a heating bath at 28–30 °C for 10 min. After this, the silicon set was perforated with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre holder (50/30 μm, 2 cm long from Supelco Ltd., Bellefonte, PA) and equilibrated for 10 min with the heating bath kept at 35 °C. Finally, the fibre was exposed for 30 min and immediately desorbed for 3 min at 250 °C into the injector of a gas chromatograph.

2.9. Gas chromatography

Volatile compounds were identified, and peak areas were integrated with a 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). 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 an ionization energy of 70 eV in the 15–250 amu mass range at 2 scan/s. Volatile compounds present in strawberries were tentatively identified basing on computer matching against commercial (NIST 1998, Wiley 6N, MassFinder 2.1 Library 2001) libraries and on our own spectral library of pure substances and literature data. Due to the choice to analyse strawberry cubes, adding an internal standard for quantification was not applicable (the standard could not be homogenized in the sample) so that data were reported as peak areas. Moreover, the aim of the present work was to compare different treatments, and thus absolute quantification was not strictly necessary.

2.10. Consumer test

Consumer acceptance tests were carried out on a laboratory scale (Stone & Sidel, 1985) in the Laboratory of Sensory Analysis of the Campus di Scienze degli Alimenti of University of Bologna, using individual booths with white neon light. As the aim of the present work was to individuate the effects of dehydrofreezing in terms of sensory acceptance level maximization, fresh samples were not analyzed.

An untrained panel of 26 consumers was used (8 males and 18 females between 30 and 40 years old, office-workers). No information about the normal fruit consumption habits of the judges was available. Fruits were thawed at controlled temperature until they reached 15 °C before serving. First, visual and odour acceptance were evaluated. Judges tasted fruits and rated the overall acceptance level. A 9-point hedonic scale was used for each descriptor with scores ranging from "1" indicating extreme dislike to "9" indicating an extremely high acceptance level, with "5" indicating indifference.

2.11. Statistical analysis

Data were analyzed using Statistica 6.1 (Statsoft Inc., Tulsa, OK) statistical software. For all analyses, the significance of differences at

Table 1
HPLC-DAD/MSD of phenolic compounds

Compound	Retention time (min)	λ_{max} (nm)	MW	Characteristic ions	
				M^+ (m/z)	M^- (m/z)
1. Cyanidin 3-glucoside	19.21	280; 520	449.38	449	–
2. Pelargonidin 3-glucoside	21.18	280; 505	433.38	433.1	–
3. Pelargonidin 3-rutinoside	22.60	275; 505	579.27	579	–
4. Unknown anthocyanin	25.58	520; 285	–	–	–
5. Unknown anthocyanin	28.17	504	–	519.3	–
6. Pelargonidin 3-acetylglucoside	33.43	504	474	475	–
7. Galloyl derivative	27.54	284	–	333	355
8. Caffeoyl glucose	9.56	250; 300sh; 330	342.1	–	341.1
9. p-coumaroyl glucoside	14.60	236; 316	326	325.1	–
10. Unknown compound	15.35	246; 296sh; 330	–	–	–
11. Unknown compound	15.91	236; 316	–	–	–
12. Unknown compound	16.83	244; 328	–	–	–
13. Ellagic derivative	31.06	254; 302; 360	–	–	–
14. Quercetin 3-glucuronide + glucoside	32.62	254; 300sh; 356	477 + 463	–	477; 463
15. Ellagic acid	33.64	254; 300sh; 360	302.2	–	301
16. Unknown compound	34.77	258; 356	–	–	–
17. Kaempferol 3-glucuronide	37.20	265; 300sh; 350	462	–	461
18. Kaempferol derivative	39.06	265; 350	–	–	–
19. Kaempferol derivative	41.11	265; 350	–	–	–

Retention time, (min); maximum of absorbance, (nm); MW, (molecular weight) and the most abundant mass fragment in positive or negative modality are reported. Abbreviation used: sh, (absorbance of shoulder spectra); m/z, indicate mass of most abundant ion/s in positive or negative mode.

95% level ($p < 0.05$) between averages was determined by a one-way ANOVA using Tukey's test. For sensory acceptance levels, the significance of differences at the 95% level ($p < 0.05$) between averages was determined with a one-way ANOVA using the LSD test.

3. Results and discussion

In osmotic processes, chemical compounds contained in fruits can be affected by different processing variables, and their content may also change through either biochemical or chemical transformations (enzymatic, hydrolytic, etc.) or leaching in the concentrated solution. In any case, the WL phenomenon causes the concentration of chemical compounds present in raw material. In contrast, SG causes an increase in sample weight with an apparent decrease of chemical compounds in fruits. Thus, at the end of the process, a final effect equal to WR = WL − SG takes place. Due to the higher value of WL with respect to SG, this effect results in a concentration that explains several of the chemical changes observed in the present study.

Osmotic dehydration processes (OD30, OD5 and VOD) presented interesting results with respect to mass transfer, while the ICF process at −19 °C did not show changes in mass transfers that may be due to the relatively short time of application (24 h) (Table 2). ICF in a concentrated solution for a short time resulted in more freezing due to direct contact with the liquid refrigerating medium and its high specific heat. It should be noted that keeping the sucrose solution in a manageable phase at low temperature is very difficult due to the high viscosity of the medium. For the same reasons, fruit cleaning from the concentrated solution necessitated an accurate washing step with water to remove all sucrose residues from fruits. This type of application would make the ICF technique particularly complicated to carry out in industrial plants. With regards to the VOD sample, it is apparent that the initial 5 min vacuum step caused a significant increase of SG with respect with the OD30 sample that was treated using the same processing variables (except for the vacuum step). At the same time, the extent of WL in the VOD sample was higher. Thus, the vacuum step applied to VOD samples improved the efficiency of mass transfer, with respect to OD30 samples, probably due to internal gases and native liquid substitution with the sucrose solution promoted by the hydrodynamic mechanism (HDM), in spite of the low porosity of strawberries (Andrés, Salvatori, Albors, Chiralt, & Fito, 2001). Sample OD5, which was treated for a longer period and at a temperature of 5 °C, had a SG comparable with OD30 samples but with a higher WL value (12.42). Thus, the WR of OD5 sample was higher than the other samples. It can be hypothesized that osmotic dehydration at a temperature of 5 °C for 24 h led to a WL to SG ratio higher than samples treated at 30 °C. This was probably due to the combination of low temperature and longer processing times, which could be confirmed by mass transfer kinetic studies. Moreover, a correlation between the influence of low temperature on fruit structure and cell membrane integrity and mass transfer likely exists.

Considering the other chemical modifications reported in Table 2, it should be underlined that there was a small range of variations

Table 2
Results of pH; TA, (titratable acidity expressed as meq NaOH/100 mL of fruit juice); DM, (dry matter expressed as g/100 g of sample); SS, (soluble solids expressed as g of sucrose/100 g of sample); WR (weight reduction), SG (solid gain); WL, (water loss) expressed as percentage variation with respect to initial sample weight of fresh and processed strawberries

	Samples						
	FR3	FR4	TQ	ICF	OD30	OD5	VOD
pH	3.43 (ab)	3.45 (ab)	3.41 (ab)	3.40 (b)	3.46 (ab)	3.55 (a)	3.41 (ab)
TA	10.80 (a)	10.76 (a)	10.80 (a)	10.10 (b)	10.08 (b)	10.94 (a)	9.90 (b)
DM	6.16 (c)	6.15 (c)	6.21 (c)	6.30 (c)	8.45 (b)	8.68 (b)	10.92 (a)
SS	5.65 (b)	5.65(b)	5.66 (b)	5.65 (b)	7.15 (ab)	7.10 (ab)	8.15 (a)
WR	–	–	–	–	9.32 (b)	10.85 (a)	8.24 (b)
SG	–	–	–	–	1.50 (b)	1.58 (b)	3.86 (a)
WL	–	–	–	–	10.82 (b)	12.42 (a)	12.10 (a)

a–c Different letters in the same row indicate statistically significantly different values (Honestly Significant Differences or HSD by Tukey $p < 0.05$).

in pH and TA of all samples, thus demonstrating that the processing techniques had little affect on these parameters. As expected, the DM increased significantly in samples VOD, OD30 and OD5 with respect with the untreated sample; the same trend was observed for SS.

In Table 3, the HPLC-DAD/MSD phenolic contents are detailed in mg/100 g of samples. The low quantity of phenolic contents found is comparable to that reported by Kosar, Kafkas, Paydas, & Husnu Can Baser (2004) for strawberries at a "pink" stage of ripening. The ICF samples presented a level of total polyphenolic (TP) content that was similar to fresh samples, while the TQ sample presented a slight decrease. This result may be due to the higher freezing rate of the ICF sample, leading to less freezing damage to fruit cells and more phenolic retention with respect to the freezing process used for the other samples. Samples OD30 and VOD had a TP level comparable to TQ samples, while OD5 presented a TP content that was similar to fresh fruits. As previously mentioned, during osmotic processes polyphenolic leaching into the treating solution occurred in addition to other chemical and biochemical reactions (chemical degradation, enzymatic oxidation, hydrolysis of polymeric compounds, polymerizations, biosynthesis, etc.) that require further characterisation. Moreover, the previously described concentration effect must also be taken into account. In the OD30 and VOD samples, the TP content appeared unchanged with respect to TQ samples. In reality, during the osmotic process a loss of phenolic compounds occurred, but a concentration effect of the same magnitude was also present. This could be easily understood if the concentration effect is not considered (last row in Table 3), and the content of total phenolic compounds in treated samples is then corrected using Eq. (1):

$$C_{xi} = \mathrm{FW_c} - \frac{\mathrm{FW_c} \times \mathrm{WR}}{100} \quad (1)$$

where C_{xi} = content of compound *xi* corrected for the concentration effect; $\mathrm{FW_c}$ = content of compound *xi* in treated sample; and WR = weight reduction.

By examining this data, the TP content in the OD30 and VOD samples was lower than in the TQ samples. Thus demonstrating a significant decrease in polyphenolic compounds during osmotic processes primarily due to phenolic losses in the treating solution (in fact, the solution became pink-coloured during processing) as previously demonstrated for apples slices (Blanda, Cerretani, Bendini, Cardinali, Scarpellini, & Lercker, 2008).

In the case of sample OD5, due to the lower temperature applied during the process major phenolic retention may have occurred with respect with the other samples. This higher content may be in part due to the neo-formation of phenolic monomeric compounds from hydrolysis of polymerized substances as previously observed (Blanda, Cerretani, Bendini, Cardinali, Scarpellini, & Lercker, 2008), or could be related to induction of metabolic synthesis due to osmotic stress (Suzuki, 1995). This effect was even more evident in OD5 samples due to the long osmodehydrating time that permitted these chemical changes.

In terms of fresh weight, we observed that some phenolic compounds mirrored the behaviour seen for TP content, while others were not affected by the different processes; some phenolic compounds were increased. The differences in behaviour of individual compounds may confirm the co-existence of different mechanisms of phenolic modification in various samples. Moreover, the variation in the distribution of phenolic compounds in the fruit matrix could explain different extent of lixiviation, i.e. compounds present in the outer part of the fruits are subject to more lixiviation than those present in the inner portion. In this case, correction for the concentration effect for each individual compound was not reported for the sake of brevity, but it further demonstrates the differential behaviour of phenolic changes in treated fruits.

In Table 4, the aroma profiles of samples are reported, and data are expressed as units of chromatogram area (ion abundance × time). As observed by other authors in treated fruits (Tovar, Garcia, & Mata, 2000; Rizzolo, Gerli, Prinzivalli, Buratti, & Torreggiani, 2007), there was a strong increase in acetaldehyde and ethanol in treated samples due to the anaerobic processing conditions used. Similarly, other volatile compounds (e.g. compound **12**) tended to drastically augment in osmotically treated samples. This effect cannot be explained by the concentration effect just described, and probably depends either on metabolic pathways related to fermentation or on the transfer of these compounds from syrup to fruits. Other compounds (compounds **10**, **13**, **14**, **19**, **22**) present in fresh samples tended to decrease or disappear altogether in treated or frozen samples (ICF and TQ). Alpha-farnesene, for

Table 3
HPLC-DAD/MSD phenolic contents of fresh and processed strawberries

Compound (mg/100g)	FR3	FR4	TQ	ICF	OD30	OD5	VOD
1. Cyanidin 3-glucoside	0.089 (ab)	0.103 (ab)	0.076 (ab)	0.093 (ab)	0.056 (b)	0.118 (a)	0.063 (b)
2. Pelargonidin 3-glucoside	3.764 (a)	4.455 (a)	3.062 (a)	3.540 (a)	2.927 (a)	4.040 (a)	2.771 (a)
3. Pelargonidin 3-rutinoside	0.209 (ab)	0.250 (ab)	0.172 (ab)	0.211 (ab)	0.147 (b)	0.258 (a)	0.143 (b)
4. Unknown anthocyanin	0.015 (abc)	0.016 (ab)	0.009 (cd)	0.012 (abcd)	0.007 (d)	0.016 (a)	0.010 (bcd)
5. Unknown anthocyanin	0.528 (ab)	0.631 (a)	0.355 (b)	0.411 (ab)	0.347 (b)	0.438 (ab)	0.333 (b)
6. Pelargonidin 3-acetilglucoside	0.031 (a)	0.046 (a)	0.027 (a)	0.038 (a)	0.034 (a)	0.035 (a)	0.028 (a)
7. galloyl derivative	2.322 (b)	2.502 (b)	2.739 (b)	3.572 (b)	2.985 (b)	5.895 (a)	2.322 (b)
8. Caffeoyl glucose	0.062 (a)	0.066 (a)	0.090 (a)	0.079 (a)	0.081 (a)	0.103 (a)	0.072 (a)
9. p-Coumaryl glucoside	0.403 (b)	0.533 (b)	0.564 (b)	0.595 (b)	0.369 (b)	1.005 (a)	0.370 (b)
10. Unknown compound	0.025 (a)	0.030 (a)	0.020 (a)	0.022 (a)	0.021 (a)	0.029 (a)	0.017 (a)
11. Unknown compound	0.067 (ab)	0.077 (ab)	0.057 (ab)	0.063 (ab)	0.048 (b)	0.086 (a)	0.044 (b)
12. Unknown compound	0.052 (ab)	0.055 (ab)	0.047 (b)	0.048 (b)	0.045 (b)	0.079 (a)	0.043 (b)
13. Ellagic derivative	0.129 (a)	0.117 (a)	0.092 (a)	0.094 (a)	0.097 (a)	0.106 (a)	0.086 (a)
14. Quercetin 3-glucuronide + glucoside	0.654 (a)	0.523 (ab)	0.235 (de)	0.169 (e)	0.277 (de)	0.451 (bc)	0.334 (cd)
15. Ellagic acid	0.257 (a)	0.195 (a)	0.118 (a)	0.180 (a)	0.147 (a)	0.228 (a)	0.169 (a)
16. Unknown compound	0.035 (a)	0.039 (a)	0.022 (a)	0.026 (a)	0.019 (a)	0.029 (a)	0.022 (a)
17. Kaempferol 3-glucuronide	0.179 (a)	0.157 (a)	0.096 (b)	0.097 (b)	0.095 (b)	0.156 (a)	0.103 (b)
18. Kaempferol derivative	0.080 (ab)	0.083 (a)	0.051 (c)	0.059 (abc)	0.047 (c)	0.076 (ab)	0.053 (bc)
19. Kaempferol derivative	0.019 (a)	0.023 (a)	0.014 (a)	0.019 (a)	0.016 (a)	0.019 (a)	0.015 (a)
Total polyphenols	**8.920** (ab)	**9.898** (ab)	**7.846** (b)	**9.330** (ab)	**7.766** (b)	**13.167** (a)	**6.995** (b)
Total polyphenols (WR corrected)	**8.920** (ab)	**9.898** (ab)	**7.846** (b)	**9.330** (ab)	**7.029** (b)	**11.739** (a)	**6.432** (c)

a–e Different letters in the same row indicate statistically significantly different values (Honestly Significant Differences or HSD by Tukey's $p < 0.05$).

Table 4
SPME-GC-MSD analysis of volatile compounds contents of fresh and processed strawberries

No.	Compound	FR	TQ	ICF	OD30	OD5	VOD
1	Acetaldehyde	8.4 (b)	3.9 (b)	7.5 (b)	30.3 (a)	29.6 (a)	34.6 (a)
2	Methyl acetate	52.0 (a)	43.3 (a)	29.8 (a)	30.1 (a)	14.1 (a)	43.9 (a)
3	Ethyl acetate	5.3 (bc)	ND	NQ	148.7 (b)	352.3 (a)	99.2 (bc)
4	Ethanol	ND	ND	ND	229.6 (a)	289.8 (a)	229.4 (a)
5	Methyl butanoate	196.7 (b)	288.2 (ab)	408.1 (a)	180.9 (b)	195.6 (b)	154.2 (b)
6	Ethyl butanoate	29.9 (b)	25.3 (b)	28.8 (b)	256.2 (ab)	342.5 (a)	132.1 (ab)
7	3-Methylbuthyl acetate	ND	ND	ND	8.8 (a)	9.7 (a)	14.3 (a)
8	2-Butenoic acid ethyl ester	ND	ND	ND	10.2 (ab)	19.0 (a)	5.4 (ab)
9	Methyl hexanoate	50.1 (a)	26.5 (a)	52.0 (a)	27.1 (a)	19.7 (a)	22.9 (a)
10	Buthyl butanoate	24.5 (a)	11.5 (b)	23.5 (a)	11.7 (b)	11.7 (b)	NQ
11	2-Hexen-1-al	5.9 (c)	47.2 (ab)	13.2 (bc)	44.8 (abc)	42.5 (abc)	63.6 (a)
12	Ethyl hexanoate	36.5 (c)	5.2 (c)	13.4 (c)	555.8 (a)	696.9 (a)	277.5 (b)
13	Hexyl acetate	437.3 (a)	28.4 (b)	43.1 (b)	34.6 (b)	58.2 (b)	28.2 (b)
14	2-Hexen-1-yl acetate	577.0 (a)	33.1 (bc)	52.2 (b)	12.1 (c)	27.8 (bc)	20.9 (bc)
15	6-Methyl-5-hepten-2-one	ND	ND	10.6 (a)	4.5 (b)	5.6 (b)	NQ
16	Ethyl-3-hexenoate	ND	ND	ND	3.1 (a)	3.7 (a)	ND
17	Nonanal	7.0 (b)	13.7 (a)	10.3 (ab)	7.8 (ab)	7.6 (ab)	9.5 (ab)
18	2-Hexen-1-ol	4.4 (a)	ND	4.5 (a)	ND	ND	ND
19	Hexyl butanoate	57.8 (a)	4.4 (b)	3.8 (b)	ND	ND	ND
20	Ethyl octanoate	ND	ND	3.1 (c)	22.5 (a)	27.7 (a)	12.0 (b)
21	Octyl acetate	ND	ND	ND	9.9 (b)	35.8 (a)	5.9 (c)
22	2-Hexen-1-yl butanoate	53.8 (a)	6.8 (b)	9.7 (b)	ND	ND	ND
23	2-Ethyl-1-hexanol	2.5 (a)	ND	1.9 (a)	2.8 (a)	2.7 (a)	5.3 (a)
24	Linalol	16.6 (a)	17.4 (a)	26.5 (a)	21.1 (a)	29.4 (a)	18.1 (a)
25	Octanol	ND	ND	3.7 (b)	3.4 (b)	12.9 (a)	ND
26	Octyl butanoate	11.2 (a)	3.1 (a)	10.7 (a)	8.7 (a)	8.7 (a)	ND
27	Ethyl benzoate	ND	ND	ND	6.3 (a)	4.4 (a)	5.7 (a)
28	Phenyl methyl acetate	5.7 (a)	3.5 (a)	6.2 (a)	5.7 (a)	11.8 (a)	4.6 (a)
29	Alpha-farnesene	16.5 (a)	ND	ND	ND	ND	ND
30	Hexanoic acid	9.9 (b)	15.4 (a)	21.5 (a)	2.6 (b)	19.2 (a)	NQ
31	Ethyl-3-phenyl propenoate	ND	ND	ND	2.8 (a)	2.8 (a)	ND
32	Gamma-decalactone	13.9 (b)	18.1 (b)	27.9 (ab)	15.9 (b)	57.5 (a)	5.9 (b)

a–c Different letters in the same row indicate statistically significantly different values (Honestly Significant Differences or HSD by Tukey's $p < 0.05$). Abbreviations used: ND – not detectable; NQ – not quantifiable. Values are expressed as peak area integration value (ion abundance × time).

example, was present in fresh samples, but was absent in all other samples. In some cases, volatile compounds that were easily detectable in fresh samples tended to decrease in TQ and ICF, perhaps due to changes induced by the freezing process. For example, 2-hexenyl butanoate decreased about 10-fold in ICF and TQ, and was not detectable in OD samples. This may be explained by volatile loss in the treating solution as observed by Rizzolo et al. (2007). Finally, other compounds were apparently unaffected by the different processes applied, and presented similar values in all samples.

Another important aim was the evaluation of the consumer acceptance levels of frozen strawberries and the advantages, in terms of sensory hedonic value, of applying osmotic dehydration as a pre-treatment for frozen fruits.

In Table 5, the acceptance test data are reported. In sensory tests with a small number of untrained consumers it is difficult to obtain statistically significant differences if samples do not present easily recognizable characteristics. It can be readily observed that the overall judgment was similar in all samples from a statistical standpoint, although certain trends can be observed. First, acceptance values of all samples were very low. One explanation resides in the size and weight of strawberries used in the present work. In preliminary analyses, we noticed a direct correlation between hedonic judgment and fruit size for obvious reasons; the shorter freezing time of smaller fruits reduced ice crystal damage and increased the impact of osmotic processes on small fruits (data not shown). Although the differences were not statistically significant, there was a tendency of OD5 and OD30 samples to be better accepted. Considering the other descriptors, the motivations of such a trend might be explained by the high impact of odour in OD5 and OD30, and to a lesser extent in VOD. The ICF and TQ samples presented a low odour acceptance level. On the other hand, TQ samples presented a higher acceptance of visual aspect, while OD30 had the lowest level. Only OD5 had a value higher than 5 (the indifference point) both in terms of visual aspect and odour acceptance. Thus, the low temperature applied has a favourable effect on the visual characteristics as perceived by consumers. As previously pointed out in Table 4, the aroma profile in OD5, OD30 and VOD was different from TQ and ICF samples, since they presented a higher content in volatile compounds formed during the process, possibly due to fermentation. In fact, compounds as ethyl butanoate and ethyl hexanoate were mostly present in dehydro-frozen samples. Other compounds (such as 3-methylbuthyl acetate) were present only in OD5, OD30 and VOD, while they were absent in FR3, TQ and ICF. These differences in the volatile compound profile may correlate with consumer acceptance. However, to confirm this quantitative descriptive sensory analysis with a trained panel would be necessary.

As previously mentioned, the presence of volatile compounds is probably correlated with the higher odour acceptance level. Nonetheless, it should also be considered that, as seen in previous studies, during thawing of fruits oxidation of phenolic compounds

Table 5
Results of laboratory scale consumer acceptance test of processed strawberries

	Samples				
	TQ	ICF	OD30	OD5	VOD
Visual aspect	6.3 (a)	5.2 (ab)	4.3 (b)	5.8 (ab)	4.9 (ab)
Odour	4.3 (b)	3.6 (b)	6.2 (a)	6.0 (a)	4.8 (ab)
Overall judgment	4.6 (a)	4.0 (a)	5.1 (a)	5.7 (a)	4.1 (a)

a–c Different letters in the same row indicate statistically significantly different values (LSD test $p < 0.05$). The 9-point hedonic scale was used with scores ranging from "1" indicating extreme dislike to "9" indicating an extremely high acceptance level, with "5" indicating indifference.

takes place, which is negatively correlated with the acceptance level of fruits (Blanda, Cerretani, Bendini, Cardinali, & Lercker, 2008). The higher the phenolic content of fruits after thawing, the higher the acceptance level. Oxidation probably occurs to a minor extent in osmodehydrated samples. As further confirmation of this hypothesis, OD5 samples presented the highest phenolic content, and were also the most pleasant samples.

As is evident, no information was presented regarding textural properties. While we did not measure this parameter, it is commonly well known that, with respect to fresh fruits, osmodehydrated fruits have a high retention of hardness after the freeze-thawing cycle. This depends on the type of solution used (Xie & Zhao, 2003). In particular, osmodehydration of strawberries permits a high retention of texture with respect to untreated samples when frozen fruits are thawed (Maestrelli, Giallonardo, Forni, & Torreggiani, 1997). This may also have accounted, at least in part, for the high values in overall judgment attributed to treated samples.

4. Conclusion

Analyses of the aroma profile in osmodehydrated frozen samples showed an increase of some compounds due to fermentation of fruits in anaerobic conditions during immersion in a concentrated sucrose solution. However, loss of native aroma compounds occurred. These changes positively affected the sensory acceptance level of fruit odour thus resulting in a high sensory acceptance level of osmodehydrated frozen samples with respect to untreated frozen samples. Among osmotic pre-treatments, the osmotic process conducted at 5 °C (a temperature not usually applied in the classical range of osmotic dehydration treatment) was very interesting in terms of both sensory and chemical characteristics. Specifically, the polyphenolic content of OD5 samples was very high, and comparable with fresh raw fruits. The reason for the high polyphenolic content was probably due to formation of compounds by hydrolysis of polymers or by metabolic synthesis induced in fruits by osmotic stress. In fact, the process at low temperature lasted for 24 h, and this longer period of time (compared with the 4 h of other osmodehydrated samples) probably allowed the induction of pathways involved in formation of phenolics. In other OD samples, lixiviation of phenolic compounds in the treating solutions prevailed, leading to a lower final content with respect to fresh raw material. Moreover, OD5 samples presented the highest WL to SG ratio, thus permitting a greater extent of dehydration without introducing excessive amounts of sucrose. In future investigations it would be interesting to examine the mechanism of phenolic formation/depletion during osmotic process and the kinetics of mass transfers at low temperature.

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Mass transfer and phenolic profile of strawberries upon refrigerated osmodehydration

Transferencia de masa y perfil fenólico de las fresas cuando son osmo-deshidratadas por refrigeración

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Whole strawberries were osmodehydrated at low temperature (5 °C) in a sucrose syrup (500 g/kg of solution) for different processing times (24, 48, 72, 96, and 120 h); after the treatment, water loss, solid gain, and weight reduction were recorded. Next, the osmodehydrated samples were frozen, and after a 5-month storage at −18 °C they were analyzed for drip loss, titratable acidity, refractometric index, pH, and sensory acceptance. The phenolic profile of the samples was characterized by means of HPLC. After thawing, osmodehydrated samples presented a high acceptability for consumers. In addition, they presented drip loss values that were inversely correlated with the processing time, with 120-h samples presenting negligible drip loss after thawing. The processing time influenced the final quality of strawberries, and samples treated for 24–48 h were very suitable for direct consumption after thawing, in substitution of fresh strawberries. The samples treated for 72–120 h also presented characteristics that may render them suitable for applications in the food industry.

Keywords: strawberry; osmotic dehydration; freezing; phenols; acceptance test

Fresas enteras se osmo-deshidrataron a baja temperatura (5 °C) en un jarabe de sacarosa (solución a 500 g/kg) para diversos tiempos de procesamiento (24, 48, 72, 96, y 120 h); después del tratamiento, la pérdida de agua, ganancia sólida y reducción de peso se registraron. A continuación, muestras osmo-deshidratadas se congelaron, y después de ser almacenadas por 5 meses a −18 °C fueron analizadas en cuanto a pérdida de agua durante descongelamiento, acidez titratable, índice refractométrico, pH y aceptación sensorial. El perfil fenólico de las muestras fue caracterizado mediante HPLC. Después de descongelar, las muestras osmo-deshidratadas presentaron una alta aceptación para los consumidores. Adicionalmente, los valores de pérdida de agua por descongelación se correlacionaron inversamente con tiempo de procesamiento, siendo las muestras con 120 h las que presentaron menos pérdida de agua después de la descongelación. El tiempo de procesamiento tuvo influencia en la calidad final de las fresas, y las muestras tratadas por 24–48 h fueron muy aceptables para consumo directo después de descongelación, en substitución de fresas frescas. Las muestras tratadas por 72–120 h también presentaron características que pueden hacerlas aceptables para aplicaciones en la industria alimentaria.

Palabras clave: fresa; deshidratación osmótica; congelación; fenoles; prueba de aceptación

Introduction

A good way to preserve strawberries (Fragaria × ananassa Duch.) from bruises and fungal attacks before processing is through the use of freezing technologies that combine low temperature and water activity (a_w) reduction associated with cryoconcentration of the fruit liquid phase during ice crystal formation. Individually quick-frozen strawberries may have potential uses as ingredients in different high-quality processed foods, such as ice creams, yogurt, jams, and jellies (Duxbury, 1992). However, several chemical–physical and sensory deteriorations take place during thawing with subsequent loss of product quality (Blanda, Cerretani, Bendini, Cardinali, & Lercker, 2008a; Martínez-Navarrete, Moraga, Martínez-Monzó, Botella, Tirado, & Chiralt, 2001).

In recent years, several studies have highlighted the importance of dehydration pre-treatment before the freezing process (dehydrofreezing) to reduce the water content and limit ice crystal damage (Chiralt, Martínez-Navarrete, Martínez-Monzó, Talens, Moraga, Ayala, & Fito, 2001). Osmotic dehydration (OD) before freezing is used to produce several kinds of fruit ingredients that can be stored for long periods with good retention of texture, color, and flavor after thawing (Dalla Rosa & Spiess, 2000; Maestrelli, Lo Scalzo, Lupi, Bertolo, & Torreggiani, 2001; Sormani, Maffi, Bertolo, & Torreggiani, 1999). OD in hypertonic

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solutions cause a flow of water from the food matrix to the liquid and flow of solute from the liquid to the food matrix. A third mass transfer involves food solutes leaching into solution. Although leaching has been generally considered to be quantitatively negligible (Dixon & Jen, 1977), it may result in loss of the nutritional content of foods (Blanda, Cerretani, Bendini, Cardinali, Scarpellini, & Lercker, 2008b; Peiro-Mena, Dias, Camacho, & Martinez-Navarrete, 2006). Solute impregnation coupled with mild partial dehydration occurs at moderate temperatures (5–50 °C), and thus OD has a minimal impact on the overall structure and composition of foods (Torreggiani, 1993).

Solute incorporation from the osmotic medium may improve the nutritional and functional properties of the food product; however, excessive solid gain (SG) can be detrimental to the quality of the food product and should be avoided (Matuska, Lenart, & Lazarides, 2006).

The ratio of water loss (WL) to SG is a useful parameter to control the final product quality in fruit osmodehydration. A higher WL/SG ratio is associated with higher processing temperatures, and this is especially true if short processing times are used (2–3 h). A high WL/SG ratio, is presumably obtained by combining long processing times and low temperatures, however this aspect needs further investigation. To the best of our knowledge, low temperature osmotic processes have not been widely studied as they are not economically favorable as the treatment solutions must be cooled and the process is time-consuming. On the other hand, we have shown that (Blanda, Cerretani, Cardinali, Barbieri, Bendini, & Lercker, 2009) these conditions lead to high quality products with a higher WL/SG ratio, higher sensory quality, and retention of healthy compounds. Thus, better understanding of the low temperature phenomena associated with these processes could be useful to obtain high quality frozen products characterized by a longer shelf life with important advantages in the formulation of processed foods.

To provide insight into low temperature OD, whole strawberries were submitted to OD at 5 °C for a processing time ranging from 1 to 5 days in an industrial scale pilot plant. Changes in chemical composition and sensory properties were determined. The aim of the article is to investigate the mass transfer occurring at low temperature for long processing time, to produce osmodehydrated strawberries for direct consumption for the consumer or for use as food ingredients in food industry.

Materials and methods

Instruments

HPLC analyses on phenolic extracts were performed using a HP 1100 instrument (Agilent Technologies, Palo Alto, CA), equipped with a binary pump delivery system, a degasser, an autosampler, a diode array UV-VIS detector (DAD), and a mass spectrometer detector (MSD). The HPLC column used was a C_{18} Luna column, 5 μm, 15 cm × 3.0 mm (Phenomenex, Torrance, CA), with a C_{18} pre-column (Phenomenex) filter.

Reagents, stock solutions, and reference compounds

p-coumaric acid, pelargonidin chloride, ellagic acid, and kaempferol were acquired from Sigma-Aldrich (Sigma, St Louis, MO). Stock solutions containing these analytes were prepared in methanol at 2.0 mg mL^{-1} for *p*-coumaric, pelargonidin, and ellagic acid and 2.5 mg mL^{-1} for kaempferol. These standard solutions were used to prepare calibration curves in a range of 1–500 μg mL^{-1}. Methanol and HPLC-grade water were from Merck (Darmstadt, Germany). Distilled water was deionized by using a Milli-Q system (Millipore, Bedford, MA).

Experimental design

Strawberries (*cv*. Camarosa) were bought at a local market. A subsample of 90 kg strawberries without damage nor fungal attacks and ranging from 23 to 27 g each was obtained from 120 kg of strawberries. After stalk removal, strawberries were accurately mixed, and divided in six aliquots of 15 kg.

Control sample

The Strawberries were immediately frozen in a freezing chamber in direct contact with dry ice pellets (2 mm diameter). After 30 min, strawberries reached a temperature of −30 °C at the core, and were then put in a conventional freezer and stored at constant temperature of −18 °C for 5 months.

The 24, 48, 72, 96, and 120 h samples

For each sample, strawberries were put in a large stainless steel tank containing sucrose syrup, prepared with 750 kg of sucrose of commercial grade (Chimab, Milan, Italy) in 750 kg of water, and were kept submerged using a grid. The syrup was kept at a constant temperature of 5 °C during the entire processing period by using a syrup chiller equipped with a pump with a flow rate of 250 L h^{-1}. At the end of each processing time, the grid was removed and the strawberries were withdrawn from the tank. Strawberries were drained, rapidly washed with tap water and dried with air, frozen in a dry ice cabinet as described above and stored at −18 °C for 5 months.

The below-described analyses were carried out in triplicate on aliquots of 19 strawberries (about 475 g). The representativeness of the aliquot dimension was

studied in a previous work (Blanda et al., 2009) where it was found that an aliquot size of 19 strawberries was a good compromise between subsample representativeness and laboratory constraints.

Mass transfer determination

SG, WL, and weight reduction (WR) were calculated as described (Giangiacomo, Torreggiani, Abbo, 1987) using the equations reported below. Changes in weight and dry matter (DM) were determined in four replicates for each processing time: for each replicate, 19 strawberries were put in a plastic net and processed in the same soaking tank with the global sample.

$$\mathrm{WL} = \frac{w_{\mathrm{wo}} - (w_{\mathrm{t}} - w_{\mathrm{st}})}{(w_{\mathrm{wo}} + w_{\mathrm{so}})} \times 100 \quad (1)$$

$$\mathrm{SG} = \frac{(w_{\mathrm{st}} - w_{\mathrm{so}})}{(w_{\mathrm{wo}} + w_{\mathrm{so}})} \times 100 \quad (2)$$

$$\mathrm{WR} = \mathrm{WL} - \mathrm{SG} \quad (3)$$

where, w_{wo} = mass of water in fruit before treatment; w_{t} = mass of strawberries after treatment; w_{st} = mass of solids (DM) in fruit after treatment; w_{so} = mass of solids (DM) in fruit before treatment.

Analyses of dry matter, pH, soluble solids, titratable acidity

DM, pH, soluble solids (SS), and titratable acidity (TA) of fresh and frozen slices were determined according to AOAC method 932 (AOAC, 2000).

Drip loss determination

Nineteen frozen strawberries for each replicate were put upon a metallic grid in plastic boxes and hermetically sealed with a lid. The plastic boxes were kept at a controlled temperature of 22 °C. After 8 h, the weight of juice lost by the strawberries was determined and expressed as percentage of fruit initial weight.

Acceptance test

Acceptance tests were carried out on a laboratory scale (Stone & Sidel, 1985) in the Laboratory of Sensory Analysis of the "Campus di Scienze degli Alimenti" at the University of Bologna using individual booths with white neon light. An untrained panel of 33 consumers was used (13 males and 20 females between the age of 25 and 40 years, office-workers). No information about the normal fruit consumption habits of the judges was available. Strawberries were thawed at controlled temperature until they reached 18 °C, and then served to judges. Firstly, visual and odour acceptance was evaluated, and then judges tasted strawberries and rated the taste acceptance level. A nine-point hedonic scale was used for each descriptor with scores ranging from 1 (extreme dislike) to 9 (extreme likeability) and 5 as the indifference point (neither like nor dislike). Each judge could freely express notes or comments on a scorecard.

Phenolic extraction and clean-up

Phenolic extracts were obtained by adapting the method reported by Blanda et al. (2009). Briefly, 500 g of strawberries (about 19 strawberries) were ground in a blender with 500 g methanol for 1 min to prevent enzymatic degradations. Next, 10 g of this homogenate was centrifuged at 22,000 rpm (39,600*g*) for 10 min at 10 °C (Avanti J25, Beckman Coulter, Fullerton, CA). The supernatant was recovered and a second extraction was performed by homogenizing the sample residue with 10 mL of methanol/water (950 mL/L) in a centrifuge tube. An Ultra Turrax blender (IKA-Werke mod. T 25 basic, Staufen, Germany) was used at 15,000 rpm for 3 min. Then, the tube was centrifuged again at 22,000 rpm. The supernatant was recovered and the two extracts were combined and evaporated in a vacuum centrifuge to complete dryness (MIVAC DUO, Genevac, Ipswich, England). The concentrated sample was dissolved in 5 mL of acidified water (30 mL/L formic acid) and then passed through a Strata C_{18}-E 55 μm 70 A cartridge (Phenomenex), previously activated with methanol followed by formic acid/water (30 mL/L). Anthocyanins and other phenolics were adsorbed onto the column while sugars, organic acids and other highly water-soluble components were eluted with 10 mL formic acid/water (30 mL/L). The anthocyanins and other phenolic compounds were then recovered with 2.0 mL of formic acid/methanol (25 mL/L).

HPLC-DAD/MSD analysis of phenols

Methanolic extracts were filtered through a 0.45 μm filter (Whatman, Clifton, NJ) and injected in HPLC 1100 Series (Agilent Technologies, Palo Alto, CA). A Luna C18 (Phenomenex, St. Torrance, CA) column (5 μm particle size, 250 mm, 3.00 mm ID) was used and 20 μL of phenolic extract were injected. Mobile phases were: A, formic acid/water (25 mL/L); and B, formic acid/methanol (25 mL/L). The elution gradient was linear: at 0 min 85% solvent A held for 5 min, from 5 to 20 min 65% A was reached and held constant until 25 min, from 25 to 35 min 50% held constant until 45 min, from 45 to 50 min 34% and finally at 59 min 85% solvent A was restored. A 10 min post run equilibration was performed. The detector wavelengths were set at 280, 320, 350, and 520 nm. Identification was also made using MSD, with an electrospray (ESI) interface operating in positive and negative mode using the following conditions: drying gas flow, 9.0 L/min; nebulizer pressure, 50 psi;

gas drying temperature, 350 °C; capillary voltage, 3000 V; fragmentor voltage, 60 V. Phenolic compounds were tentatively identified based on their UV-VIS and mass spectra obtained by HPLC-DAD/MSD (Table 3) and comparison with data from the literature (Lopes da Silva, De Pascual-Teresa, Rivas-Gonzalo, & Santos-Buelga, 2002; Määttä–Riihinen, Kamal–Eldin, & Törrönen, 2004).

For quantification in HPLC-DAD, five standard calibration curves were constructed using four commercial reference compounds, *p*-coumaric acid, pelargonidin chloride, ellagic acid, and kaempferol. Anthocyanins (compounds 1–8 in Table 3) were quantified using the calibration curve of pelargonidin chloride at 520 nm ($r^2 = 0.9952$). Phenolic acids (compounds 9–13) were quantified on the basis of a *p*-coumaric standard calibration curve at 320 nm ($r^2 = 0.9926$); ellagic acid (compound 17) and an ellagic derivative (compound 15) were quantified using an ellagic acid curve at 350 nm ($r^2 = 0.9998$); finally, flavonols (compounds 14, 16, and 18–21) were quantified using a kaempferol calibration curve at 350 nm ($r^2 = 0.9890$).

Statistical analysis

The data were analysed using Statistica 7.0 (Statsoft, Tulsa, OK) statistical software. For all analyses, the significance of differences at 5% level between averages was determined by one-way ANOVA using Tukey's test. For sensory acceptance levels, the significance of differences at the 5% level between averages was determined by one-way ANOVA using the LSD test.

Results and discussion

Mass transfer

The WL and SG behavior agree with the kinetic models developed by other authors (Fickian diffusion laws) (Rastogi, Raghavarao, & Miranjan, 1997) reporting a higher WL and SG rate during the first days of treatment and a slow down in the latter days. The influence of processing time on mass transfers in osmodehydrated strawberries is reported in Figure 1. The WL to SG ratio showed a remarkable increase during the first 2 days of the osmodehydrating treatment (from 4.9 to 6.2); after 3 days the WL/SG ratio reached a plateau until the 5th day (6.2 to 6.8).

In a previous study (Blanda et al., 2009), we evaluated the nutritional and sensory quality of strawberries submitted to different combinations of OD processes and freezing. Moreover, the samples treated at lower temperature presented the highest WL/SG ratio, which is in apparent contrast with previous findings (Lazarides, Katsanidis, & Nickolaidis, 1995; Sereno, Moreira, & Martinez,, 2001) that reported higher WL/SG values at higher processing temperatures. The reason behind these discrepancies may be

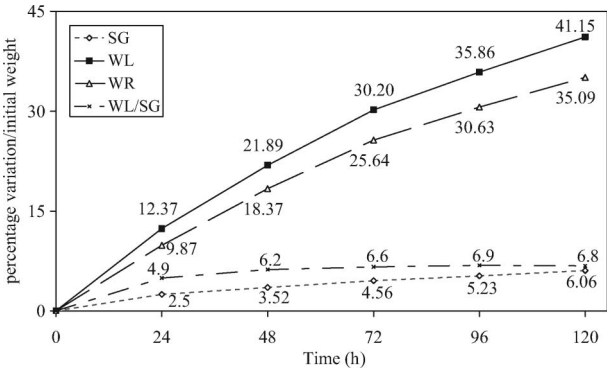

Figure 1. Weight reduction (WR), water loss (WL), solid gain (SG), and WL/SG, at different processing times.

Figura 1. Reducción en peso (WR), pérdida de agua (WL), ganacia sólida (SG) y WL/SG, a diferentes tiempos de procesamiento.

related to the processing time used in our experiment, which was very long compared with other OD processes that have been reported. In fact, the rate of WL (and SG) depends on several factors such as the solution concentration, processing temperature, processing time, level of agitation, sample size and geometry, solution to solid volume ratio and operating pressure, and particularly the use of vacuum (Moreno, Chiralt, Escriche, & Serra, 2000; Rastogi et al., 1997; Shi & Fito, 1994). In fact, the WL/SG ratio calculated according to the kinetic model described by (Sereno et al., 2001) using a 24 h processing time and 5 °C processing temperature provides support for the supposition that the value is comparable with that obtained at higher temperature and shorter processing times.

Finally, a higher solute uptake is likely present in the first stages of the process, depending on the morphological structure of the fruit.

Dry matter, pH, soluble solids, titratable acidity

In Table 1 the values of DM, pH, SS, and TA are reported. DM and SS increased with the processing time as expected. As these variables change significantly, it was interesting to note that pH values in fruit juice did not vary with the treatment time, in accordance with previous data (Blanda et al., 2009). On the other hand, TA increased significantly in the first 2 days of treatment, while it decreased in the following days. Because this behavior is difficult to understand, the observed variations could be explained by the concentration of organic acids during the process due to the decrease of water content, the higher mobility of H^+ with respect to K^+ and other cations during osmodehydration or the *ex-novo* formation of organic acids induced in the fruit maintained the pH constant at later times. In a previous work (Blanda et al., 2009), large amounts of volatile

④ Table 1. Average values ($n = 3$) of pH.

Tabla 1. Valores promedio ($n = 3$) de pH.

	Control	24 h	48 h	72 h	96 h	120 h
pH	3.66[a]	3.60[a]	3.61[a]	3.65[a]	3.61[a]	3.69[a]
TA	4.97[c]	5.32[b]	5.87[a]	5.77[a]	4.73[c,d]	4.55[d]
DM	97.8[f]	143.7[e]	162.9[d]	192.8[c]	208.4[b]	244.1[a]
SS	88.0[f]	130.0[e]	151.0[d]	181.0[c]	198.0[b]	235.0[a]

TA, titratable acidity expressed as meq NaOH; DM, dry matter expressed as g/kg; SS, soluble solids expressed as g/kg of sucrose of processed strawberries.
a–d different letters in the same row indicate statistically significant differences (honestly significant differences or HSD by Tukey, $p < 0.05$).

TA, acidez titratable expresada en meq NaOH; DM, materia seca expresada en g/kg; SS, sólidos solubles expresados en g/kg de sacarosa de fresas procesadas.
Letras diferentes a–d en la misma fila indican diferencias estadísticamente significativas (Diferencias honestamente significativas o HSD por Tukey, $p < 0.05$).

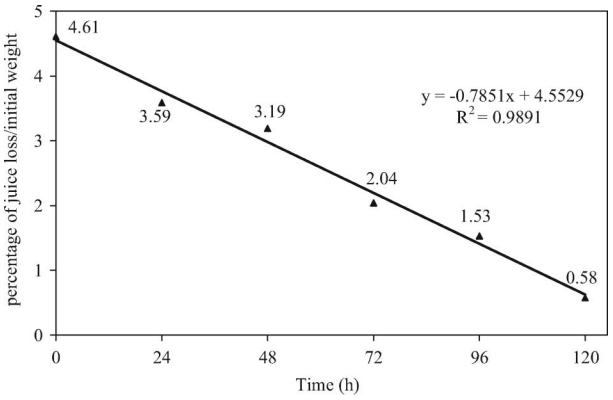

Figure 2. Average values of drip loss (DL) of untreated samples and processed strawberries ($n = 3$).

Figura 2. Valores promedio de pérdida de contenido de agua por descongelación (DL) de las muestras no tratadas y fresas procesadas ($n = 3$).

compounds (such as alcohols and acetaldehyde) deriving from fermentation were detected in osmodehydrated strawberries. Thus, fermentative metabolism could be activated in fruit, induced by the process itself causing changes in the organic acid profile. The decrease of TA in the subsequent days of treatment could be explained in the same way, by changes in the organic acids profile or by leaching of acids or protons into the treatment solution. These two factors could be active in strawberries at the same time during the process, with the latter being more important at 3–5 days of treatment. Better knowledge of metabolism pathways may be needed to better explain this behavior.

Drip loss

In Figure 2, the results of drip loss determinations are reported. A linear relationship between processing time and the amount of drip loss can be observed, and a significant reduction in juice loss from fruit after thawing after 1 day of treatment was already evident. The reduction in drip loss was very high in samples treated for 3–4 days and accounted for only 0.58 g of juice in 100 g of strawberries after 5 days of treatment. Drip loss reduction in treated strawberries was supposedly due to less ice crystal formation during freezing of strawberries promoted by the reduction on free water after osmotic treatment and by the cryoprotective effect of sucrose impregnated in strawberries that increased the non-freezable water fraction.

Acceptance test

The results of the sensory acceptance test are presented in Table 2. An inverse correlation was found between visual acceptance scores and processing time. This could be substantially ascribed to the dry and rugged appearance of osmotically-treated fruit, particularly when processed for longer times. However, the high drip loss of control samples and of less treated samples did not appear to affect the acceptance level. With regards to the odour acceptance level, judgments were more variable and there were no statistically significant differences among treated samples; generally, untreated strawberries presented the lowest odour acceptance score. In any case, osmotic treatment seemed to improve the odour acceptance level of the fruits, as already reported by other authors (Blanda et al., 2009; Dalla Rosa & Spiess, 2000).

Analysis of taste acceptance levels provided interesting results. As it is already known, osmotic treatment allows for partial water removal from strawberries thus preventing damages caused by freezing (dehydrofreezing) and ice crystal formation

Table 2. Average values ($n = 3$) of the consumer's acceptance test of processed strawberries.

Tabla 2. Valores promedio ($n = 3$) de la prueba de aceptación del consumidor de fresas procesadas.

	Samples					
	Control	24 h	48 h	72 h	96 h	120 h
Descriptors						
Visual aspect	6.31[a]	6.06[a,b]	5.72[a,b]	5.22[b,c]	4.72[c,d]	4.15[d]
Odour	4.31[b]	5.78[a]	6.22[a]	5.56[a]	5.62[a]	5.18[a,b]
Taste	2.31[c]	4.91[a,b]	5.31[a]	4.81[a,b]	4.31[b]	4.47[a,b]

a–d different letters in the same row indicate statistically significant differences (Honestly significant differences or HSD by Tukey, $p < 0.05$).
A nine-point hedonic scale was used.

Letras diferentes a–d en la misma fila indican diferencias estadísticamente significativas (Diferencias honestamente significativas o HSD por Tukey, $p < 0.05$).
Se usó una escala hedonística de nueve puntos.

that causes disruption of cell structures with dramatic changes in both texture and enzymatic activation. These phenomena cause off-flavor development, phenolic oxidation and overall declines in the sensory quality (Blanda et al., 2008a,b, 2009; Chiralt et al., 2001; Dalla Rosa et al., 2000; Dixon et al., 1977; Maestrelli et al., 2001; Matuska et al., 2006; Peiro-Mena, Camacho, & Martinez-Navarrete, 2007; Peiro-Mena et al., 2006; Sormani et al., 1999; Torreggiani, 1993). This is quite evident in the differences between the taste acceptance level of untreated and treated samples. The very low score of untreated samples is likely due to the very low hardness level and atypical taste (as reported by the judges in their scorecards). Samples treated for 48 h were the most accepted, probably because the treatment had only minimal effects on the characteristics of the raw material, and limited the damages caused by freezing. In fact, samples treated for 48 h had an acceptability score higher than the indifference point. Samples treated for 24, 72, and 120 h were very similar (and not statistically different) to the 48 h sample, while the 96 h sample seemed to be the less accepted, although the score was still higher than untreated samples. The low score given to samples treated for 96 and 120 h are probably due to the low juiciness of strawberries and the gummy texture due to high level of dehydration, but were not statistically different from samples treated for 24 or 72 h; thus, there was not a trend towards lower acceptance levels that could be attributed to the low juiciness. Considering the chemical physical results and the comments given by panellists regarding taste, strawberries processed for 4–5 days could be used as an ingredient in food preparation (e.g. fruit cakes) as they showed low drip loss and very sweet taste.

HPLC-DAD/MS phenolic analysis

As reported in a previous study (Blanda et al., 2009) on osmotic processes, the natural components of strawberries can be affected by different processing variables, and their content may also change due to either biochemical or chemical transformations (enzymatic, hydrolytic, etc.) or leaching in the concentrated solution. In any case, the WL phenomenon causes the re-concentration of the constituents present in the fresh strawberries. In contrast, SG causes an increase in sample weight with an apparent decrease of the natural components of strawberries. Thus, at the end of the process, a final effect equal to $WR = WL - SG$ takes place. Because of the higher value of WL with respect to SG, this effect results in concentration.

Table 3. HPLC-DAD/MSD of phenolic compounds.

Tabla 3. HPLC-DAD/MSD de los compuestos fenólicos.

Compound	Retention time (min)	λ max (nm)	MW	Characteristic ions	
				M^+ (m/z)	M^- (m/z)
1. Unknown anthocyanin	14.60	270; 510	–	–	–
2. Cyanidin 3-glucoside	19.21	280; 520	449.38	449	–
3. Pelargonidin 3-glucoside	21.18	280; 505	433.38	433.1	–
4. Pelargonidin 3-rutinoside	22.60	275; 505	579.27	579	–
5. Unknown anthocyanin	25.58	520; 285	–	–	–
6. Unknown anthocyanin	28.17	504	–	519.3	–
7. Pelargonidin 3-acetylglucoside	33.43	504	474	475	–
8. Unknown anthocyanin	40.07	280; 500	–	–	–
9. Galloyl derivative	27.54	284	–	333	355
10. *p*-coumaroyl glucoside	14.60	236; 316	326	325.1	–
11. *p*-coumaroyl derivative	16.06	–	–	–	–
12. Cinnamic compound	15.35	246; 296sh; 330	–	–	–
13. Cinnamic compound	15.91	236; 316	–	–	–
14. Cinnamic compound	16.83	244; 328	–	–	–
15. Ellagic derivative	31.06	254; 302; 360	–	–	–
16. Quercetin 3-glucuronide + glucoside	32.62	254; 300 sh; 356	477 + 463	–	477; 463
17. Ellagic acid	33.64	254; 300 sh; 360	302,2	–	301
18. Kaempferol 3-glucuronide	37.20	265; 300sh; 350	462	–	461
19. Kaempferol derivative	38.50	350	–	–	–
20. Unknown compound	39.06	345	–	–	–
21. Kaempferol derivative	41.11	265; 350	–	–	–

Retention time, (min); maximum of absorbance, (nm); MW, (molecular weight) and the most abundant mass fragment in positive or negative modality are reported.

Abbreviation used: sh: absorbance of shoulder spectra.

The pseudomolecular masses of the most abundant ion/s in positive or negative mode are reported.

Tiempo de retención, (min); absorción máxima, (nm); MW, (peso molecular) y se reportaron los fragmentos de masa más abundante en modalidad positiva y negativa.

Abreviaciones usadas: sh: absorbancia de espectros de hombro.

Se reportaron las masas pseudomoleculares de los iones más abundantes en modalidad positiva o negativa.

Table 4. HPLC-DAD/MSD phenolic contents of fresh and processed strawberries with (cor) and without (uncor) the correction for the concentration effect (see the Equation 4).

Tabla 4. HPLC-DAD/MSD contenidos fenólicos de fresas frescas y procesadas con (cor) y sin (uncor) la corrección del efecto de concentración (vea la Ecuación 4).

Compound (mg/kg)	Control	24 h		48 h		72 h		96 h		120 h	
		uncor	cor	uncor	cor	uncor	cor	uncor	cor	uncor	cor
1. Unknown anthocyanin	0.18 (c-A)	0.22 (bc)	0.20 (A)	0.26 (ab)	0.21 (A)	0.19 (c)	0.14 (B)	0.24 (ab)	0.17 (AB)	0.30 (a)	0.20 (A)
2. Cyanidin 3-glucoside	4.65 (c-B)	6.66 (a)	6.00 (A)	6.20 (bc)	5.06 (B)	4.61 (c)	3.43 (C)	6.52 (a)	4.52 (B)	5.37 (bc)	3.48 (C)
3. Pelargonidin 3-glucoside	57.7 (c-AB)	68.5 (a)	61.7 (A)	68.1 (ab)	55.6 (BC)	63.3 (bc)	47.0 (DE)	73.5 (a)	51.0 (CD)	66.2 (ab)	43.0 (E)
4. Pelargonidin 3-rutinoside	5.92 (b-B)	7.86 (a)	7.09 (A)	7.03 (ab)	5.74 (BC)	6.27 (bc)	4.66 (C)	8.36 (a)	5.80 (B)	7.46 (ab)	4.84 (BC)
5. Unknown anthocyanin	0.14 (c-B)	0.19 (a)	0.17 (A)	0.18 (b)	0.14 (B)	0.11 (d)	0.08 (D)	0.19 (ab)	0.13 (B)	0.17 (b)	0.11 (C)
6. Unknown anthocyanin	0.22 (d-C)	0.35 (b)	0.31 (A)	0.32 (bc)	0.26 (BC)	0.30 (c)	0.22 (C)	0.40 (a)	0.28 (AB)	0.34 (b)	0.22 (C)
7. Pelargonidin 3-acetilglucoside	0.36 (d-C)	0.61 (c)	0.55 (B)	0.59 (c)	0.48 (B)	0.80 (a)	0.59 (A)	0.67 (b)	0.46 (B)	0.69 (b)	0.44 (BC)
8. Unknown anthocyanins	0.05 (c-D)	0.17 (b)	0.15 (A)	0.20 (a)	0.17 (A)	0.17 (b)	0.12 (B)	0.17 (b)	0.12 (B)	0.14 (b)	0.09 (C)
9. Galloyl derivative	15.8 (c-B)	19.5 (b)	17.6 (A)	22.4 (a)	18.3 (A)	15.5 (c)	11.5 (D)	19.2 (b)	13.3 (C)	18.7 (b)	12.2 (CD)
10. *p*-Coumaroyl glucoside	25.5 (b-B)	38.8 (a)	34.9 (A)	38.5 (a)	31.4 (AB)	34.3 (a)	25.5 (B)	36.1 (a)	25.0 (B)	38.8 (a)	25.2 (BC)
11. *p*-Coumaroyl derivative	4.21 (a-A)	5.06 (a)	4.56 (A)	5.37 (a)	4.38 (A)	4.71 (a)	3.50 (A)	3.98 (a)	2.76 (A)	5.86 (a)	3.80 (A)
12. Cinnamic compound	1.56 (a-A)	1.93 (a)	1.74 (A)	1.81 (a)	1.47 (A)	1.56 (a)	1.16 (A)	1.46 (a)	1.01 (A)	2.35 (a)	1.52 (A)
13. Cinnamic compound	1.19 (a-A)	1.19 (a)	1.08 (A)	1.74 (a)	1.42 (A)	1.54 (a)	1.14 (A)	1.18 (a)	0.82 (A)	0.88 (a)	0.57 (A)
14. Cinnamic compound	3.73 (a-AB)	4.12 (a)	3.72 (AB)	4.94 (a)	4.03 (A)	3.73 (a)	2.77 (C)	4.66 (a)	3.23 (B)	4.51 (a)	2.93 (B)
15. Ellagic derivative	3.52 (bc-A)	3.30 (cd)	2.97 (B)	3.36 (bcd)	2.75 (BC)	3.16 (d)	2.35 (D)	3.70 (ab)	2.57 (CD)	3.98 (a)	2.58 (CD)
16. Querc. 3-glucur. + glucos.	13.2 (ab-A)	13.7 (a)	12.35 (A)	13.1 (ab)	10.71 (B)	9.7 (c)	7.20 (D)	12.2 (b)	8.46 (C)	13.8 (a)	8.93 (C)
17. Ellagic acid	4.00 (a-A)	2.65 (b)	2.39 (BC)	3.39 (a)	2.76 (B)	3.82 (a)	2.84 (B)	2.65 (b)	1.83 (C)	3.98 (a)	2.58 (BC)
18. Kaempferol 3-glucuronide	4.38 (a-A)	3.29 (d)	2.97 (B)	3.92 (bc)	3.20 (B)	3.95 (b)	2.94 (B)	3.48 (cd)	2.41 (C)	4.54 (a)	2.95 (B)
19. Kaempferol derivative	0.52 (a-A)	0.35 (b)	0.32 (C)	0.59 (a)	0.48 (AB)	0.51 (a)	0.38 (C)	0.31 (b)	0.21 (D)	0.61 (a)	0.40 (BC)
20. Unknown compound	0.84 (bc-A)	0.75 (c)	0.68 (B)	0.97 (a)	0.79 (A)	1.07 (a)	0.79 (A)	0.96 (a)	0.67 (B)	0.98 (a)	0.64 (B)
21. Kaempferol derivative	0.21 (e-E)	0.28 (d)	0.25 (D)	0.41 (b)	0.34 (B)	0.50 (a)	0.37 (A)	0.38 (c)	0.26 (CD)	0.43 (b)	0.28 (C)
Total polyphenols	148 (c-B)	179 (b)	162 (A)	183 (a)	150 (B)	160 (bc)	119 (C)	180 (ab)	125 (C)	180 (ab)	117 (C)

Different apogee letters (A–E for cor and a–e for uncor) in the same row indicate statistically significant differences (honestly significant differences or HSD by Tukey, $p < 0.05$).

Diferentes letras de apogeo (A–E para cor y a–e para uncor) en la misma fila indican diferencias estadísticamente significativas (Diferencias honestamente significativas o HSD por Tukey, $p < 0.05$).

Of course, solid loss (SL) may also occur, i.e. leaching of chemical compounds in the treatment solution.

The Table 3 shows different information (retention time, maximum of absorbance and the most abundant mass fragments in positive or negative modality) useful to identify the 21 considered phenolic compounds. The values of these phenolic compounds expressed in mg/kg of untreated and processed samples with (''cor'') and without (''uncor'') correction for the concentration effect using equation 4 (see below) are reported in Table 4.:

$$Cx_i = \mathrm{FW_c} - \frac{\mathrm{FW_c} \cdot \mathrm{WR}}{100} \qquad (4)$$

where, Cx_i = content of compound x_i corrected for the concentration effect; FWc, content of compound x_i in treated sample; WR, weight reduction.

The data relative to ''cor'' values will be used to discuss the absolute variations in strawberries after the treatment.

We have previously found that the phenolic compounds in osmodehydrated strawberries at 5 °C for 24 h showed an interesting behavior (Blanda et al., 2009). Under the conditions used, strawberries presented an absolute increase in the polyphenolic content, principally ascribed to an increase in anthocyanins. Such behavior was also found in the present study, and from examination of the data in Table 4 and Figure 4, after the first 2 days of treatment, the total phenolic (sum of HPLC detected compounds) content increased. Although the phenolic content in the treatment solution was not determined, it is obvious that a certain extent of phenolic leaching in the treating solution will occur (also demonstrated by the pink color of the solution within a few hours after fruit immersion). Thus, the absolute increase can be explained by the neo-formation of phenolic compounds derived by hydrolysis of polymeric compounds or by activation of anabolic pathways. As reviewed by Stintzing and Carle (2004), anthocyanins play different roles in plant physiology, and appear to be important as monosaccharide transporters and osmotic adjusters during periods of drought and low temperatures. Strawberry cells submitted to osmotic stress induce anthocyanin synthesis (Suzuky, 1995), and thus it is not unexpected that strawberries osmodehydrated at low temperature had an increase in the anthocyanin content. This increase was evident in the first day of treatment, while the anthocyanin content undergoes a continuous decrease in the subsequent days. In fact, apart from the neo-synthesis effect, a leaching effect in the concentrated solution is present and probably becomes more important, prevailing in the latter stages of the process. This behavior was particularly evident for the most abundant compound, pelargonidin-3-glucoside, as shown in Figure 4. It is evident that in addition to anthocyanins, other phenolic compounds also had a similar behavior, such as the *p*-coumaroyl-glucoside (compound 10). Compound 9 (a tentatively identified galloyl derivative) showed an absolute increase in the first day of treatment that continued until the second day. Not only anthocyanins, but also other phenolic compounds in strawberries, may possess functional properties as osmotic regulators. Other compounds appeared to be less affected by the process itself, and their content did not change significantly during the process. Other phenols decreased constantly as only leaching was present (compounds 11 and 16).

By examination of Table 4 and Figure 3, where data are expressed referring to the fresh weight of samples, it can be noticed that the combination of three

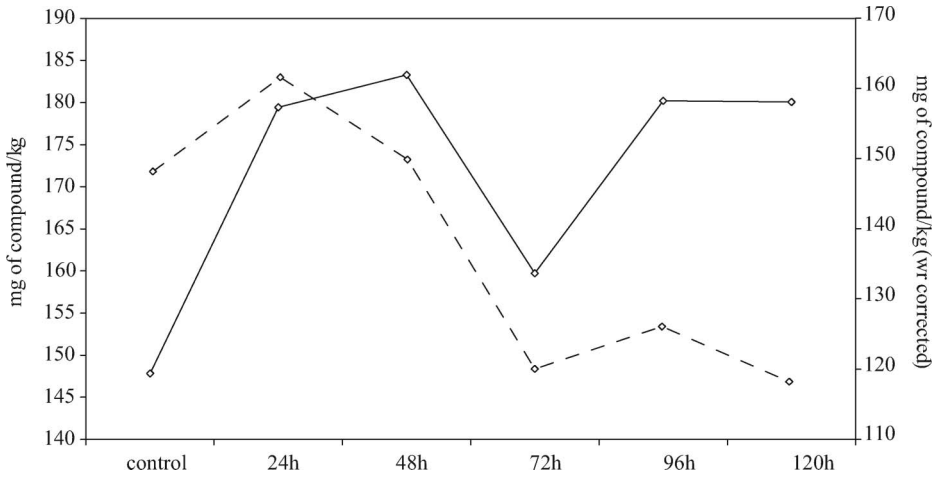

Figure 3. HPLC-DAD/MSD total phenolic contents of untreated and processed strawberries (continuous line) and of untreated and processed strawberries corrected for the concentration effect (dotted line) (see Equation 4).

Figura 3. HPLC-DAD/MSD contenidos fenólicos totales de fresas no tratadas y procesadas (línea continua) y de fresas no tratadas y procesadas, corregidas por el efecto de concentración (línea punteada) (vea Ecuación 4).

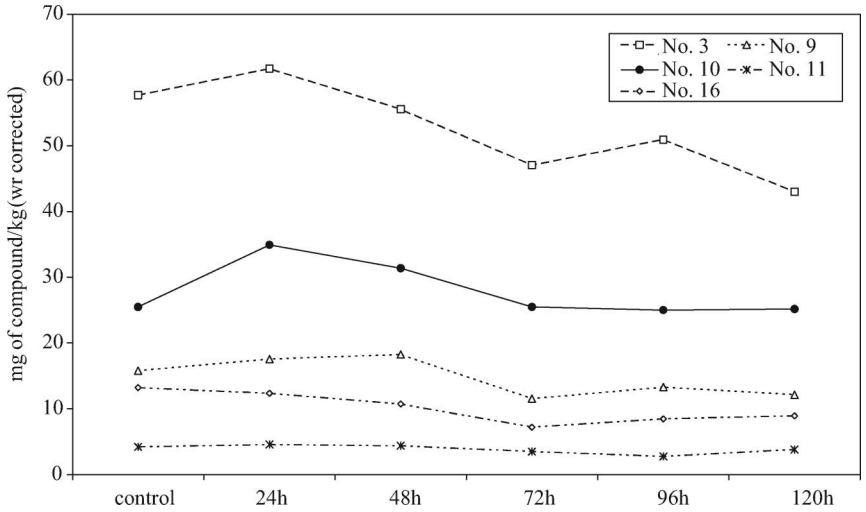

Figure 4. HPLC-DAD/MSD content of selected phenolic compounds of untreated and processed strawberries corrected for the concentration effect (see Equation 4).

Figura 4. HPLC-DAD/MSD contenidos fenólicos totales de fresas no tratadas y procesadas, corregidas por el efecto de concentración (vea Ecuación 4).

effects (leaching in the treating solution, neo-formation and re-concentration caused by WL) caused a higher content of phenolic compounds in all samples with respect to the control. In particular, during the first 2 days of treatment, when the neo-synthesis effect was high and the concentration effect was important, the total phenolic content was significantly higher and remained constant in subsequent days. Considering the behavior of individual compounds (Table 4), it can be seen that on the third day of treatment the leaching effect prevailed over the other two effects, while it was less important in the following days.

It is difficult to precisely understand the underlying reasons for the observed changes in polyphenolic content in osmodehydrated samples. The mechanism should involve the alteration of the cell walls and membranes causing an irreversible increase of permeability particularly after 2 days of treatment. The reaction of strawberries to OD is interesting and not only polyphenols, but also other compounds may be implicated in modifications during the process (e.g. organic acids or pectins). A better understanding of metabolic reactions and the chemical modifications would allow optimization of osmotic processes and production of high quality frozen fruits under the sensory and the nutritional point of view.

Conclusions

From a technological point of view, it is important to underline the high quality of samples treated for 96 and 120 h, and that they may potentially be employed as ingredients in foods (such as frozen desserts and cakes) due to the very low drip loss values and high acceptance level. At the same time, the phenolic content of treated samples was very high and comparable to fresh strawberries, and thus the product could be considered as healthy as fresh strawberries (with obvious marketing implications). The low temperature process probably induces a protective mechanism in strawberries that permits improvements in their freezing performance. This hypothesis needs to be confirmed by more in-depth studies on the textural, sensory, and chemical characteristics of fruits.

In the present study, we have provided an initial insight in the low temperature osmodehydro-freezing of whole strawberries. At the temperature used (5 °C), a few hours of treatment are not sufficient to obtain a substantial WL extent, thus making the technique relatively slow with respect to osmodehydration at higher temperatures. On the other hand, at low temperature the process has two major advantages: it permits a higher sensory acceptance level and a higher polyphenolic content with respect to osmodehydration at higher temperatures (as demonstrated in previous works) also with respect to the same fresh raw material. This last supposition is supported by the neo-synthesis of low molecular weight phenolic compounds, probably induced by the combination of osmotic stress and low temperatures. The increase in the phenolic content of strawberries occurs in the first day of treatment, and decreases slightly in subsequent days, when leaching in the osmotic solution prevailed. In any case, strawberries treated for 1–2 days at 5 °C presented a high acceptance level for consumers and a very high polyphenolic content (in particular anthocyanins), while strawberries treated for 3–5 days presented a reasonable acceptance level, very low drip loss and high phenolic content. Thus, the processing time influences the final quality of strawberries. The strawberries treated for 1–2 days are very suitable for direct consumption after thawing in substitution of

fresh fruits, and samples treated for 2–5 days may have interesting applications as ingredients in the food industry.

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FIRMNESS CHANGES OF IMPREGNATED WHOLE PEELED PRICKLY PEAR

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ABSTRACT

*The firmness of green prickly pear fruits (*Opuntia ficus indica*) impregnated with a sucrose isotonic solution (IS) was evaluated. Whole fresh-peeled prickly pears were processed under different combinations of vacuum pressure (p_v), vacuum application time (t_v) and relaxation time (t_r). Puncture test was applied to impregnated and fresh whole prickly pears. The maximum force (F_{max}), average force (F_{av}) and the work (W_p) required to puncture 3 cm in the fruits were measured. Second order models provided good fits to the experimental data of F_{max} ($R^2 = 0.754$), F_{av} ($R^2 = 0.788$) and W_p ($R^2 = 0.792$). Impregnation factors p_v, t_r, t_v–t_r significantly affected firmness parameters ($P \leq 0.05$). Firmness parameters of fresh-peeled prickly pear were $F_{max} = 10.934 \pm 1.571$ N, $F_{av} = 2.152 \pm 0.270$ N and $W_p = 5.930 \times 10^{-2} \pm 0.0054$ Nm. Firmness reduction of impregnated fruits was linked to the IS impregnation levels and deformation-relaxation phenomena.*

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PRACTICAL APPLICATIONS

The aim of this study was to assess the influence of impregnation conditions on firmness of whole peeled prickly pear, as excessive softening is the main factor limiting consumer acceptability. This study helps to identify the better impregnation conditions for maintaining firmness. This information could be useful for the application of vacuum impregnation in the development of fruits and vegetables products, in which the impregnating solution is used as a carrier of active agents. To date, very limited studies have been conducted in whole fruits involving only peeling and impregnation operations.

KEYWORDS

Firmness, prickly pear, puncture test, vacuum impregnation

INTRODUCTION

Fruits and vegetables become softer after harvesting, during storage or minimal processing (Soliva-Fortuny and Martín-Belloso 2003; Lana *et al.* 2005). Firmness is a relevant property for consumer acceptability and quality control (Camps *et al.* 2005).

Examples of minimal processing are impregnation and osmotic dehydration-impregnation, which are accomplished by immersing fruits or vegetables pieces in an isotonic solution (IS), in the former case, and in a hypertonic solution, in the later case (Mújica-Paz *et al.* 2003a,b). In recent years, a vacuum pulse has been applied on these processes for a better control of the mass transfer phenomena and compositional modification of the products (Alzamora *et al.* 2000; Zhao and Xie 2004).

A limited number of studies have been carried out to evaluate the effect of vacuum pulse application on firmness of fruits and vegetables subjected to osmotic dehydration treatments (Taiwo *et al.* 2003; Moreno *et al.* 2004). Other works have been designed with the specific purpose of increasing tissue strength through immersion of samples in hypertonic or ISs containing calcium salts and a vacuum pulse application (del Valle *et al.* 1998; Ortiz *et al.* 2003; Anino *et al.* 2006). Among the few existing reports, there are studies regarding the effect of impregnation treatment with IS on the firmness of kiwi halves (Muntada *et al.* 1998), mushrooms (Ortiz *et al.* 2003), whole jalapeño peppers (Mújica-Paz *et al.* 2004) and apple slices (Anino *et al.* 2006).

With the increasing applications of vacuum pulse for minimal processing of fruits and vegetables (Zhao and Xie 2004), the assessment of firmness

should be considered for evaluating the effect of these treatments on the quality of fruits and vegetables. Thus, the aim of this research was to study the effect of impregnation parameters on firmness of green-skinned, whole peeled prickly pear (*Opuntia ficus-indica*) using a sucrose IS.

Prickly pear is an oval elongated berry produced by the cactus *Opuntia*, which is native of Mexico. This fruit consists of a thick peel, covered with small thin spines, enclosing a sweet juicy pulp intermixed with many hard seeds. The prickly pear contains about 84–87% water and 11–16% soluble solids. It has a titratable acidity of 0.015–0.049 g citric acid/100 g pulp and a pH of 6.5–7.5 (Pimienta-Barrios 1994; El-Gharras *et al.* 2006).

MATERIALS AND METHODS

Material Preparation

Green-skinned prickly pear fruits (*Opuntia ficus-indica var* Villanueva) of homogeneous size and maturity and with no external injuries were selected. A 12% sucrose solution was prepared for impregnation trials. Such solution had the same water activity of fresh prickly pear (0.990 ± 0.001), which is the condition to be an IS. Water activity of the fruit and IS was measured with a hygrometer (Novasina, Lachen, Switzerland), which was calibrated with saturated salt solutions of NaCl ($a_w = 0.754$), KCl ($a_w = 0.851$) and K_2SO_4 ($a_w = 0.976$) at 20C (Ayranci and Duman 2005).

Firmness Measurement

The firmness of fresh and impregnated peeled prickly pears was determined by puncture test using a texture analyzer TA-XT2 (Texture Technologies Corp., Scardale, NY). A 2-mm-diameter stainless steel probe with a flat tip was driven radially into the whole peeled fruits at a speed of 10 mm/s at 11 different positions. The TAXT2 was set to automatically reverse the plunger travel direction when it reached the pre-established depth (30 mm). At the end of each test, the force–displacement curves, the maximum force (F_{max}, N), the average force (F_{av}, N) and the work required to penetrate the samples (W_p, Nm) were recorded and used for the evaluation of the firmness. Two peeled whole prickly pears were tested for each impregnation condition, performing seven punctures at different locations on each fruit. The same procedure was used with fresh fruits.

Impregnation Experiments

Impregnation treatments were performed by immersing weighed whole peeled prickly pears in a sucrose IS ($a_w = 0.990 \pm 0.001$) contained in a vacuum

TABLE 1.
IMPREGNATION CONDITIONS, IMPREGNATED VOLUMETRIC FRACTION AND FIRMNESS PARAMETERS OF WHOLE PEELED PRICKLY PEAR

Run	p_v (cm Hg)	t_v (min)	t_r (min)	X (cm^3 IS/cm^3 fruit)	F_{max} (N)	F_{av} (N)	$W_p \times 10^{-2}$ (Nm)
1	32.5	60.0	61.5	0.0718	7.483	1.363	4.114
2	22.0	14.5	96.2	0.0421	5.866	1.244	3.686
3	32.5	31.5	61.5	0.0548	6.660	1.443	4.323
4	22.0	14.5	26.7	0.0260	6.992	1.813	5.349
5	32.5	3.0	61.5	0.0419	6.548	1.400	4.256
6	32.5	31.5	120.0	0.0593	4.660	1.020	3.068
7	32.5	31.5	3.0	0.0238	4.915	1.355	4.099
8	42.9	48.4	96.2	0.0787	5.958	1.294	3.451
9	32.5	31.5	61.5	0.0530	5.630	1.355	3.825
10	50.0	31.5	61.5	0.0912	6.489	1.310	3.202
11	32.5	31.5	61.5	0.0547	5.822	1.691	4.887
12	32.5	31.5	61.5	0.0512	5.620	1.385	4.124
13	42.9	48.4	26.7	0.0691	4.810	1.274	3.565
14	32.5	31.5	61.5	0.0442	5.643	1.500	4.401
15	22.0	48.4	26.7	0.0317	5.314	1.277	3.636
16	42.9	14.5	96.2	0.0883	5.644	1.195	3.432
17	32.5	31.5	61.5	0.0490	5.846	1.565	4.658
18	15.0	31.5	61.5	0.0365	6.894	1.781	4.926
19	22.0	48.4	96.2	0.0478	5.638	1.448	3.998
20	42.9	14.5	26.7	0.0498	6.203	1.189	3.592

IS, isotonic solution.

desiccator, using a fruit-to-IS weight ratio of one to five. Prickly pears were kept dipped until consecutive time intervals under vacuum (t_v) and atmospheric conditions (t_r) elapsed. Then, impregnated samples were taken out of the IS, drained for 3 min, weighed and immediately punched. The experimental combinations and values of vacuum pressure (p_v) and time (t_v and t_r) were established using a central composite design (Table 1) (Cornell 1990).

In each of the impregnation treatments, the volumetric fraction of prickly pear impregnated with IS (X, cm^3 IS/cm^3 fruit) was calculated, using the density of the IS (ρ_{IS}), the initial volume of the fruit (V_0), and the weight of the fresh (m_0) and impregnated (m_{im}) fruit:

$$X = \frac{m_{im} - m_o}{\rho_{IS} V_o} \tag{1}$$

This equation was used under the assumptions that there is no deformation of the fruit during the whole impregnation process, and that the gas filling the voids of the tissue is replaced by an external solution as a result of the pressure changes (Zhao and Xie 2004).

Statistical Analysis

Regression analysis and analysis of variance (ANOVA) were carried out to examine the results of the firmness parameters (F_{max} F_{av}, and W_p and impregnation parameter X), using the Design-Expert software v. 6.0.6 (Stat-Ease, Inc., Minneapolis, MN). These response variables were related to the independent variables by linear or quadratic models (Montgomery 2001):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum \sum_{i<j} \beta_{ij} x_i x_j \quad (2)$$

where y represents the measured response (F_{max}, F_{av}, W_p, X); x_i represents the independent variables (p_v, t_v, t_r), in coded value; β_0 is the value of the fitted response at the center point of design; β_i, β_{ii} and β_{ij} are linear, quadratic and interaction regression coefficients of the model, respectively.

RESULTS AND DISCUSSIONS

Firmness Tests

Typical force–displacement curves are presented for fresh (Fig. 1) and impregnated (Fig. 2) peeled prickly pears. They show the force changes as the

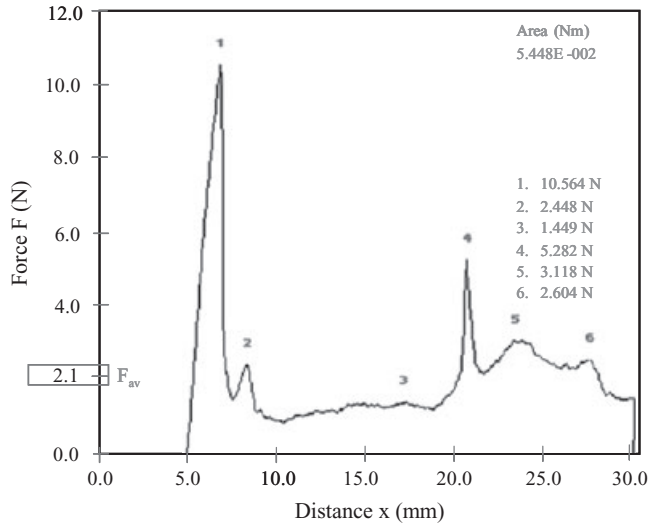

FIG. 1. SAMPLE FORCE–DISPLACEMENT CURVE RECORDED FOR FRESH WHOLE PEELED PRICKLY PEAR

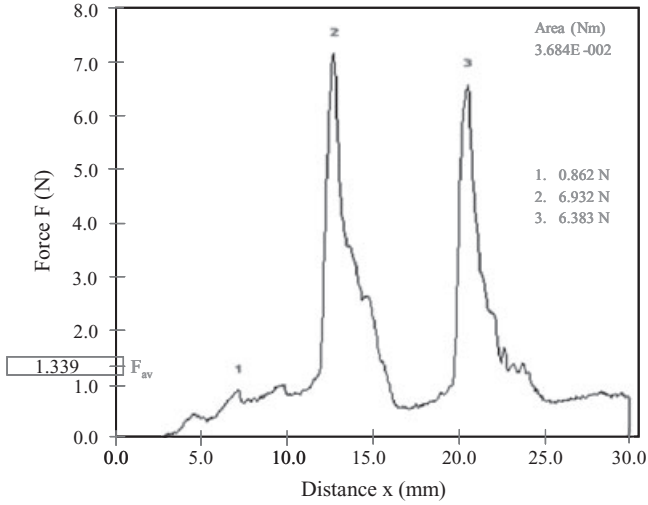

FIG. 2. SAMPLE FORCE–DISPLACEMENT CURVE RECORDED FOR IMPREGNATED WHOLE PEELED PRICKLY PEAR, AT p_v = 50 cm Hg, t_v = 31.5 MIN AND t_r = 61.5 MIN

probe passed through the prickly pear tissue and the total energy consumed during puncture (work of puncture, W_p), which is given by the area under the force–displacement curve (Camps *et al.* 2005). These figures also give the mean value of the forces involved in the puncture test (F_{av}) on the ordinate.

The graph of fresh fruit shows at the beginning of the penetration a sudden linear rise in force (Fig. 1) until the higher force value (peak) is reached. This point gives the maximum force of penetration (F_{max}), which is defined as the puncture force, and indicates the failure of the tissue (Camps *et al.* 2005). When the probe contacts a seed, the readings of the force increase, generating a small peak. This peak has an elevation proportional to the contact area between the probe and the seed, and the integrity of the tissue that surrounds the seed. A separation of the force–distance curve from the abscissa other than the peaks is observed. These sections of the curve represent the friction forces ($F \sim 1$–3 N) between the probe and the tissue of the fruit as the probe is moving down (Jackson and Harker 1997).

Marked differences in the pattern of force changes are observed in the force–distance curves obtained with impregnated fruit (Fig. 2). There is no initial peak during the first 10 mm and the frictional drag on the probe ($F < 1$ N), and the elevation of the peaks are lower than in the fresh prickly pear (Fig. 1). These variations have a direct influence on the puncture energy value (W_p) of the impregnated samples, which indicates that softening occurred in whole prickly pear subjected to impregnation treatments.

Firmness and Impregnation Parameters

From puncture tests on fresh peeled prickly pear, mean values of $F_{av} = 2.152 \pm 0.270$ N, $F_{max} = 10.934 \pm 1.571$ N and $W_p = 5.930 \times 10^{-2} \pm 0.005$ Nm were needed to puncture 30 mm into the flesh of fresh fruits.

The experimental design matrix and obtained responses (X, F_{max}, F_{av} and W_p) are shown in Table 1.

Significant regression models ($P < 0.05$) were obtained for X, F_{max}, F_{av} and W_p. Table 2 summarizes the regression analysis and the ANOVA and gives the estimated coefficients of Eq. (2), for the factors (p_v, t_v and t_r) in coded units.

The values of the lack of fit for the four obtained models were not significant if related to the pure error, which means that the models adequately represent the impregnation and firmness parameters variability (Montgomery 2001). A relatively high proportion of the firmness parameters variation (75.4 to 79.3%) could be explained by their fitted models, considering the normal spreading extent of texture measurements, ascribed to the inherent variability of biological materials (Rizvi and Tong 1997). The determination coefficient for the response X indicates that 95.2% of the total variations are explained by the experiment factors. According to the R^2 values, F_{av} and W_p seem to be more accurate parameters for discussion of prickly pear firmness.

The Effects of Impregnation Factors

Regarding the vacuum pressure and relaxation time (Table 2), the negative sign of their coefficients indicates that both factors induced firmness loss

TABLE 2.
SUMMARIZED STATISTICAL ANALYSIS OF THE IMPREGNATION AND FIRMNESS PARAMETERS OF WHOLE PEELED PRICKLY PEAR

Factors	X (cm^3/cm^3)	F_{max} (N)	F_{av} (N)	$W_p \times 10^{-2}$ (Nm)
β_o	+0.051	+5.88	+1.49	+4.37
p_v	+0.017*	–0.120	–0.120*	–0.400*
t_v	$+5.223 \times 10^{-3}$*	–0.085	–0.015	–0.110
t_r	+0.010*	–0.065	–0.068***	–0.240**
p_v^2	$+4.487 \times 10^{-3}$**	+0.220	+0.014	–0.120
t_v^2	$+1.995 \times 10^{-3}$	+0.330**	–0.043	–0.090
t_r^2	-3.404×10^{-3}**	–0.460*	–0.110*	–0.290**
$p_v\ t_v$	-2.070×10^{-4}	+0.130	+0.064	+0.170
$p_v\ t_r$	$+1.984 \times 10^{-3}$	+0.140	+0.053	+0.130
$t_v\ t_r$	-3.613×10^{-3}	+0.360***	+0.094***	+0.260***
Lack of fit	ns	ns	ns	ns
R^2	0.952	0.754	0.788	0.793

* P value < 0.01; ** P value < 0.05; *** P value < 0.1.
ns, not significant.

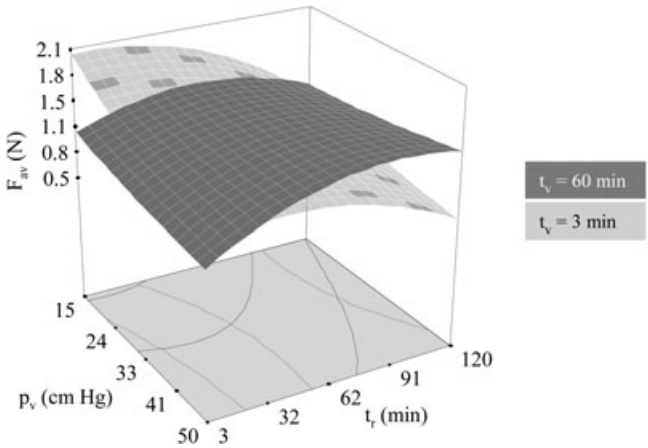

FIG. 3. EFFECT OF VACUUM PRESSURE AND RELAXATION TIME ON F_{av} OF IMPREGNATED WHOLE PEELED PRICKLY PEAR

of prickly pear fruits, as they increased. However, when these factors increase, higher impregnation levels can be reached, as stated by the positive sign of their coefficients in the model.

The predicting models of Table 2 allowed plotting of the response surfaces for F_{av} (Fig. 3) and W_p (Fig. 4), at short (3 min) and long (60 min) vacuum application times. Under these conditions, similar graphs were obtained for F_{av} and W_p, but for each firmness parameter, significant differences can be noticed at the two t_v levels.

At $t_v = 3$ min, firmness parameters decreased continuously from 2.04 to 0.50 N for F_{av} (Fig. 3) and from 5.82×10^{-2} to 1.18×10^{-2} Nm for W_p (Fig. 4), over the given intervals of p_v and t_r. This softening behavior may likely be attributed to a leaching process and deformation-relaxation phenomena, even if vacuum application time is very short to cause significant deformation. Thus, as t_r increases, water-soluble pectins may partially dissolve, as they represent around 32–75% of the total pectins in prickly pear (Goycoolea and Cárdenas 2003). Because of the high calcium content of prickly pear pulp (93 mg/100 g fresh weight; Stintzing and Carle 2005), leaching of calcium ions into the IS might also occur, gradually reducing the number of cross bridges between Ca^{++} and the carboxyl groups of pectic acids (Poovaiah 1986). These assumptions would be supported by the increasing trend of X with t_r and p_v, at $t_v = 3$ min, as shown in Fig. 5. Thus, slightly reduced levels of soluble pectin and calcium ions inside the prickly pear could be responsible for the observed general pattern of firmness reduction (Figs. 3 and 4) at $t_v = 3$ min.

On the other hand, long vacuum application time (60 min) drastically decreased the firmness of prickly pear ($F_{av} = 1$ N, $W_p = 2.9 \times 10^{-2}$ Nm), but as

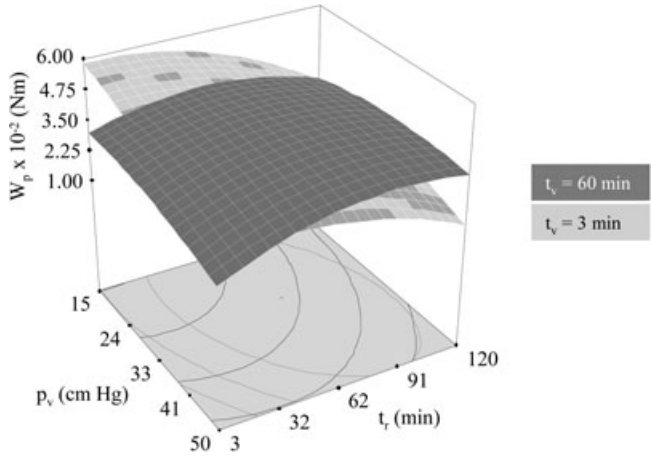

FIG. 4. EFFECT OF VACUUM PRESSURE AND RELAXATION TIME ON W_p OF IMPREGNATED WHOLE PEELED PRICKLY PEAR

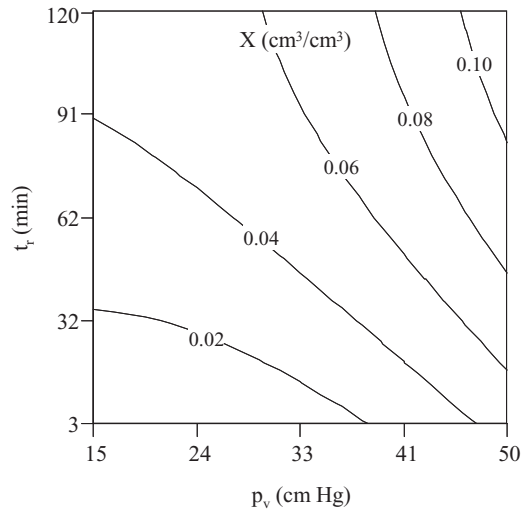

FIG. 5. VOLUME FRACTION OF WHOLE PEELED PRICKLY PEAR IMPREGNATED WITH ISOTONIC SOLUTION, AS A FUNCTION OF VACUUM PRESSURE AND RELAXATION TIME, AT $t_v = 3$ MIN

soon as the relaxation period started, F_{av} and W_p changed according to the quadratic effect of t_r (Table 2, Figs. 3 and 4). Although prickly pear tissue is under vacuum conditions, dissolving and leaching phenomena probably occur; this may explain the observed drastic change of F_{av} and W_p at the end of the

vacuum application time. After this deformation period, the prickly pear tissue relaxes and the IS gradually fills the intercellular spaces during the first 52 min. Along this stage, the levels of IS in the pores could still be low but significant for opposing resistance to the probe, while it is descending through the fruit flesh, producing the observed increasing trend of W_p and F_{av} (Figs. 3 and 4).

In longer relaxation times ($t_r > 52$ min), F_{av} and W_p of the impregnated samples show a decreasing trend. This behavior might be linked to severe deformation-relaxation phenomena of the soft sensitive prickly pear pulp and loss of tissue integrity and aggregation. Under these conditions, the existing pressure gradient would lead to higher levels of IS in the fruit porous structure, which in turn could have favored partial leaching of soluble pectins and cross-linking agents, which are naturally present in the prickly pear structure (Goycoolea and Cárdenas 2003). It is well documented that pectins and cross-linking agents are essential for maintaining the cell wall rigidity and integrity of the tissue (Reyes-Agüero *et al.* 2005; El-Gharras *et al.* 2006).

The combined effect of deformation-relaxation phenomena, soluble pectin loss and calcium leaching could be particularly relevant in the outer pericarp of prickly pear, where tissue zones are more exposed to the IS. The overall effect of these factors would cause the softening of tissue as observed in the early stage of the force–distance curve in Fig. 2.

The strong decrease of prickly pear firmness caused by impregnation treatments can be slightly limited by selecting the least severe impregnation conditions. For instance, performing impregnation at $t_r = 65$ min, $p_v = 28$ cm Hg and $t_v = 60$ min would reduce prickly pear firmness parameters around 35% at an acceptable impregnation level ($X = 0.043$ cm^3 IS/cm^3 fruit). A better alternative to counteract the overall softening effect of impregnation factors on prickly pear could be the addition of calcium salts, a well-known firming agent, to the impregnating solutions (Soliva-Fortuny and Martín-Belloso 2003). Thus, specific studies should be carried out to evaluate the effect of calcium salts on prickly pear subjected not only to impregnation, but also to impregnation-dehydration treatments. The beneficial role of calcium in these treatments could be influenced by the type and concentration of solute, and the interactions between calcium ions and free carboxyl groups of pectin chains.

CONCLUSIONS

Whole prickly pear resulted as very sensitive to vacuum impregnation treatments. The firmness of impregnated prickly pear, mainly evaluated as mean average force and work of puncture, decreased significantly with relax-

ation time and vacuum pressure. The major change in firmness was observed after long vacuum application times. Favorable impregnation conditions cause firmness reduction of around 35%. The softening of impregnated prickly pear tissue was explained in terms of deformation-relaxation, pectin solubilization and calcium leaching phenomena. Further studies should be carried out to evaluate the effect of calcium salts in retaining the firmness of impregnated prickly pear.

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

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Running title: Aroma Enrichment of Apple Sticks

Abstract. Food industry often needs flavour enriched semi-finished food products to employ as ingredients in more complex preparations. To the best of our knowledge there are not methods to produce minimally processed food items added with flavourings.

In this investigation apple sticks were enriched with a green apple aroma, applying different techniques; vacuum impregnation (VI), ultrasound technology (USI) and the combination of these two techniques (VUSI) were tested and compared with atmospheric pressure impregnation (AI).

An isotonic solution of fructose, containing ascorbic acid and green apple flavouring was used for the impregnation of apple sticks. Different treatment times (2.5, 5.0 and 12.5 min) were investigated and the concentration of the major compounds of impregnation flavouring was recorded.

Significant differences between treatments were detected: VI and VUSI gave the highest aroma enrichment at 5.0 min of treatment. Different impregnation behaviours were recorded for alcohols and esters: the first increased even after 5.0 min of treatments, the others components instead rose until 5.0 min and then decreased, mainly when ultrasounds were applied (US and VUSI). Some possible causes of such results were supposed, but further studies are necessary to explain the mechanisms involved.

Keywords. Apple sticks, aroma enrichment, impregnation techniques, ultrasound, vacuum impregnation.

Introduction

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.

Dairy products, like flavoured milks, yogurts, and frozen desserts, represent a major market for flavouring materials (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.

The production of convenient, fresh and healthy foods has been widely investigated in the last few years and has included fortified foods with physiologically active compounds (PAC), like probiotics (Alzamora et al. 2005), calcium (Mújica-Paz et al. 2002; Torres et al. 2006; Saxena et al. 2009) or zinc salts (Zhao and Xie 2004); osmo-dehydrofreezed fruits enriched with cryoprotectants (Talens et al. 2002a,b); or also 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 which 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 ultrasounds have been used to accelerate the mass transfer kinetics involved in osmotic dehydration (Fernandes et al. 2008). The high intensity of the 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 transfers (Mason et al., 1996).

In this study the methodologies used for the manufacturing of functional food enriched with PAC, as vacuum and atmospheric impregnation, and the innovative technology of ultrasounds will be employed to promote the flavouring enrichment of apple sticks. This investigation will compare different impregnation techniques, such as impregnation at atmospheric pressure (AI), vacuum impregnation (VI), impregnation assisted by ultrasounds (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 the analysis. Fruits were cut in regular sticks (45 x 9 x 9 mm^3) with an handle cutter; core sticks, peel and seeds were removed. Each sample, obtained from 6 apples, was shared in 4 aliquots of 50 g and submitted to the impregnation treatments described below.

Impregnation treatments

The experiments were carried out at room temperature; a fructose isotonic solution (14.0-15.0°Bx), containing ascorbic acid (0.5% w/w) and dry green apple flavouring (0.5% w/w) of food grade 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. Step 1 and 2 lasted respectively 5.0 and 1.5 min, instead, during step 3 different times (2.5, 5.0 and 12.5 min) were tested.

- AI (Atmospheric Impregnation): Apple sticks were maintained all the time at atmospheric pressure.
- VI (Vacuum Impregnation): 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). Then atmospheric pressure was restored in 1.5 min (step 2) and apple sticks were maintained immersed for 2.5, 5.0 or 12.5 min (step 3).
- USI (Ultrasound Impregnation): Samples were maintained at atmospheric pressure during the first two steps and then they were put in a 35 kHz ultrasonic bath (Liarre s.r.l., Bologna, Italy) during step 3.
- VUSI (Vacuum plus Ultrasound Impregnation): The vacuum pressure (280 mbar) was applied during step 1, then atmospheric conditions were restored (step 2) and the samples were placed inside the ultrasonic bath (step 3) to complete the treatment.

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

Volatiles 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 14000 rpm (Ultra Turrax, IKA-Werke T25 basic, Staufen, Germany). The mixture was centrifuged at 3500 rpm (1880 × *g*), for 10 min. 1 g of supernatant was recovered

and diluted 50 times with distilled water. 50 µL of butan-1-ol (0.04 µL*100 mL^{-1}) were added as internal standard.

The HS-SPME devices and the fused silica fiber coated with DVB/CAR/PDMS (50/30 µm, 2 cm) were purchased from Supelco (Bellefonte, PA, USA).

5 g of the diluted sample was placed in a 10 mL vial hermetically closed with a PTFE/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. A 30 min extraction at 50°C was performed. After sampling, the fiber was desorbed for 5 min at 250°C.

Volatile compounds were analyzed by gas chromatograph GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan), coupled to Shimadzu's GC-MS solution software (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, USA). 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 then was increased up 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°C and 280°C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy in the 20-250 amu mass range (2 scan sec^{-1}). Volatile compounds present in flavour enriched apple sticks 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 and literature data.

After identification of the major components of the green apple flavouring, samples 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, Torrance, CA, USA). 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.

Response factor

The response factor (RF) of the analytes to the internal standard was calculated. RF was expressed as normalized peak area on initial weight of samples [g^{-1}], according to the following equation:

$$RF = \frac{A_{vol}}{A_{i.s.} * W_i}$$

where A_{vol} is peak area of volatiles [µV s], $A_{i.s.}$ is peak area of internal standard [µV s] and W_i is the initial weight of the sample [g], before the impregnation treatment.

RF was determined for the individual compounds of green apple flavouring and for the overall aroma, by summing the RFs of ethyl 2-methylbutanoate, 3-methylbutylacetate, hexyl acetate and hexan-1-ol.

Statistical analysis

Data were analysed using Statistica 8.0 (Statsoft Inc., Tulsa, OK); two ways ANOVA was carried out and Fisher's least significant differences test was applied ($p<0.05$).

Results and discussion

Preliminary experiments

Several experiments were made to develop and optimize the flavouring impregnation techniques, the volatile extraction and the GC analytical conditions.
It was chosen to carry out magnetic agitation during SPME extraction because an improvement of the signal to noise ratio of about 1.35 was noted.
The linearity range of SPME/GC response was evaluated by analyzing different aqueous dilutions of the flavour enriched apple samples; in particular, 4 dilutions were tested, 1:10, 1:25, 1:50 and 1:100. The correlation coefficients between RFs and dilution employed were calculated for the major compounds of green apple flavouring and they resulted respectively 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 flavouring

A typical gas chromatographic trace obtained by analyzing flavour enriched apple sticks is reported 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 number five is butan-1-ol which was added to the samples before SPME/GC analysis as internal standard.
All these compounds were components of the external flavouring because, owing to the dilution used, the native volatiles of apples were not detectable, as observed in preliminary experiments.

Overall green apple flavouring impregnation

Significant differences ($p<0.05$) were detected between treatments and times for green apple flavouring impregnation. As reported in Table 2, VI and VUSI gave the highest impregnation, compared to AI and USI, mainly at 5.0 min. USI treatments, instead, were not different from AI, for this reason it was supposed that the higher RFs obtained for VUSI were closely related to the vacuum effect. The higher volatiles impregnation obtained in VI and VUSI was due to the fraction of isotonic solution penetrated inside the apple sticks by hydrodynamic mechanism (HDM), that is the pressure gradient developed when the atmospheric pressure is restored. In fact, VI and VUSI samples after treatment had a weight variation of about 14%; on the other hand, no significant weight variations were detected for AI and USI samples.

Evolution of esters and alcohols concentration

The RFs of the major components of green apple flavouring (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 concentration of ethyl 2-methylbutanoate, 3-methylbutyl acetate and hexyl acetate increased until 5.0 min of impregnation with all treatments.

In AI, esters concentration increased until 5.0 min of treatment and then reached a maximum level. An exception is ethyl 2-methylbutanoate whom concentration gradually rose until 12.5 min. We believe that apple sticks reached and equilibrium state with the impregnation medium after some min of treatments. No weight variations (before and after AI treatment) were detected, so the volatiles transfer was mainly due to selective diffusion mechanism of aromatic compounds.

Also in VI esters RF increased until 5.0 min, then a stabilization occurred. These findings highlighted that, in the experimental conditions used, a 5.0 min relaxation time determined the maximum impregnation of the product; the increase of the relaxation phase up to 12.5 min did not determine an improvement of the volatile concentrations. The same weight variation (15%, $p<0.05$) was recorded for the three different relaxation steps, 2.5, 5.0, and 12.5 min. Based on these results we hypothesized that 2.5 min of relaxation time were sufficient to determine the inlet of liquid inside apple tissues, but volatiles transfers continued until 5.0 min thanks to a diffusion mechanism. For this reason the 5.0 min relaxation time caused a higher RF for VI samples, although the volume of isotonic solution penetrated into apple sticks did not change.

The RF obtained with USI was due mainly to the diffusion mechanism, because no significant weight variations were detected for USI samples after treatment. Esters RF increased until 5.0 min

of impregnation, then 3-methylbutyl acetate and hexyl acetate concentration decreased; the RF of ethyl 2-methylbutanoate, instead, was constant.

Hexyl acetate concentration was always higher in USI respect to AI. As regard ethyl 2-methylbutanoate and 3-methylbutyl acetate, the maximum US concentration, obtained at 5.0 min, was the same as AI; the ultrasound application until 12.5 min, instead, caused a reduction of such compounds.

The application of VUSI technique caused an elevated impregnation of esters during the first 5.0 min of treatments, with a trend similar to VI; after 5.0 min, instead, a significant decreasing of esters concentration was noted.

In our view until 5.0 min, the main US effect was mixing the solution and maintaining an high gradient of concentration at the surface of the product. After 5.0 min, instead, the reduction in concentration was related to an acceleration of the evaporation rate. In fact, at 5.0 min of treatment the increase in temperature was about of 0.8°C; at 12.5 min, instead, the temperature of the isotonic solution increased of about 2.1°C, promoting a major evaporation of the most volatile components.

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

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

$$Fm = ks(Css - Cs)$$

where ks was the mass transport coefficient, Css was the aroma compound concentration at the surface of the solution and Cs was 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 flavour concentration and the mass transport took place from the solution to air to restore the phase equilibrium. In the analytical conditions used, the flavour depletion 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 function of the diffusion mechanism. In stagnant systems, mass transport can take place only by molecular (static) diffusion, caused by the random movement of the molecules (De Roos, 2006). Dynamic systems are characterized by eddy or convective diffusion, that is transport of the fluid elements and the dissolved solutes, from one location to another.

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 esters concentration 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 flavouring enrichment was HDM, but in VI the relaxation was realized in static conditions, at atmospheric pressure, and the esters concentration did not vary significantly; in VUSI, instead, the application of ultrasounds during relaxation, caused a notable flavouring depletion for all esters at 12.5 min.

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

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

Conclusions

The findings of the present investigation were consistent with those reported in the literature regarding the efficacy of vacuum application in flavour impregnation: VI and VUSI treatments gave the highest aroma enrichment at 5.0 min; USI, instead, was not significantly different from AI.

Esters and alcohols had different impregnation behaviours: further studies are necessary to explain such results, as well as the reduction of ethyl 2-methylbutanoate, 3-methylbutyl acetate and hexyl acetate after some min of treatment.

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Tables legend

Table 1 Impregnation conditions of apples with green apple flavouring (AI: Atmospheric Impregnation, VI: Vacuum Impregnation, USI: Ultrasound Impregnation, VUSI: Vacuum plus Ultrasound Impregnation).

Table 2 RF obtained for green apple flavouring impregnation with different treatments and times. Values are means ± standard deviations (n = 3).

Figures legend

Fig. 1 SPME-GC/MS chromatogram of flavouring enriched apple sticks with VI technique. Peaks: 1=Ethyl butanoate, 2=Ethyl-2-methylbutanoate, 3=Butyl acetate, 4=3-Methylbutyl acetate, 5=Butan-1-ol (I.S.), 6=3-Methylbutan-1-ol, 7=3-Methylbutyl butanoate, 8=Hexyl acetate, 9=(E)-Hex-3-enyl acetate, 10=Hexan-1-ol.

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).

Table 1 Impregnation conditions of apples with green apple flavouring (AI: Atmospheric Impregnation, VI: Vacuum Impregnation, USI: Ultrasound Impregnation, VUSI: Vacuum plus Ultrasound Impregnation).

Treatment	Impregnation steps		
	Step 1 (5 minutes)	Step 2 (1.5 minutes)	Step 3 (2.5/5.0/12.5 minutes)
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

Table 2 RF obtained for green apple flavouring impregnation with different treatments and times. Values are means ± standard deviations (n = 3).

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

[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. 1 SPME-GC/MS chromatogram of flavouring enriched apple sticks with VI technique. Peaks: 1=Ethyl butanoate, 2=Ethyl-2-methylbutanoate, 3=Butyl acetate, 4=3-Methylbutyl acetate, 5=Butan-1-ol (I.S.), 6=3-Methylbutan-1-ol, 7=3-Methylbutyl butanoate, 8=Hexyl acetate, 9=(E)-Hex-3-enyl acetate, 10=Hexan-1-ol.

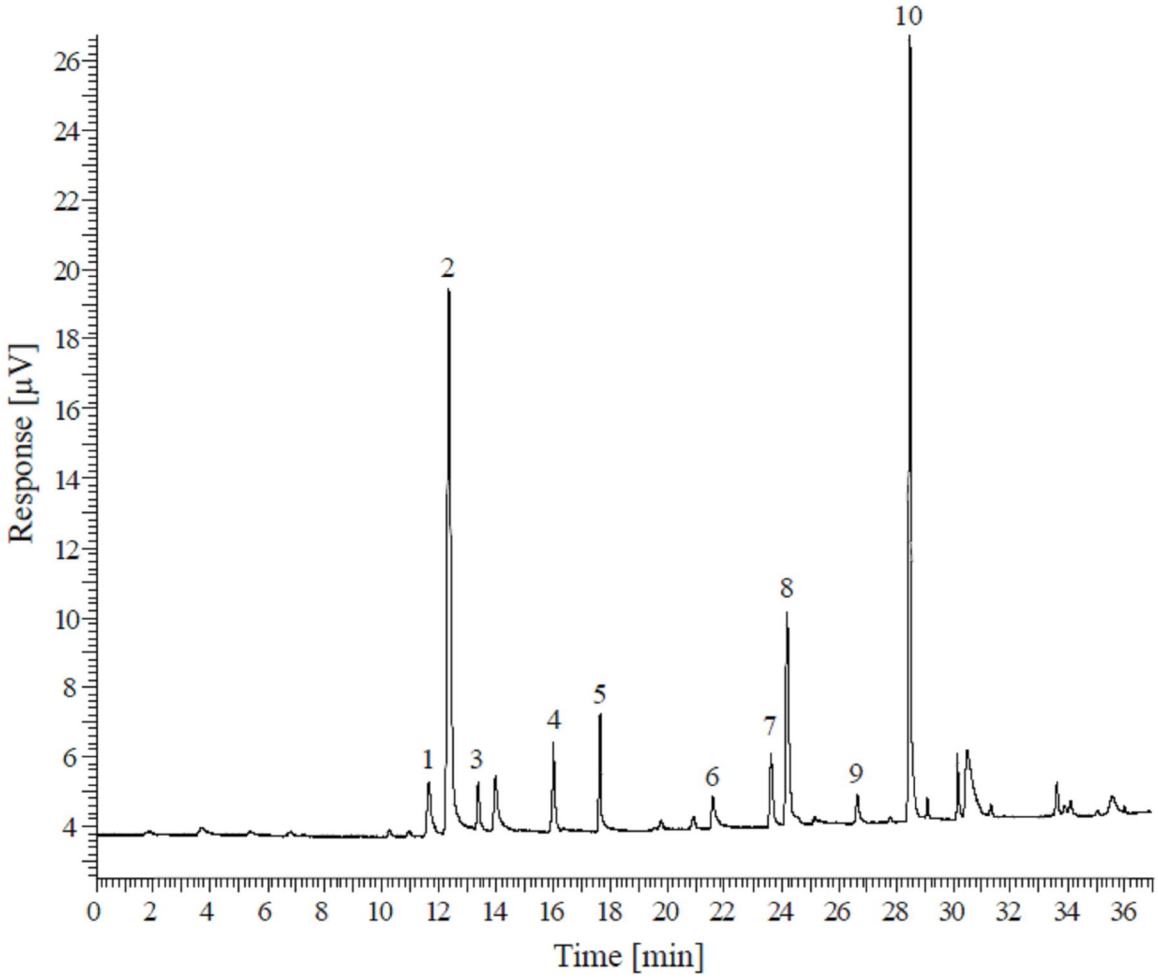
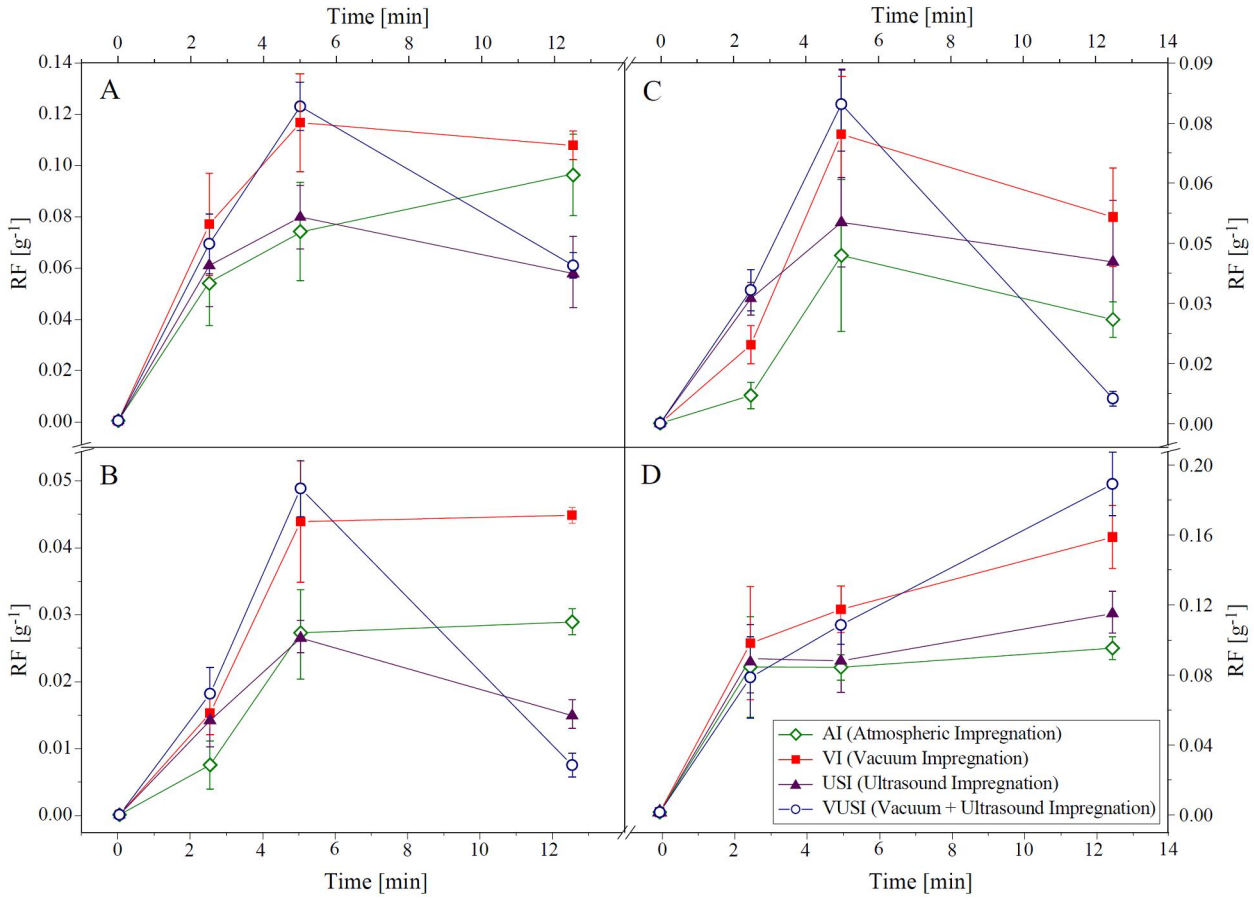


Fig. 2 Impregnation of ethyl 2-methylbutanoate (A), 3-methylbutyl acetate (B), hexyl acetate (C) and hexan-1-ol (D) at different relaxation times (2.5, 5.0 and 12.5 min).



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Original Paper

CZE separation of strawberry anthocyanins with acidic buffer and comparison with HPLC

Anthocyanins, the major colourants of strawberries, are polar pigments that are positively charged at low pH. Herein, we have assessed a new analytical method for the separation of anthocyanins using CZE. Acidic buffer solutions (pH <2) were employed in order to maintain pigments in the cation flavylium form and achieve high molar absorptivity at 510 nm. These spectral properties enabled us to identify strawberry anthocyanins in a preliminary stage by detection in the visible range, although the method was optimised at 280 nm to obtain the best S/N. The effects of buffer composition highlighted the necessity of adding an organic modifier to the running buffer to obtain a suitable separation. The electrophoretic method permitted the separation of the three main anthocyanins of strawberry extracts, namely pelargonidin 3-glucoside (Pg-glu), pelargonidin 3-rutinoside and cyanidin 3-glucoside. The electrophoretic results, expressed as retention time and separation efficiency of the major anthocyanin (Pg-glu), were compared to those achieved in HPLC, the analytical technique traditionally used for the investigation of anthocyanins in vegetable matrix. The content of Pg-glu in strawberries (*cv.* Camarosa), calculated with HPCE and HPLC methods, resulted respectively in 11.41 mg/L and 11.37 mg/L.

Keywords: Anthocyanins / CZE / Food analysis / HPLC / Strawberry

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1 Introduction

Anthocyanins are a large class of water-soluble pigments found in most plant species. These polyphenols provide orange, red, violet, purple and blue colouration in leaves, stems, roots, flowers and fruits such as roses and carnations, eggplant and cauliflowers, apples, red grapes, strawberries, cranberries, blackcurrants and many others [1]. Due to their bright colouration, anthocyanins play an important role in reproduction by attracting pollinators and promoting dispersion of seeds. These pigments occur not only in vacuoles of flowers and fruits, but are also present in storage and vegetative tissues, such as roots and leaves. Their role in the latter is not well established, but a protective function from UV light seems to be the most reasonable explanation [1], moreover in plant organs that are not exposed to light anthocyanins act as transport vehicles for monosaccharides and as osmotic adjusters during periods of drought and low temperatures [2].

The therapeutic effects of anthocyanins in humans are related to their antioxidant capacity, which is why foodstuffs containing flavonoids have functional properties [3]. Analysis of anthocyanins may permit qualitative evaluation of fresh and processed food products [4], and have also been employed in establishing the varietal and geographical origin of red wines [5]. In jams and fruit juices containing red fruits, the compositional profile of anthocyanins can be used to evaluate adequacy with information reported on labelling [6, 7]. A major limitation to the use of anthocyanins as food colourants is their susceptibility to environmental factors such as light, pH and temperature [8].

Anthocyanins are amphoteric compounds: they can exist as either a weak acid or base depending on the pH. Pigment stability and colouration are related to the concentration of hydronium ions. According to the pH in solution, anthocyanins may occur in four different chemical forms: flavylium cation, carbinol (pseudo)base or hemiacetal, chalcone or quinoidal base (Fig. 1). The flavy-

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Abbreviations: Cy-glu, cyanidin 3-glucoside; **DAD,** diode array UV–Vis detector; **MSD,** mass spectrometer detector; **Pg-Cl,** pelargonidin chloride; **Pg-glu,** pelargonidin 3-glucoside; **Pg-rut,** pelargonidin 3-rutinoside

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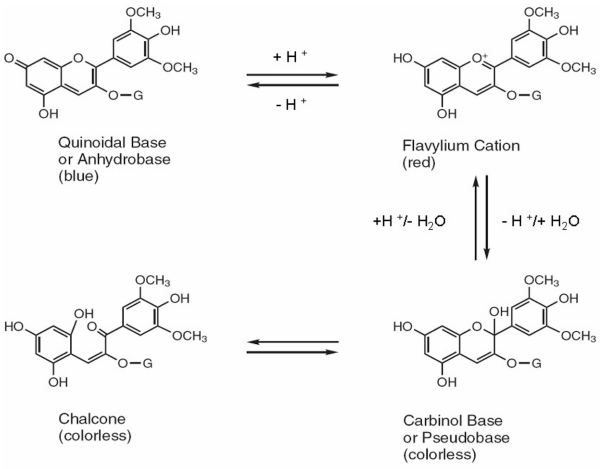

Figure 1. Anthocyanin equilibrium in aqueous solution; G: glucose (adapted from ref. [9, 10]).

lium cation is the most stable structure; it is responsible for orange–red colouration of anthocyanins under strong acidic conditions (pH <2); these hues fade when the pH increases, until complete discolouration is achieved. Around neutrality, extracts present blue–violet colouration with decreasing intensity. Shifting the pH to higher values may denature these molecules in an irreversible manner, with a complete loss of colour [8].

RP-HPLC is the most widely used analytical technique for the separation of anthocyanins [1, 4, 11, 12]. In the last few years, electrophoretic techniques, such as high performance CE (HPCE), have been extensively utilised due to their numerous advantages such as the small amounts of sample and reagents needed, limited costs and high efficiencies. To date, electrophoretic separation of anthocyanins in red fruits has been poorly reviewed [13–16]. To the best of our knowledge, there are no publications on the electrophoretic separation of anthocyanins in strawberry in acidic BGEs.

In 1997 Bridle and García-Viguera [14] reported on the separation of strawberry and elderberry anthocyanins in CE with borate running buffer at pH 8. They obtained a reduced analysis time (less than 6 min), but the applicability of their method was limited by the instability of anthocyanins in basic media; moreover, concentrated samples had to be analysed due to the decreased absorptivity of the predominant ionic species at 580 nm [4]. More recently Watson *et al.* [16] developed a capillary electrophoretic method to analyse cranberry anthocyanidins. After hydrolysis, they were able to obtain acidic separation (phosphate buffer at pH 2.11) of the analytes in 17.5 min.

Building upon the research of Da Costa *et al.* [15], who published a new method for the separation of anthocyanins of blackcurrants under acidic conditions, we have optimised a similar method using CZE for the separation of coloured pigments in strawberry extracts. With this study it was our intention to reduce the retention time of anthocyanins and improve the efficiency by lowering electromigration dispersion (EMD) due to the strongly acidic conditions employed. The results of CZE analysis (migration time, efficiency, LOD, LOQ and solvent consumption) were compared with those obtained in HPLC adapting a method previously pointed by Blanda *et al.* (2008, unpublished data).

2 Experimental

2.1 Reagents

Pelargonidin chloride (Pg-Cl) standard was acquired from Sigma (St. Louis, MO, USA), HPLC-grade methanol and water, HPCE-grade water, monobasic sodium phosphate dihydrate ($NaH_2PO_462H_2O$, puriss.), 85% *ortho*-phosphoric acid (H_3PO_4, p.a.), formic acid (CH_2O_2, p.a.), ACN (CH_3CN, assay ≥99.9%) and *iso*-propanol ($CH_3CH(OH)CH_3$, assay ≥99.9%) were purchased from Merck (Darmstadt, Germany). Sodium chloride (NaCl, p.a.), sodium hydroxide in pellets (NaOH, assay ≥99%) were from Carlo Erba (Milan, Italy). Deionised water was obtained from an Elix 10 water purification system from Millipore (Bedford, MA, USA).

2.2 Strawberry samples

Strawberries (*cv.* Camarosa) were purchased from a local market in Cesena (Forlì-Cesena, Italy). From the stock bought, about 30 kg, only fruits without bruises, damages or fungal attacks were selected. The fruits were deep-frozen at −47°C and stored until extraction of anthocyanins.

2.3 Strawberry extract preparation for CZE and HPLC analyses

Strawberry extracts were obtained by adapting the method from Blanda *et al.* (2008, unpublished data) and Gil *et al.* [17]. Briefly, 100 g of strawberries were ground in a blender with 100 mL of methanol for about 1 min. Next 10 g of homogenate was centrifuged at 22 000 rpm (39 600 × *g*) for 10 min at 10°C (Avant J25, Beckman Coulter, Fullerton, CA, USA). The supernatant was recovered and a second extraction was performed on the sample residue with 10 mL of 95% aqueous methanol. The solid residue and the hydro-alcoholic mixture were homogenised with an Ultra Turrax (IKA-Werke mod. T 25 basic, Staufen, Germany) at 13 000 rpm (rotor speed) for 2 min and then centrifuged at 39 600 × *g* for 10 min at 10°C (Avant J25, Beckman Coulter) before separating the second amount of supernatant. Next, the two extractions were combined and evaporated in a vacuum centrifuge

(35°C) to complete dryness (MIVAC DUO, Genevac, Ipswich, England). The concentrated extract was dissolved in 5 mL acidified water (3% formic acid) and then passed through an SPE Strata C_{18}-E 55 μm 70 A cartridge (500 mg/3 mL) (Phenomenex, Torrance, CA, USA), previously activated with methanol, followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column while sugars, organic acids and other highly water-soluble components were eluted with 3% aqueous formic acid. The anthocyanins and other phenolic compounds were recovered with 2 mL of methanol containing 3% formic acid. Methanolic extracts were filtered through 0.20 μm cellulose acetate syringe filters (Orange Scientific, Braine-l'Alleud, Belgium) and injected in HPLC and HPCE for the analysis of anthocyanins as described below.

2.4 CZE determination of anthocyanins

2.4.1 Equipment

CZE analyses were performed with a CE instrument P/ACE 5500 from Beckman equipped with a single wavelength UV–Vis detector and a diode array UV–Vis detector (DAD). Processing and data acquisition were performed using software from Beckman (Beckman P/ACE Station – CE Software, version 1.21). The capillary cartridge contained a polyimide-coated fused-silica tube (375 μm od, 50 μm id) supplied by Beckman. The total capillary length was 57 cm with an effective length of 50 cm.

2.4.2 Running buffer and washing solutions

The optimised running buffer was 250 mM monobasic sodium phosphate containing 30% v/v ACN (pH 1.4). The solution was prepared dissolving 3.9 g of the salt in 100 mL of HPLC-grade water, titrating to pH 1.5 with *ortho*-phosphoric acid and adding 30% ACN v/v. Finally, the buffer was adjusted to pH 1.4 with *ortho*-phosphoric acid, filtered through 0.20 mm nylon filters (Whatman, Florham Park, NJ, USA) and sonicated for 10 min. Capillary conditioning and washing solutions (NaOH 1 and 0.1 M; HCl 1 N; H_3PO_4 0.1 M) were prepared in HPLC-grade water.

2.4.3 Electrophoretic conditions

New capillaries were conditioned, at 25°C, by flushing with HCl 1 N (5 min), HPCE-grade water (5 min), NaOH 1 M (5 min), NaOH 0.1 M (5 min), HPCE-grade water (5 min) and running buffer (5 min). At the beginning of each day, the capillary was rinsed with running buffer for 5 min and before each run with H_3PO_4 0.1 M for 2 min, HPCE-grade water for 2 min and running buffer for 2 min. The choice of acidic washing was related to the necessity of avoiding hysteresis of the wall charge generated by the use of acidic run buffers. At the end of each run, the capillary was rinsed for 2 min with HPCE-grade water to prevent BGE crystallisation.

Each injection was performed hydrodynamically at the anodic end; the sample was loaded onto the capillary for 2 s at low-pressure mode (0.5 psi, 1 psi = 6894.76 Pa), whereas all the conditioning and washing steps were performed at high-pressure mode (20 psi). The electrophoretic runs were carried out at 23 kV for 22 min at 25°C, and the resulting current ranged from 140 to 150 μA.

The running buffer was changed after each run. The overall run time was 30 min. The detection was performed at 280 nm (UV–Vis detector); other instrumental parameters like rise time, ramp time and data rate were set at 0.2 s, 0.17 s and 10 Hz, respectively.

2.5 HPLC determination of anthocyanins

2.5.1 Equipment

HPLC analysis was performed using an HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump delivery system, degasser, autosampler, DAD and mass spectrometer detector (MSD). An analytical HPLC column was used (C_{18} Luna column; 5 μm, 25 cm × 3 mm id; Phenomenex).

2.5.2 Mobile phase

The separation of anthocyanins was performed with a mobile phase composed of a solvent A: 2.5% v/v formic acid in HPLC-grade water and a solvent B: 2.5% v/v formic acid in HPLC-grade methanol. The solutions were filtered on 0.20 μm cellulose acetate filter discs (Albet, Barcelona, Spain) and sonicated for 10 min.

2.5.3 Chromatographic conditions

The HPLC system was conditioned with the mobile phase at least for an hour or until a stable baseline was obtained.

The following linear elution gradient was employed: at 0 min 85% solvent A held for 5 min, from 5 to 20 min 65% A was reached and held constant until 25 min, from 25 to 35 min solvent A decreased to 50% and held constant until 45 min, from 45 to 50 min decreased to 34% and finally at 59 min 85% solvent A was restored. A 10 min postrun equilibration was performed. The total run time was 70 min. An injection volume of 20 μL and a flow rate of 0.5 mL/min were used. Absorption spectra were recorded in the range of 230–650 nm, while the detector wavelength was set at 510 nm.

2.5.4 HPLC anthocyanins standards collection

The three main strawberry anthocyanins (pelargonidin 3-glucoside (Pg-glu), cyanidin 3-glucoside (Cy-glu) and pelargonidin 3-rutinoside (Pg-rut)) were collected in HPLC with a C_{18} Luna column (10 μm, 25 cm × 10 mm id; Phenomenex). The mobile phase and the gradient

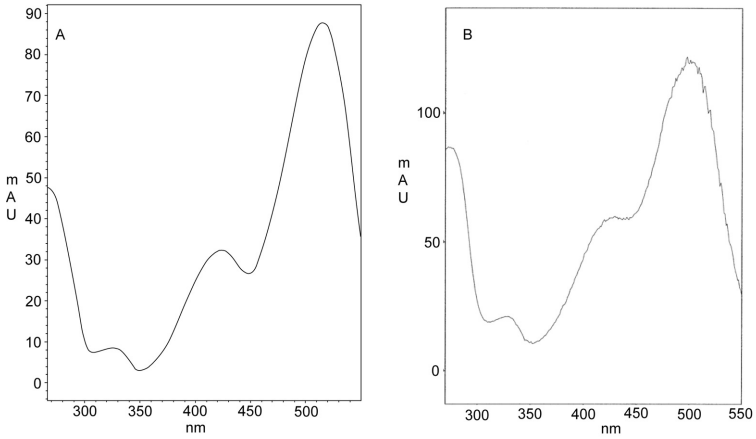

Figure 2. Absorbance spectra of (A) Pg-Cl and (B) Pg-glu.

Table 1. HPLC-DAD/ESI-MSD of the three most abundant anthocyanins in strawberry extracts (adapted from ref. [14])

Compound	Retention time (min)	λ_{max} (nm)[a)]	MW[b)]	Characteristic ions M^+ (m/z)[c)]
1. Cy-glu	19.21	280; 520	449.38	449
2. Pg-glu	21.18	280; 505	433.38	433.1
3. Pg-rut	22.60	275; 505	579.27	579

a) λ_{max}, maximum absorbance.
b) MW, molecular weight.
c) M^+ (m/z) indicates the mass of the most abundant molecular ion/s in positive mode.

employed in the isolation were the same as those used for pigment separation, as described in Section 2.5.3. The injection volume and flow rate were 20 μL and 3 mL/min, respectively.

2.5.5 HPLC anthocyanins identification and quantification

Pg-glu, Cy-glu and Pg-rut were identified based on their mass spectra obtained by HPLC-DAD/ESI-MSD, their order of migration and relative retention time, as reported in Table 1. The results were substantiated by the literature data [18] and agreement with UV–Vis spectra of the separated anthocyanins. The identification of Pg-glu was also confirmed by the comparison of its absorbance spectrum acquired by DAD with those of a standard solution of Pg-Cl (Fig. 2).

The anthocyanin standards collected in HPLC, as described in Section 2.5.4, were dried and dissolved in acidified methanol (3% formic acid) obtaining stock solutions of Pg-glu, Pg-rut and Cy-glu. A standard solution of Pg-Cl was prepared in acidic methanol at a concentration of 500 mg/L and employed for the construction of a calibration curve in HPLC. Pg-glu in the stock solution was quantified by rearranging the expression of the Pg-Cl regression equation on the basis of the different molecular weights of Pg-Cl and Pg-glu (306.70 and 433.38 g/mol, respectively).

The Pg-glu standard solution, with a concentration of 96.6 mg/L, was diluted to obtain several solutions employed for the construction of the calibration curve (from 96.6 to 16.1 mg/L). The regression equation achieved for Pg-glu was $A = 199.78c$ ($r^2 = 0.990$), where A is the Pg-glu peak area and c the Pg-glu concentration (mg/L). Pg-rut and Cy-glu standard solutions collected were more diluted with respect to Pg-glu, and were only employed for anthocyanins identification.

2.5.6 HPCE identification and quantification of anthocyanins

In order to distinguish anthocyanins from other compounds present in strawberry extracts, CZE detection was initially performed with a DAD; it allowed the acquisition of absorption spectra of the analytes, and discrimination of anthocyanins was possible due to their high molar absorptivity at 510 nm at the extremely low pH used.

The three peaks separated in HPCE at 510 nm were presumed to be the three most abundant coloured flavonoids separated in HPLC and previously collected. The HPCE analysis of the pigments isolated in HPLC, as described in Section 2.5.4, confirmed the identification based on migration times. Moreover, the injection of collected anthocyanins showed that the compounds separated in HPLC were pure and that the chromatograms were unaffected by coelution. In fact, in CZE analysis each pigment collected produced a single peak. After identifying anthocyanins and verifying the absence of interfering compounds in the range of migration of the examined compounds, we detected anthocyanins at 280 nm with a single wave detector, owing to high instrumental sensitivity and the higher S/N obtained.

The Pg-glu standard solutions employed in HPLC for the construction of the calibration curve were also injected in HPCE. The Pg-glu regression equation

obtained was $A = 1756.4c$ ($r^2 = 0.9989$), where A is the Pg-glu peak area and c the Pg-glu concentration in mg/L.

2.6 Method evaluation

During the optimisation phase, separation capacity and sensitivity were determined for Pg-glu, the most abundant anthocyanin in strawberry extracts. The LOD and LOQ were, respectively, three and ten times the S/N recorded in electropherograms and chromatograms. These values were extrapolated by plotting S/N *versus* the concentration of Pg-glu in different standard solutions at known concentration. Separation capacity was evaluated in terms of migration time and separation efficiency: the latter calculated as the number of theoretical plates (N) with the expression $N = 5.54 \times (M_t/W_{1/2})^2$, where M_t is the migration time of the anthocyanin and $W_{1/2}$ is the peak width at half height.

The repeatability of the methods was evaluated on strawberry extracts by injecting it ten times consecutively on the same day (intra-assay, $n = 10$). The precision was expressed as the mean, SD and RSD% of migration time and peak area of Pg-glu.

3 Results and discussion

3.1 Preliminary studies

In preliminary experiments, strawberry extracts, prepared as described in Section 2.3, were processed differently to determine the best method for the electrophoretic separation of anthocyanins. In addition to methanolic extracts previously described, we tested two other kinds of samples obtained by drying the original strawberry extracts and mixing them with aqueous or methanolic formic acid solutions. Any manipulation of the extracts was considered unacceptable because these operating procedures led to a reduction in signal intensity, probably due to a loss of pigments during the drying step. Moreover, we noted a worsening in baseline and frequent drops in system current. These observations led us to employ early strawberry extracts.

3.2 Analytical parameters investigated during CZE method optimisation

3.2.1 Capillary length

An initial aim of this research was to reduce the analysis time of anthocyanins with respect to the previously reported values. Da Costa *et al.* [15] used an uncoated fused-silica capillary of 75 cm (id 50 μm), and achieved separation of blackcurrant anthocyanins after 27–33 min in optimised conditions. We determined the separation capacity of shorter fused-silica capillaries with lengths of 37, 47 and 57 cm.

Table 2. Separation capacity and currents through fused-silica capillaries of different lengths

Capillary length (cm)	Separation efficiency Pg-glu (N)[a)]	Pg-glu M_t (min)[b)]	Current (μA)
37	5 784	3.5	118
47	27 539	8.2	92
57	55 464	12.2	77

a) N, number of theoretical plates ($N = 5.54 \times (M_t/W_{1/2})^2$).
b) Pg-glu M_t, migration time of Pg-glu.

Employing the 57 cm capillary, the separation of the three major strawberry anthocyanins was satisfactory and the migration times were halved with respect to those previously published. Analysis times were particularly reduced with the 37 cm capillary, but under the same analytical conditions, a poorer separation efficiency and a higher current in the capillary were also recorded. The 47 cm capillary did not provide good separation of Pg-glu and Cy-glu.

Based on these results, reported in Table 2, we gave high importance to the efficiency of the separation and stability of the system rather to the maximum reduction in duration analysis; the 57 cm capillary was utilised for the optimisation step.

3.2.2 Organic modifiers

As previously outlined [15], it was necessary to add an organic modifier to the running buffer to get a good separation. The buffer additives tested were ACN, methanol and *iso*-propanol; the latter was employed only in a preliminary stage as it has no significant effect on the separation. By comparing the two other organic modifiers, the best results were achieved with ACN.

The effects of ACN were evaluated by adding increasing concentrations in the range from 10 to 90%; up to 30%, we noted an enhancement in separation efficiency with a better peak shape; the electropherograms recorded with 40% ACN were comparable to those at 30%; larger volumes of ACN led to significant worsening in separation capacity and a reduced stability in the baseline of electropherograms. The improvements obtained by adding 30% of methanol and ACN with respect to running buffer without organic additives are shown in Fig. 3.

Till date, the effects of such modifiers were not completely comprised. They modify the compositional characteristics of running buffer and can affect several aspects as pK_a of silanol groups on the capillary wall and dissociation equilibriums of analytes.

We attempted to explain our results by considering the chemical–physical properties of ACN. Due to the low

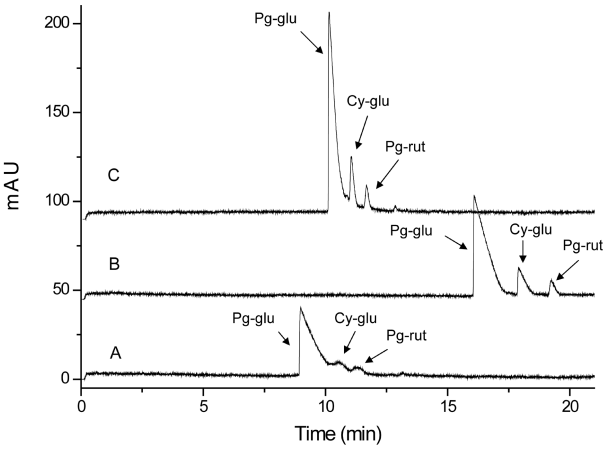

Figure 3. Effect of organic modifiers on running buffer. (A) Buffer solution without organic modifiers; (B) buffer solution containing 30% v/v methanol; (C) buffer solution containing 30% v/v ACN. Separation conditions (not optimised): fused-silica capillary 47 cm (id 50 μm); applied voltage 20 kV; capillary temperature: 25°C; injection time: 2 s; BGE: 19 mM monobasic sodium phosphate and 9 mM bibasic sodium phosphate adjusted to pH 1.55 with *ortho*-phosphoric acid; detection with a diode array detector at 510 nm.

pH range employed, in our view the peak dispersion was essentially caused by an EMD phenomenon. The high concentration of hydronium ions and their high mobility gave the buffer solution a higher conductivity than those of anthocyanins in the sample zone. The addition of 30% of an organic modifier resulted in the largest viscosity of the running buffer and in the lowest proton mobility; in fact, by plotting viscosity *versus* ACN concentration a parabolic graph with a maximum near 30 – 40% of organic modifier was obtained. Other effects promoted by adding ACN were an increase in separation times and a reduction of the current in the system. For these reasons all running buffers employed during optimisation contained 30% v/v ACN.

3.2.3 Time of injection

In hydrodynamic injection, the sample volume loaded onto the capillary depends on the duration of injection and the pressure employed. As the latter parameter was fixed at 0.5 psi and was not modifiable, the sample volume was adjusted by varying the duration of injection from 1 to 6 s (1, 2, 4 and 6 s). To obtain high efficiencies, injection plug lengths should be less than 1 – 2% of the total length of the capillary. In this way field nonhomogeneities and peak broadenings would be avoided, but a lower sensitivity may result if diluted samples were analysed. Pg-glu separation efficiencies were 19 875 N (injection time 1 s), 21 673 N (injection time 2 s), 18 386 N (injection time 4 s) and 16179 N (injection time 6 s).

The strawberry extracts we employed were rather concentrated, and so after the analysis of separation efficiencies obtained with the different injection time, we used 2 s in successive phases. By applying the Hagen – Poiseuille equation with 2 s injection time, a volume of 1.24×10^{-3} mm^3 was obtained, which corresponded to a plug length of 0.6 mm (about 0.1% of the total length of the capillary).

By analysing the Pg-glu peak areas obtained at different injection times, there was no analyte adsorption on the capillary wall. In fact, the Pg-glu areas achieved by increasing the time of injection were linearly correlated to injection times ($A = 11\,441\ \ I_t + 4712.8$, $r^2 = 0.990$, where A is Pg-glu peak area and I_t is the injection time).

3.2.4 Running buffer optimisation: Phosphate concentration, NaCl addition and pH

Optimisation of the CZE method was performed on strawberry extracts prepared as described in Section 2.3 with a running buffer containing 30% v/v ACN. Samples were loaded by hydrodynamic injection for 2 s at 0.5 psi.

The choice of phosphate buffer was due to its good buffering capacity at the low pH values employed (pH <2). During BGE optimisation, which was related to phosphate concentration, NaCl addition and pH adjustments, the voltage and temperature used were 25 kV and 25°C, respectively.

The first aspect we considered was the concentration of NaH_2PO_4 in running buffer. Six different solutions (pH 1.5) were tested, from 100 to 300 mM. When BGE concentration was increased, rises in retention time and separation efficiency of anthocyanins were seen, as shown in Fig. 4. These effects were due to the lower value of zeta potential and to the higher currents generated. Based on these aspects, 250 mM phosphate BGE was selected; higher concentrations promoted more elevated efficiencies, but also led to excessive increases in the capillary current.

The addition of NaCl to the optimised buffer had no positive effects. In fact, increase in the concentration of NaCl from 25 to 100 mM led to an increase in migration time and peak broadening. The latter effect was due to excessive Joule heating and to the formation of a temperature gradient in the capillary. For these reasons, the ionic strength of running buffer was not changed by the addition of salt. Furthermore, the reduced efficiency obtained by adding NaCl demonstrated the absence of Coulombic interactions of analytes (anthocyanins in form of flavylium cations) with the capillary surface. The adsorption effects, due to ionic interactions of the charged pigments with the capillary wall, were also limited by the low pH range employed which maintained the silanol groups mainly in the protonated form.

The aim of this work was to analyse anthocyanins in the native form of flavylium cation, and for this reason

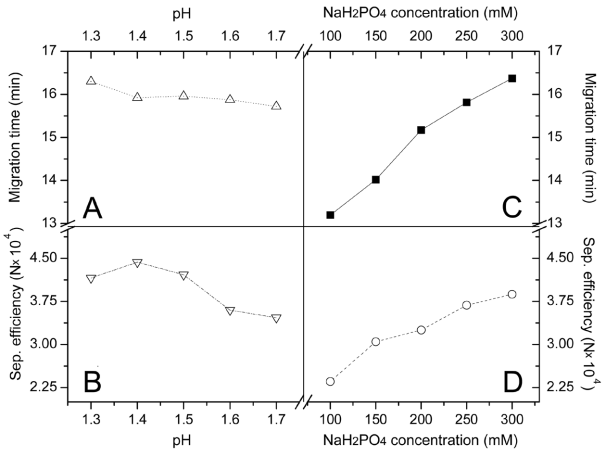

Figure 4. Effect of running buffer pH and concentration of monobasic sodium phosphate on migration time and separation efficiency of Pg-glu. (A) pH *versus* migration time, (B) pH *versus* separation efficiency, (C) concentration of NaH_2PO_4 *versus* migration time, (D) concentration of NaH_2PO_4 *versus* separation efficiency.

the pH had to be low. We prepared several phosphate buffers (NaH_2PO_4 250 mM, without NaCl) whose pH ranged from 1.3 to 1.7, adjusted by progressive addition of *ortho*-phosphoric acid. On the whole, the pH change caused a reduction in migration time of anthocyanins, which was attributed to the higher deprotonation of silanol groups on the inner wall of the capillary and the subsequent greater electroosmotic flux. At high pH, a reduction in separation efficiency was also observed caused by shifting the equilibrium of anthocyanins towards the formation of carbinol pseudobases (neutral forms). The characteristic effects caused by pH were nonlinear. The study of migration times and efficiencies led us to choose a buffer with pH 1.4, which also allows for maximum separation efficiency (Fig. 4).

3.2.5 Instrumental parameters optimisation: Voltage and temperature

With 250 mM phosphate buffer (pH 1.4), different voltages and temperatures were tested. The voltages employed ranged from 20 to 30 kV (20, 23, 25 and 30 kV). The optimal voltage, which permitted a reduction in migration time and a minimal dispersion of analytes, was 23 kV.

The effects of temperature were studied keeping voltage at 23 kV and maintaining the other parameters constant. Increases in temperature (from 20 to 40°C) led to a reduction in migration time due to the lower viscosity of buffer, and an increase in separation efficiency. A temperature of 25°C was selected as higher values resulted in elevated currents. In Fig. 5, an electropherogram recorded with the optimised method is shown.

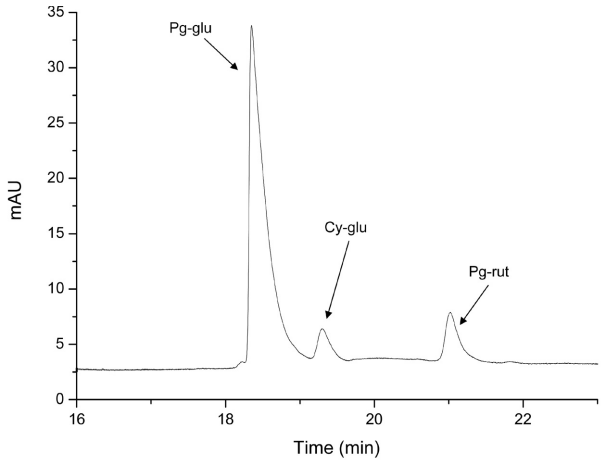

Figure 5. Electropherogram of a strawberry extract recorded with the optimised method. Electrophoretic conditions: fused-silica capillary 45 cm (id 50 μm); applied voltage 23 kV; capillary temperature: 25°C; injection time: 2 s; BGE: 250 mM monobasic sodium phosphate containing 30% v/v ACN and adjusted to pH 1.4 with *ortho*-phosphoric acid; detection at 280 nm with a UV–Vis detector. The peak at 12.5 min was not identified.

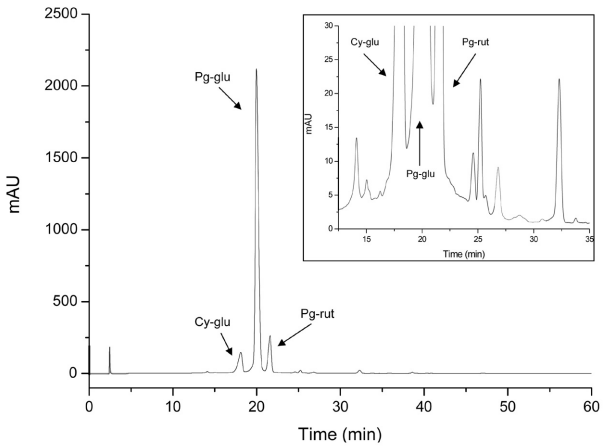

Figure 6. Chromatogram of a strawberry extract. The square on the right shows enlargement of the chromatographic trace between 10 and 35 min. Separation conditions: column C_{18} Luna 250 × 3 mm id, 5 μm particle size; mobile phase (A) 2.5% formic acid in water, (B) 2.5% formic acid in methanol; flow rate 0.5 mL/min; injection volume: 20 μL; detection performed at 510 nm.

3.3 Comparison of CZE and HPLC

Strawberry extracts were analysed in HPLC with the method previously developed by Blanda *et al.* (2008, unpublished data) and suitably re-arranged; the chromatographic technique led to separation of the three main anthocyanins after 17–22 min, and it also allowed for the separation of many other compounds, probably anthocyanins, not detectable on electropherograms, as reported in Fig. 6 (inset).

Table 3. Sensitivity, analysis time and solvent consumption of HPCE and HPLC

	HPCE	HPLC
LOD (mg/L)	2.06	0.04
LOQ (mg/L)	6.87	0.13
Strawberry extracts A_t (min)[a)]	30	70
Sample volume analysed (nL)	1 – 50	20×10^3
Running buffer/mobile phase employed for 100 analysis (mL)	50	3500

[a)] Strawberry extracts A_t: analysis time of strawberry extracts plus system re-equilibration time.

Table 4. Repeatability study on quantification and migration time of Pg-glu of CZE

Area[a)]			M_t (min)[b)]		
Mean	SD	%RSD	Mean	SD	%RSD
50 1137	46 318	9.24	18.35	0.09	0.47

The instrumental precisions are evaluated on a strawberry extract.

[a)] Area: peak area of Pg-glu.

[b)] M_t: migration time of Pg-glu.

As shown in Table 3, the Pg-glu LOD and LOQ of the optimised CZE method were ten times higher than those obtained in HPLC (2.06 and 6.87 mg/L in HPCE *versus* 0.04 and 0.13 mg/L in HPLC). These discrepancies were due to the different sensitivities of the detectors employed, UV–Vis in HPCE *versus* DAD in HPLC and to the different sample volumes loaded. Moreover, in CZE anthocyanins were detected at 280 nm and their molar absorptivity was notably reduced compared to those at 510 nm, the wavelength employed in HPLC.

The electrophoretic method presented a high reproducibility in migration time, with an RSD% less than 0.5%, as reported in Table 4. Moreover, the RSDs of Pg-glu peak area obtained in CZE, despite the lower repeatability (%RSD = 9.47%), were highly acceptable for a hyphenated analytical technique. Such values were probably due to the hydrodynamic injection mode and the high incidence of the rise time (0.2 s) on the total duration of the injection (2 s).

The main advantages of HPCE in this particular application were the small volumes of samples and solvents employed, leading to lower laboratory costs for purchase and dismantlement and the reduced analysis time.

The methods developed in HPCE and in HPLC were used to determine the concentration of Pg-glu in strawberries of the *cv.* Camarosa. This analysis gave a content of 11.41 mg/L of Pg-glu in HPCE and 11.37 mg/L in HPLC.

4 Concluding remarks

In this investigation, a new electrophoretic separation technique was developed that allows the separation of the main anthocyanins from a matrix not previously analysed in acidic conditions, such as methanolic strawberry extracts.

The optimised method presents the traditional benefits of CE analysis, such as high separation efficiency with a low consumption of solvents and samples; it also results in considerable reduction in analysis time of anthocyanins, with respect to that reported by Da Costa *et al.* [15]. However, the major volumes injected in HPLC and the different detector employed (diode array detector *vs.* single wavelength UV–Vis detector in HPCE) enabled to reach lower LOD and LOQ in HPLC, than HPCE.

The results obtained denote the high potential of electrophoretic applications, although further studies are required in order to improve its quantification repeatability, and make CZE an effective alternative to HPLC, the technique traditionally employed in the separation of anthocyanins.

The authors declared no conflict of interest.

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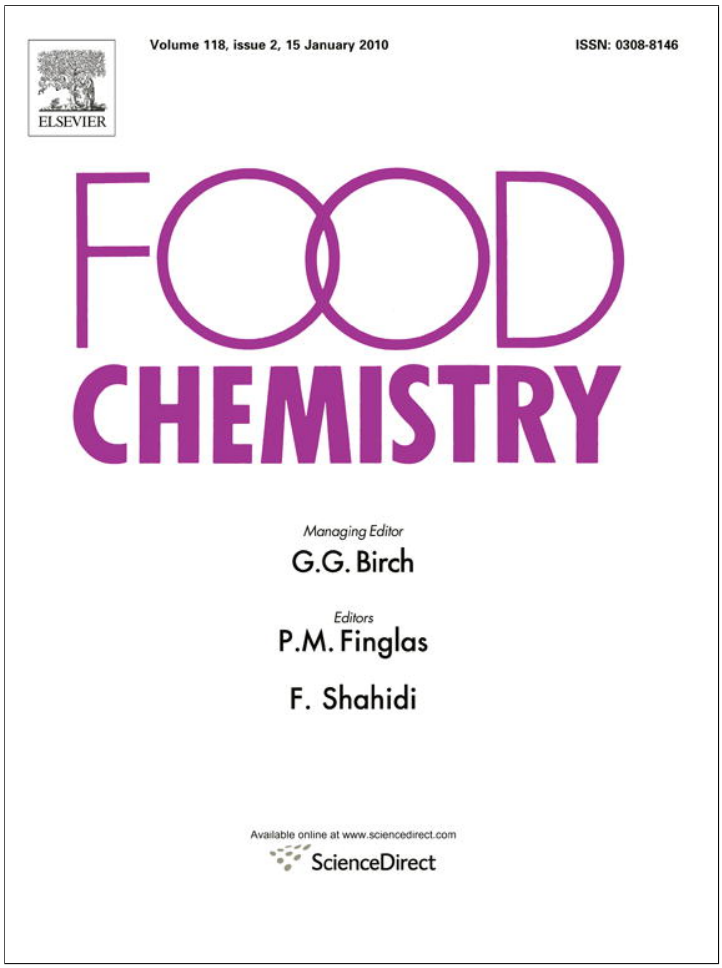

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Investigation of off-odour and off-flavour development in boiled potatoes

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Volatile compounds

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.

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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)

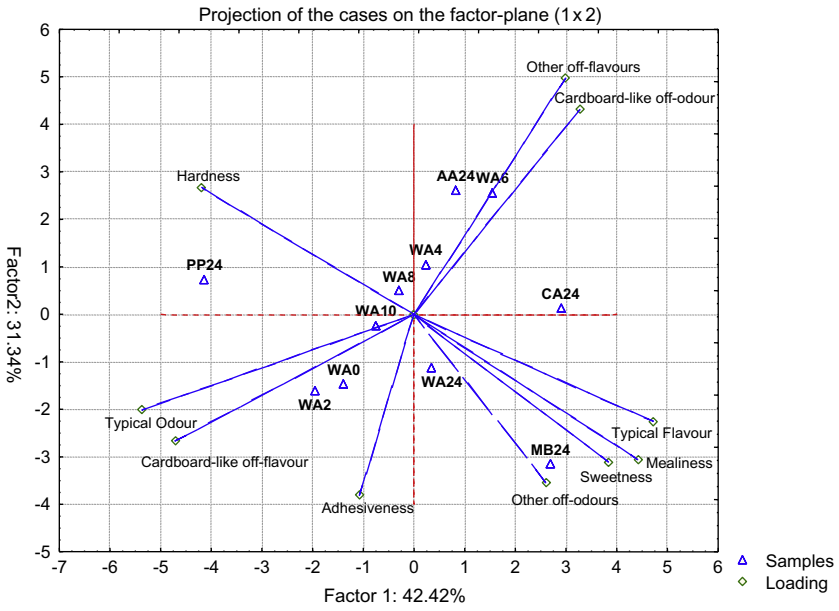

Fig. 1. Principal component analysis of sensory analysis results of boiled potato slices.

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.

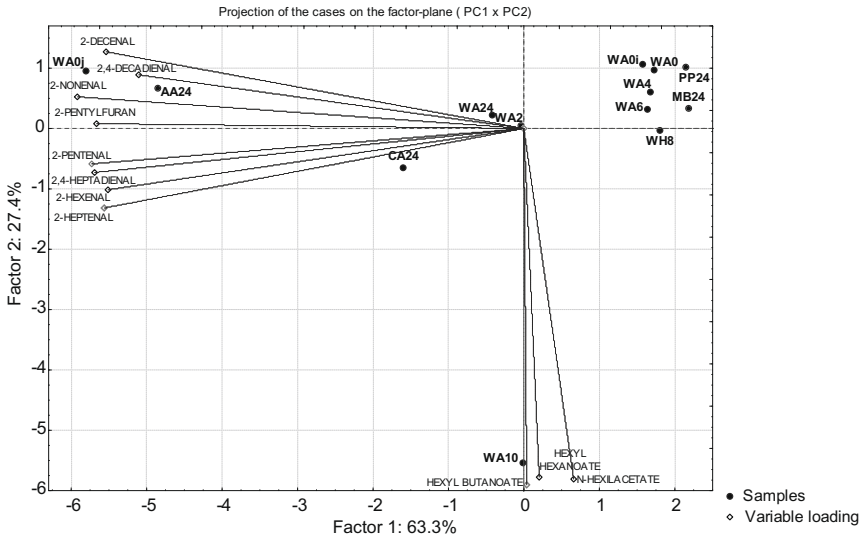

Fig. 2. Principal component analysis of solid-phase microextraction gas chromatography–mass spectrometry analysis results of boiled potato slices.

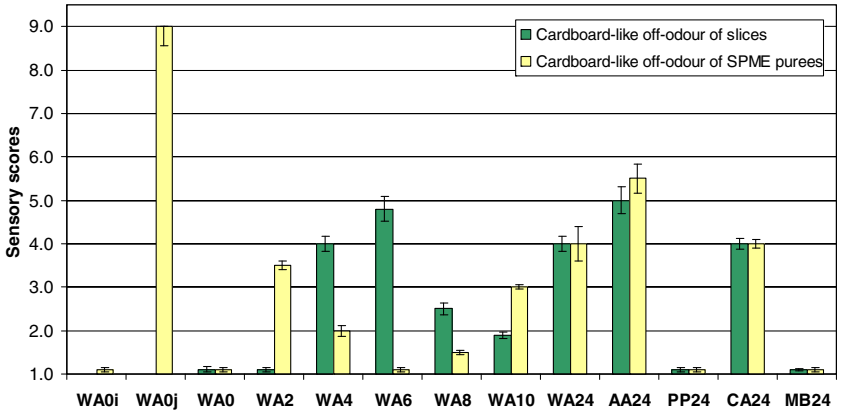

Fig. 3. Quantitative descriptive analysis of cardboard-like off-odour on boiled potato slices and potatoes pureed immediately before solid-phase microextraction analysis.

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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