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Development of innovative technologies for fruit products processing

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Abstract

Most developments in food technology have been aimed toward processing food products more conveniently, more efficiently, at lower cost, and with higher quality and safety levels.

This PhD thesis research project dealt with the identification and development of innovative technologies for processing of different type of fruit-based products in order to maintain and/or increase their quality and stability. The evaluation of qualitative, nutritional and functional characteristics of differently obtained products have been assessed after the processing and the application of innovative solutions also during shelf-life tests.

In particular, the technologies studied during PhD project were: pulsed electric field (PEF), ohmic heating (OH), high pressure of homogenization (HPH) and innovative edible coating.

Different experimental procedures were set up by performing the following independent case of studies: i) PEF pre-treatment of osmo-dehydrated strawberries in order to improve their stability; ii) PEF pre-treatment (with or without osmotic dehydration - OD) before air-drying in order to accelerate mass transfer phenomena and obtain high quality dried strawberry and kiwifruit products; iii) PEF and OH treatments to enhance the juice yield, the release of selected compounds from carrot and apple mash and to control the enzyme activity on the obtained juices; iv) HPH application and its effect on quality, functional properties and stability of mandarin and organic kiwifruit juice; v) application of innovative edible-coating formulations to improve some qualitative characteristics of blueberry fruits during storage.

Based on the obtained results, the application of PEF treatment at low field strength and the use of trehalose for the osmo-dehydration process seems to be an optimal combination for obtaining a semi-dried strawberry product with quality characteristics similar to the fresh one. Thermal treatment followed by PEF or conducted by OH can improve the cell disintegration and juice yield; moreover, a controlled application of high temperature may contribute to the faster inactivation of enzymes, reflected by improved colour values and the release of polyphenols. The application of HPH on fruit juice production contributes to guarantee the protecting effect of trehalose on mandarin juice structure during storage and allows obtaining stable kiwifruit juice for more than 40 days under refrigerated storage by increasing its shelf-life. Instead, for what concern the innovative packaging solutions, the use of different kinds of edible coatings showed positive effects on firmness, colour retention and contributed to the increase of antioxidant activity of blueberry fruits. Results from this study suggest the possibility of using edible coatings to develop high quality ready-to-eat fresh blueberries without compromising their stability, or even increasing it.

The results of this PhD research work can greatly contribute to obtain high quality fruit/vegetable processed products in an efficient and environmentally sustainable way, thus determining an added value for food companies.

List of papers

This thesis is based on the following Papers, referred to in the text by their Roman numerals. The Papers are attached at the end of the thesis.

I Betoret E., **Mannozzi C.**, Dellarosa N., Laghi L., Rocculi P., Dalla Rosa M. (2017) Metabolomic studies after high pressure homogenization processed low pulp mandarin juice with trehalose addition. Functional and technological properties. Journal of Food Engineering, 200, 22-28.

II Tylewicz U., Tappi S., **Mannozzi C.**, Romani S., Dellarosa N., Laghi L., Ragni L., Rocculi P., Dalla Rosa M. (2017). Effect of pulsed electric field (PEF) pre-treatment coupled with osmotic dehydration on physico-chemical characteristics of organic strawberries, Journal of food Engineering 213, 2-9.

III **Mannozzi C.**, Cecchini J.P., Tylewicz U., Siroli L., Patrignani F., Lanciotti R., Rocculi P., Dalla Rosa M., Romani S. (2017) Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life, LWT-Food Science and Technology 85, 440-444.

IV **Mannozzi C**., Tylewicz U., Chinnici F., Siroli L., Rocculi P., Dalla Rosa M., & Romani S. (2018). Effects of chitosan based coatings enriched with procyanidin by-product on quality of fresh blueberries during storage. Food Chemistry, 251, 18-24.

V **Mannozzi C**, Fauster T, Haas K, Tylewicz U, Romani S, Dalla Rosa M., Jaeger H (2018). Role of thermal and electric field effects during the pre-treatment of fruit and vegetable mash by pulsed electric fields (PEF) and ohmic heating (OH). Innovative Food Science and Emerging Technologies, 48, 131-137.

VI **Mannozzi C**., Rompoonpol K., Fauster T., Tylewicz U., Romani S., Dalla Rosa M., Jaeger H. (2018). Influence of pulsed electric field (PEF) and ohmic heating (OH) pre-treatment on enzyme and antioxidant activity of fruit and vegetable juices. Food Chemistry, (submitted).

VII Patrignani F., **Mannozzi C.**, Tappi S., Tylewicz U., Pasini F., Castellone V., Riciputi Y., Rocculi P., Romani S., Caboni M.F., Gardini F., Lanciotti R., Dalla Rosa M. Effect of (ultra)-high pressure homogenization on the shelf-life and functionality of organic kiwifruit juice. Frontiers in Microbiology (accepted).

Contents

I. Introduction and Objective

Processed fruits represent one of the major growing segments in food production due to the consumer great interest in food with high nutritional (antioxidants, vitamins, bioactive compounds) and sensory properties (colour, taste, texture, etc.). Food processing can be defined as the set of operations, which allows manufacturing, preservation and distribution of food products from suitable raw materials. The improvement of the food products is now directed towards ensuring nutritional and specific functional benefits. Regarding the process improvement it is directed to ensure the quality and safety of environmentally friendly food products, prepared optimizing the resources used, minimally affecting or even enhancing their nutritional and beneficial characteristics.

Another important aspect that must be taken into account is the reduction of the energy needs for the processes, thereby decreasing both environmental and financial costs.

In this sense, the application of different innovative technologies can be performed on different type of fruit-based products in order to improve food quality, sustainability and extend shelf-life.

Pulsed electric field (PEF) and osmotic dehydration (OD) can be used in combination to modulate specific structural features of plant tissue. PEF treatment is able to alter the cell permeability (Tylewicz et al., 2017) by inducing reversible or irreversible electroporation on the cell membrane; thus, PEF treatments can increase the mass transfer phenomena and the release of intracellular compounds from plant tissues. Moreover, an osmotic solution applied to a vegetable tissue promotes the water migration from the inner compartments of the cells toward the external solution. At the same time, solutes from the solution migrate to the treated tissue.

Moisture removal from the food materials allows to minimize substantially the microbial activity and deteriorative chemical reactions (Barbosa-Canovas & Vega-Mercado, 1996). Commonly, freeze-drying or conventional drying have been used to obtain stable products; however, these techniques are limited by high-energy consumption and long drying time. Moreover, drying processes at elevated temperatures produce undesirable changes in pigments, vitamins and flavouring agents (Aguilera et al., 2003). In general, the drying processes consume an appreciable part of the total energy used in food industry, and therefore it is very important to develop new hybrid drying technologies for energy saving and preservation of food quality (Chou and Chua, 2001). The PEF treatment at high and moderate fields applied as a pre-treatment has been proposed for enhancement of drying process efficiency (Toepfl 2006; Lebovka et al., 2007b).

Moreover, PEF pre-treatment of plant tissue facilitates juice release by increasing extraction efficiency and allows producing the fruit juice with high quality characteristics. It has been proposed as an alternative to the enzymatic treatment, showing high potential not only in the increasing yield of juice production but also to extract selected valuable compounds from the plant matrix.

Ohmic heating (OH) could also be used to increase extraction yield, as an alternative thermal method, since it allows heating in a rapid and uniform way, which is especially suitable for high viscous, particulate products such as fruit or vegetable mash (Jaeger et al., 2016). This technique may allow to improve the retention of vitamins, pigments and nutrients due to an optimized heating profile resulting in less thermal damage of heat sensitive substances.

The use of edible coatings, produced from edible biopolymers and food-grade additives, represents an alternative and/or additional way for fruit preservation, because of their ability to improve the mechanical resistance of food products, visual and tactile characteristics of product surface, reduce moisture migration, gas exchange, microbial growth and oxidative reactions, as well as reduce or even suppress physiological disorders (Rojas-Graü et al. 2009). Edible coatings enhance the quality of food products, protecting them from physical, chemical and biological deteriorations. Therefore, the maintenance of quality is directly related to the shelf-life extension and safety of the products. In order to protect functional characteristics and generally preserve the quality of food, it is important also to consider the possibility of the enrichment of coating formulations with active carrier substances such as antimicrobials, antioxidants, flavours, nutraceutical compounds and colorants.

This PhD thesis research project deals with the identification and development of innovative technologies for transformation/processing of different type of fruit-based products in order to maintain and/or increase their quality and stability.

The combined applications of pulsed electric field for improving the moisture removal during the osmotic dehydration as well as the convective drying on strawberry and kiwifruit slices respectively were investigated. Moreover, pulsed electric field, ohmic heating and high pressure of homogenization were applied in juicing technology of carrot, apple, mandarin and kiwifruit.

Concerning the application of innovative coating solutions, the influence of different formulation on fresh blueberry quality was investigated.

The effects of the studied innovative technologies have been assessed on the nutritional and functional and other quality characteristics of differently obtained fruit-based products.

II. Innovative processing technologies

Most developments in food technology have been aimed toward processing food products more conveniently, more efficiently, at lower cost, and with higher quality and safety levels.

Food processing is in constant evolution in response to different challenges. The changes in consumer tastes and the need to produce safe and high quality foods are responsible for the evolution of the established food processes or the development of the new ones. In this sense, the introduction of new technologies could lead to a reduction of the processing time or an improvement in operating conditions. These aspects are closely linked to the search for high quality products that preserve their natural characteristics.

In recent years, with the aim to improve or replace conventional processing technologies in order to deliver higher quality and better consumer targeted food products, different innovative technologies have been investigated and developed. In this sense, different innovative technologies could be used such as vacuum impregnation (VI) (Betoret et al., 2012a), ohmic heating (OH) (Jaeger et al., 2016), pulsed electric field (PEF) (Donsì et al., 2010), ultrasound (US) (Nowacka et al., 2018), high-pressure (HP) (Dede et al., 2007), ultraviolet light (UV) (Sastry et al., 2000), etc.

VI is used in order to make easier the introduction of solutes inside vegetable tissues. PEF and US are employed to accelerate mass transfer, by modifying the physical, structural and functional properties of food product, and the enrichment of plant tissues in combination with VI or osmotic dehydration process. OH, HP and UV light have used for food preservation by the inactivation of microorganisms and enzymes.

In this PhD thesis, the application of pulsed electric field, ohmic heating and high pressure homogenization on different fruit-based products has been studied and implemented in order to obtain high quality products.

1. Pulsed electric field (PEF)

1.1 Introduction

Pulsed electric field (PEF) is considered a promising technology that in the last years received considerable attention in food and biotechnology fields. The PEF treatment consists in the application of electric pulses with high voltage and short duration to plant or animal biomaterials placed between two electrodes. PEF could alter the cell permeability (Knorr et al., 2001; Dellarosa et al., 2016; Tylewicz et al., 2017) by inducing reversible or irreversible electroporation on the biological cell membrane (Figure 1).

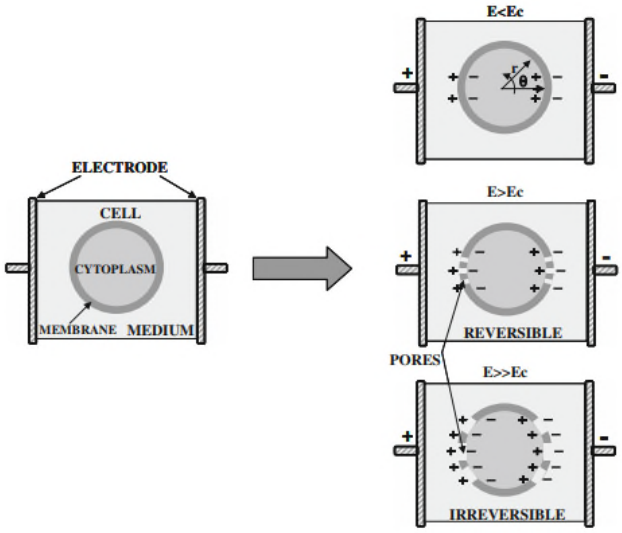


Figure 1. Scheme of dielectric breakdown of cell membrane and pore formation (Donsì et al., 2010).

1.2 Principles of PEF

The biological cells, when exposed to an external electric field will accumulate the charges, due to movement of ions along the electric field lines. This phenomenon will increase their natural transmembrane potential. Exceeding a transmembrane potential in a range of 1 V has been shown to cause formation of a pore (Toepfl et al., 2006a) (Figure 2).

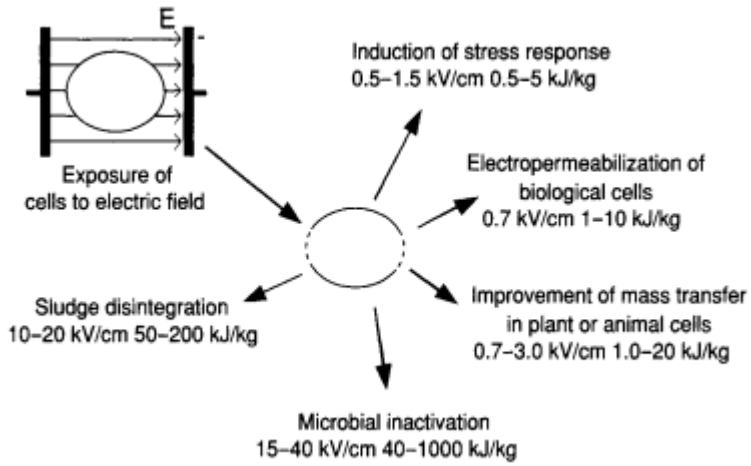


Figure 2. Exposure of biological cell to an electric field and application in food with typical electric field strength and energy input requirements (Toepfl et al., 2006a).

The electroporation consists of different phases. The first one is the pore formation, which is the response of the cell membranes to the induced threshold membrane potential. The second step is a time-dependent expansion of the pore size and the last phase is membrane recovery, which takes place after PEF treatment and is related to pore resealing. Depending on the intensity of the treatment

applied (external electric field, single pulse duration, treatment time) and on the cell characteristics (size, shape and orientation in the electric field), the viability of the electroporated cell can be preserved by recovering the membrane integrity, or the electroporation can lead to cell death (Donsì et al., 2010). Low-intensity treatment induces stress reactions in plant cells, resulting in the promotion of a defence mechanism by increased production of secondary metabolites.

PEF treatment is used also to increase permeability of cell membrane causing an enhancement of mass transport out of the cells, maintaining unchanged the structure of the product (Knorr et al., 1994; Fincan et al., 2004). In fact, for large size cells, as animal and plant, permeabilization of membrane is obtained with low electric field intensities and lower energy requirements.

Due to the induced permeabilization of plant cell, PEF technology can be used as a pre-treatment to increase the yield in the juice production, or to accelerate the transfer of water during drying or osmotic dehydration, as well as to enhance the extraction of selected compounds (such as antioxidants, colorants or flavors) from the inner parts of the cells (Knorr & Angersbach, 1998; Bouzrara & Vorobiev, 2003; Donsì et al., 2010).

1.3 Processing parameters

The process parameters characterizing PEF technology are amplitude of electric pulses (U), electric field strength (E), treatment time (t), pulse width (τ), number of pulses (n), pulse specific energy (W) and pulse repetition frequency (Raso et al., 2016).

Electric field strength refers to the field strength locally present in the treatment chamber during the sample treatment. It depends on the voltage applied between the electrodes, geometry of the treatment chamber and the distribution of dielectric properties of the matrix between the electrodes. For parallel plate, the electric field (E) is homogenous within electrode that it can be estimated by the following Eq (1):

$$E = \frac{U}{L} \quad (1)$$

where:

U=voltage (V)

L=electrode distance (cm)

The treatment time (t) reports the number of pulses (n) applied multiplied by the pulse duration (τ), which depended on the pulse shape:

$$t = n \times \tau \quad (2)$$

As shown in Fig. 3, generally in PEF treatment the pulse shapes are either exponential or square-wave pulses, unipolar or bipolar.

The pulse width or pulse duration for the square pulse is the time that the voltage reaches the maximum value (Reberšek et al., 2014). In the case of exponential decay pulses, the pulse width is defined as the time needed to decrease the voltage to 37 % of its peak value (Fig 3).

Frequency indicates the number of pulses applied by unit of time and it is reported in Hz (number of pulses s^{-1}). It determines the amount of electrical energy delivered per unit of time on the product placed in the treatment chamber, which affects the increased temperature of the processed product due to Joule effect.

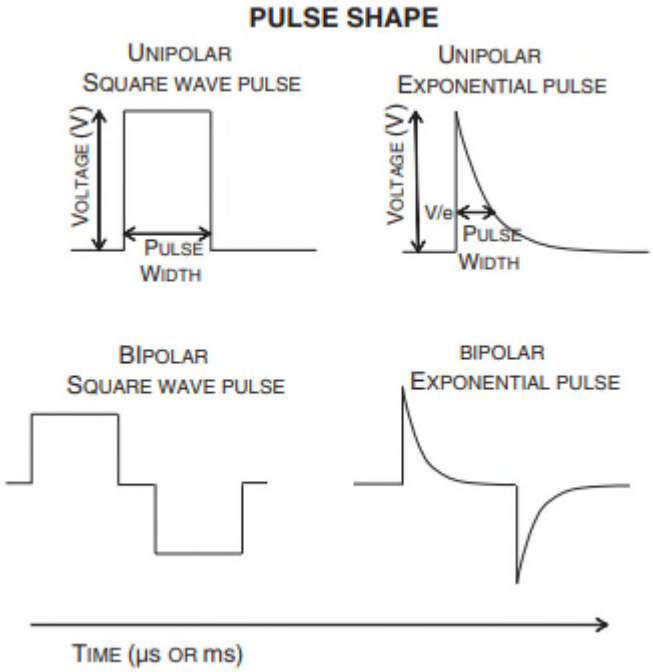


Figure 3. Pulse shapes used in PEF treatment (Raso et al., 2016).

The specific energy per pulse (W) expressed in kJ/kg/pulse represents the electrical energy received by the treated product in the PEF chamber per each pulse. It depends on the electrical properties of the treated matrix and on the pulse shape. The electrical properties of the matrix depend on the conductivity that increases due to the membrane electroporation and to the diffusion of ions from the cells to the water, usually with low conductance at the beginning of treatment.

Due to the losses through the connections, the specific energy per pulse should be considered by the integral over time of the recorded voltage and electrical current (Raso et al., 2016).

The total specific energy input W_t (kJ kg^{-1}) can be calculated by multiplying W by the number of pulses (n) (Eq 3):

$$W_t = W \times n \quad (3)$$

1.4 Exploitation of PEF in plant-based food processing

PEF promotes structural modifications on a cellular level and water redistribution between different cellular compartments, thus resulting in changes in tissue material properties. In fact, several studies reported the changes in texture and colour parameters of different plant tissues subjected to the PEF processing, which could consequently affect the final product quality characteristics (Siemer et al., 2014).

Combination of PEF treatment with vacuum infusion was applied to impregnate the intercellular spaces of spinach leaves with cryoprotectant such as trehalose (Phoon et al., 2008). This allowed a substantial improvement of the freezing tolerance of spinach leaves. Recent studies have also shown a potential of PEF pre-treatment to reduce freezing time. Wiktor et al. (2015a) showed that the application of PEF pre-treatment was able to reduce total freezing time by 3.5–17.2 % on apple compared to conventional freezing process, moreover, the total thawing time was reduced by 71.5 %. This means that PEF treatment modified the mechanical properties of the plant tissue, due to the loss of membrane semi permeability that consequently reduced the native turgor pressure of the treated sample and induced softer tissues. Due to the loss of turgor pressure and tissue softening the processes steps such us cutting and peeling are facilitated. Fauster et al. (2018) reported that after the application of PEF (1 kV/cm and 0.2 kJ/kg) a positive effect on the cutting behaviour and the resulting smoother cutting surface of potato sticks were observed. The application of PEF below 1 kV/cm and 1 kJ/k reduced the energy for peeling of whole tomatoes compared to a conventional peeling process (Pataro et al., 2018).

In this PhD research work, PEF has been used as pre-treatment both before the osmotic dehydration (OD) of fruits and before the pressing step for the juice production, in order to enhance the mass transfer, the yield of juice and the extraction of selected compounds from vegetable and fruit matrixes. Moreover, in this project PEF has been also coupled with drying process.

1.4.1 Osmotic dehydration

Osmotic dehydration (OD) treatment is a partial dewatering process by immersion of cellular tissue in hypertonic solution. The driving force for the water removal is the concentration gradient between

the solution and the intracellular fluid (Rahman, 2008). The diffusion of water from the vegetable tissue to the solution is usually accompanied by the simultaneous solutes diffusion into the tissue and natural solutes present in the cell (vitamins, organic acids, minerals, etc.) can also be leached into the osmotic solution (Lerici et al., 1985). In general, OD occurs when products such as fruits and vegetables, are placed in a hypertonic sugar or salt solution presenting a high osmotic pressure and a low water activity. There are two major mass transfer phenomena involved in osmotic dehydration: the movement of solute into the matrix and the flow of water out of the tissue. These mechanisms lead to water loss (WL) and solid gain (SG) in the food. However, the cellular membrane employs a high resistance to transfers, thus slowing down the OD rate.

Since OD treatment, especially when applied at room temperature, is a time-required process, other pre-treatments could be used before OD in order to increase the velocity of mass transfer kinetics. One of these pretreatments could be the application of PEF, which damages mainly the cell membranes; while other structural changes, that could be induced in plant tissue by PEF, remain limited.

Recently, the PEF has been successfully applied for enhancing OD of different food plants, such as apples (Amami et al., 2006; Dellarosa et al., 2016), kiwifruits (Traffano-Schiffo et al., 2016), carrots (Amami et al., 2007), potatoes (Fincan & Dejmek, 2003). While the effect of PEF pre-treatment on enhancing the water loss of OD treated tissues seems to be clearly stated, its effect on the solid gain is not well understood.

Osmotic dehydration with PEF already at 100 V/cm for 60 pulses is an effective aid in removing water from apple tissue and increasing solute concentration, due to the alteration of the membrane permeability (Dellarosa et al., 2016).

The application of PEF (100, 250 and 400 V/cm) as a pre-treatment of the osmotic dehydration in kiwifruit increased the water mass transfer and reduced the final sugar concentration comparing with not pre-treated samples (Traffano-Schiffo et al., 2016).

Moreover, also Ade-Omowaje et al. (2003) observed similar results for red bell peppers pretreated with PEF. In fact, a significantly enhanced rate of transfers (around 11–25 % in water loss and 2–5 % in solid gain) during osmosis was observed.

New findings

In ***Paper II*** PEF has been evaluated as a pre-treatment of osmo-dehydrated strawberries in order to study its effect on mass transfer phenomena, water distribution and some physico-chemical parameters.

The application of lowest electric field intensity (100 V/cm) resulted sufficient to increase the water loss by 12 % and 6 %, after one hour of OD, respectively for strawberries dehydrated in sucrose and trehalose solutions. Concerning the solid gain results, while the solid gain was favoured by the application of all the tested PEF intensities in samples dehydrated in sucrose solution, the treatment at 200 and 400 V/cm reduced the trehalose uptake due to a lower initial mass transfer rate. TD-NMR permitted to separately observe two main water populations located in vacuoles and cytoplasm plus extra-cellular spaces of strawberry tissues. During OD treatment it was possible to observe a decrease of the signal intensity, related to the water protons located in the vacuole, throughout 120 min. Consequently, the shrinkage of vacuole led to an increase of the intensity of the water protons through the cytoplasm and extracellular space as shown in Figure 4a. Figure 4b shows the effect of water distribution due to the application of PEF on the strawberry tissue before immersion in the hypertonic solution. The electroporation induced by the treatment led to a loss of compartmentalization highlighted by a single proton population. Similar results were reported by Dellarosa et al. (2016) that after the application of PEF treatment at 150 V/cm with 60 pulses observed a no-reversible behaviour on apple tissue. Figure 4c shows the transverse relaxation time (T2) of the water population throughout 120 min of osmotic treatment. As expected, the T2 decrease during OD could be due to the water removal and the different water-solutes-biopolymers interactions.

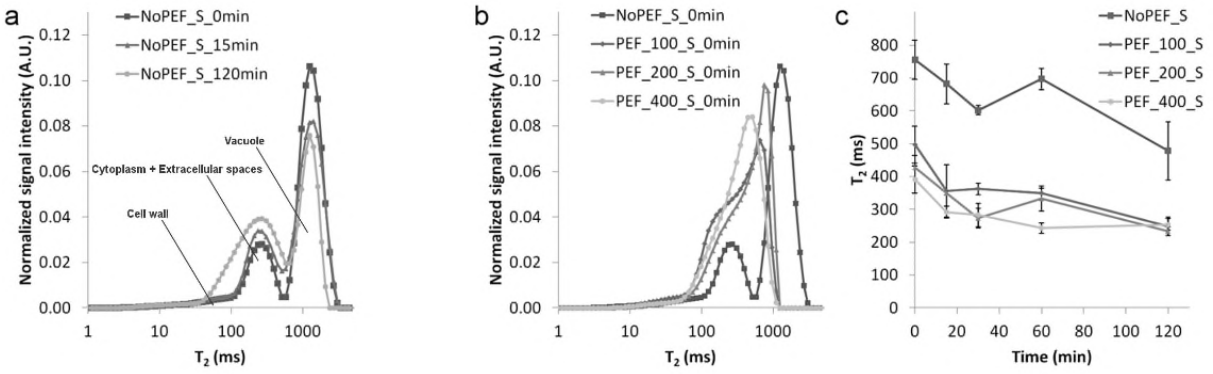


Figure 4. T2- weighted signal distribution, of a) OD samples with sucrose, b) samples immediately after PEF pre-treatment and c) transverse relaxation time (T2) of PEF pre-treated and control strawberries during 120 min from immersion into the sucrose solution (corresponding to Figure 3 in ***Paper II***).

Moreover, cell viability was not completely lost for strawberry samples treated with the lowest electric field strength (100 V/cm), maintaining at the same time the fresh-like characteristics of fruits (Figure 5).

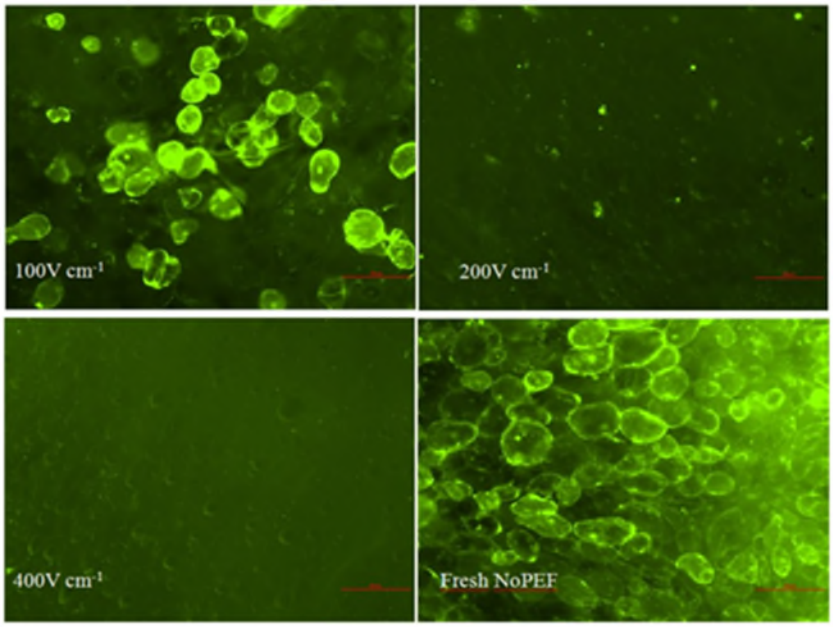


Figure 5. Microscopy images of fresh strawberry tissue and after PEF treatment followed by staining with FDA (corresponding to Figure 4 in ***Paper II***).

1.4.2 Drying

Moisture removal from the food materials allows to substantially minimize the microbial activity and deteriorative chemical reactions (Barbosa-Canovas & Vega-Mercado, 1996). Commonly freeze-drying or conventional drying have been used to obtain stable products, however, these techniques are limited by high energy consumption and long drying times. Moreover, drying process at elevated temperatures produced undesirable changes in pigments, vitamins and flavouring agents (Aguilera et al., 2003). In general, the drying processes consume an appreciable part of the total energy used in food industry, and it is very important to develop new hybrid drying technologies for energy saving and food quality preserving (Chou and Chua, 2001). The PEF treatment at high and moderate fields have been proposed for the enhancement of drying process (Toepfl 2006; Lebovka et al., 2007b). Electrically assisted drying allows decrease processing time, temperature, and energy consumption.

The permeabilization of vegetable tissues caused by PEF application induces an increase of mass and heat transfer rates between the cells and their surroundings, which can be also used for enhancing the efficiency of a subsequent drying process. In particular, PEF was configured as a pre-treatment of the drying process, in order either increase the drying rates or to obtain a stable product whit high quality. Lebovka et al. (2007b) showed an influence of PEF treatment at 300 and 400 V/cm on drying (30-70 °C) of potato disks. The effective moisture diffusivity increased by increasing the PEF treatment time, thus reducing the drying process duration. Moreover, for potato tissue the PEF treatment allowed decreasing the drying temperature approximately by 20°C, therefore the PEF pre-treatment seems to be promising for drying thermal sensitive product at moderate temperature.

Amami et al. (2008) demonstrated that it was possible to reduce the drying time of the carrot treated by PEF compared to the untreated samples. The addition of osmotic dehydration in 65 % w/w sucrose solution for 240 min at 40 °C after PEF treatment and before drying allowed a reduction of drying time by 70 min, for instance drying time at 60 °C was reduced by 81 % with subsequent retention of thermolabile compounds present in the product.

New findings

In another case of study (work to be submitted) PEF has been used as a pre-treatment of drying, with or without the osmotic dehydration (OD) process, in order to obtain dried kiwifruit products (see on M&M, section 1.2) by accelerating mass transfer phenomena.

This research work was aimed to find new solutions in order to reduce the negative effect of dehydration on biomolecules, even increasing the functional value of some compounds. The scope was to apply and assess the combination of the two non-thermal technologies such as OD and PEF as pre-processing steps to drying, in order to obtain a novel dried kiwifruit product with very high quality (crispness) and nutritional characteristics (antioxidant activity, total polyphenol and vitamin C content).

The results of research work showed that the application of OD process reduced the drying time of kiwifruits, which was even more enhanced by the application of PEF, especially at the lowest drying temperature. The enhancement of mass transfer by PEF application observed in the present study has been previously reported for fruits and vegetables (Ade-Omowaye, Rastogi, et al., 2003; Wiktor et al., 2014; Tylewicz et al., 2017; Yu et al., 2018). Toepfl and Knorr (2006b) reported a 25% reduction of drying time after the application of PEF treatment (2 kV / cm and 20 pulses) on red bell pepper. They also reported that the combined application of PEF and OD led to a faster initial moisture loss indicating the enhanced mass transfer rate. Changes during drying could be associated to the electroporation occurred during PEF pre-processing that promotes the water transfer from the sample to the environment (Wiktor et al., 2015b).

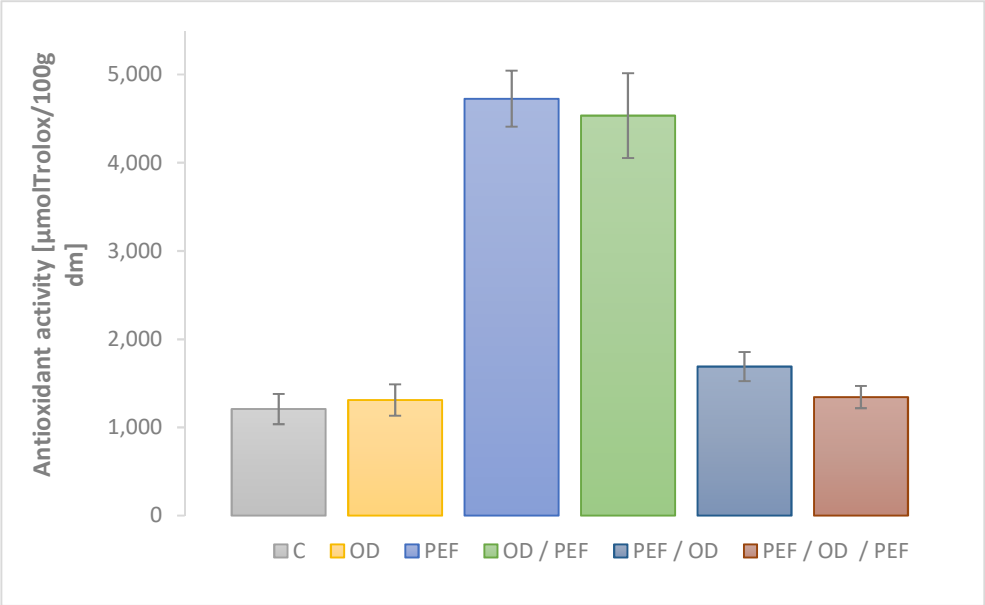


Figure 6. Antioxidant activity of kiwifruit snacks dehydrated at 70 °C upon different pre-treatments (C: control; OD: osmotic dehydration; PEF: pulsed electric field).

The antioxidant activity, measured by the ABTS method, of differently obtained kiwifruit samples is shown in Figure 6. The highest antioxidant activity values were obtained for kiwifruit slices pre-treated with PEF and OD respectively (PEF and OD/PEF) at 70 °C. Similar results were also observed at 50 and 60 °C dehydration temperatures. PEF and OD treatment allowed to increase moisture removal due to electroporation and dehydration process respectively, that consequently allowed the reduction of drying time and temperature. Moreover, the changes on cellular membranes allowed the increased release of bounded antioxidant compounds making them more accessible during the extraction. Similar results were reported by Dermesonlouoglou et al. (2018), which investigated the application of PEF (2.8kV / cm, 750 pulses) and OD (55 °C, 60 min) prior to the drying process at 60 °C in a goji berry product.

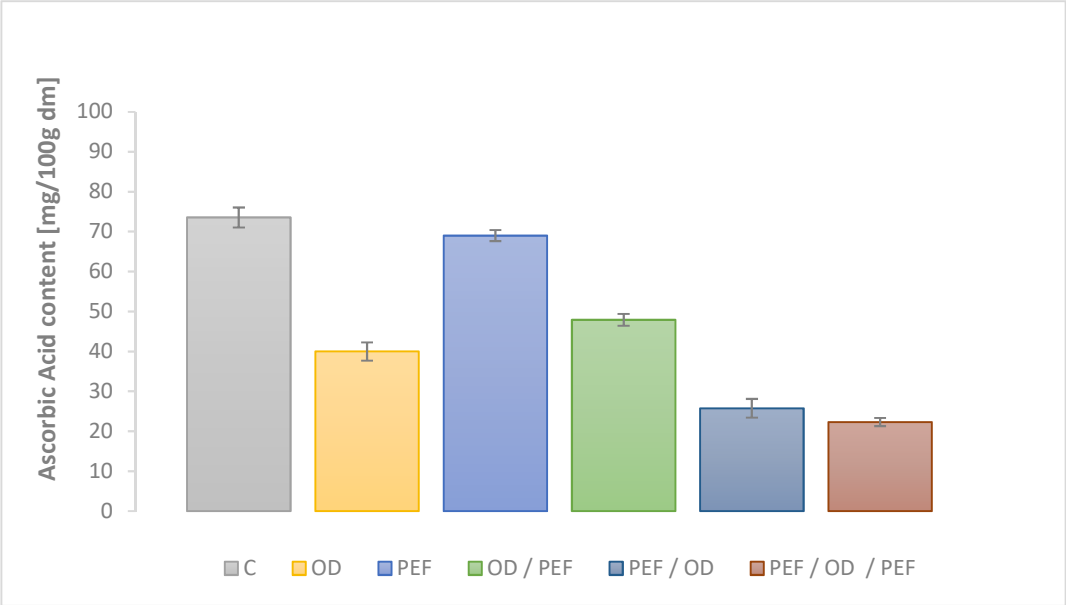


Figure 7. Ascorbic acid content of kiwifruit snacks dehydrated at 70 °C upon different pre-treatments (C: control; OD: osmotic dehydration; PEF: pulsed electric field).

For what concern the ascorbic acid content, PEF treated and control air-dried kiwifruits showed higher values (Figure 7) compared to the other treated samples when they were dried at 60 and 50 °C. In this way, it was possible to observe better retention of thermolabile compounds by the application of the lowest drying temperature. The degradation of ascorbic acid is due to the enzymatic and non-enzymatic oxidation that were accelerated by the highest drying temperature (Miura and Araki, 1988). An increase in temperature promote higher degradation of this sensitive bioactive compound, which may be triggered due to the high temperature and presence of oxygen.

All treated samples maintained a good crispiness comparable to non-treated kiwifruits (control) (Figure 8). OD, OD/PEF and PEF treatments decreased the crispness of air-dried kiwifruits probably due to the disruption of cell membranes and the loss of turgor pressure (Alam et al., 2018). PEF pre-treatment followed by OD, instead, provoked an increase of kiwifruit crispness probably due to the alteration of membrane permeability and consequently high loss of water.

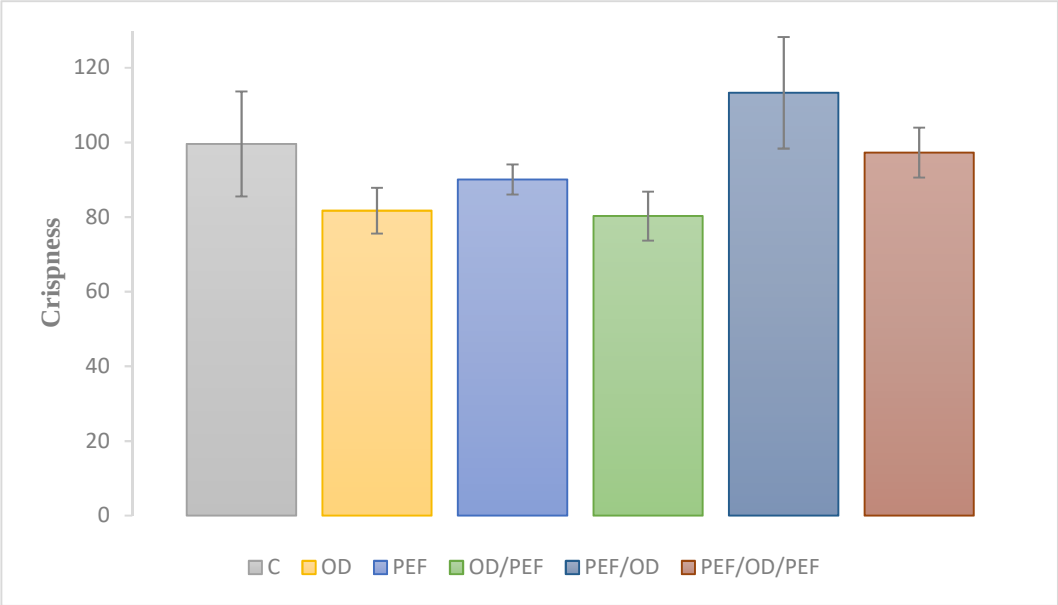


Figure 8. Crispness of kiwifruit snacks dehydrated at 70 °C upon different pre-treatments (C: control; OD: osmotic dehydration; PEF: pulsed electric field).

Based on the obtained results the combination of PEF and OD allowed to reduce drying time for the production of kiwifruit slices whit high quality characteristics and level of nutrient retention. Moreover, PEF processing has proven to be highly effective for improving the drying rates of kiwifruit slices, for this reason could be used as a pre-drying processing step as well as for its potential for continuous operations and short treatment time (Odriozola-Serrano et al., 2013).

1.4.3 Extraction

PEF pre-treatment of plant tissue facilitates juice release by increasing extraction efficiency and allow producing the fruit juice with high quality characteristics. It has been proposed as an alternative to the enzymatic treatment, showing high potential not only in the increasing yield of juice but also to extract selected valuable compounds from the plant matrix.

Jaeger et al. (2012) applied PEF treatment ($W_{specific}$ = 2 kJ/kg and 12 kJ/kg) to apple and carrot mashes and compared the total juice yield, carotenoid content and total polyphenols after applying four different de-juicing systems. The total polyphenol content increased on apple juice pretreated with PEF for all de-juicing systems and carotenoid contents increased as compared to the untreated samples in three of the four de-juicing systems.

PEF treatments carried out at field strengths of 1, 3, and 5 kV/cm and an energy input of 10 kJ/kg achieved a cell disintegration index of 0.70, 0.80, and 0.87, respectively. Mechanical pressing of PEF-treated berries (1, 3, and 5 kV/cm at 10 kJ/kg) significantly increased the juice yield (+28 %) compared with the untreated sample. The juice obtained from PEF pre-treated berries also had a

significantly higher nutritional characteristic such as total phenolic content, total anthocyanin content and antioxidant activity (Bobinaitė et al., 2015).

Wiktor et al. (2015a) studied the impact of PEF on different bioactive compounds on carrot and apple tissues. For this purpose tissues were treated by PEF at 0, 1.85, 3, 5 kV/cm and 0, 10, 50 and 100 pulses, which corresponded to the specific energy input of 0–80 kJ/kg. The application of PEF at 1.85 kV/cm at each applied pulse number increased the total carotenoid content, while the total polyphenol content and antioxidant activity increased in the apple tissue treated at 1.85 kV/cm and 10 pulses. Conversely, the increased field strength at 5 kV/cm and 100 pulses decreased total polyphenol content and antioxidant activity above 35.93 and 32.95 % respectively.

New findings

Pre-treatment of plant tissue before PEF is also used in order to change cell properties, particularly pre-treatment that can cause extra stress on the cell membrane as this can facilitate PEF-induced permeabilization process.

In ***Paper V*** the application of PEF treatment was investigated for apple and carrot mash in order to enhance the juice yield and the recovery of bioactive compounds such as polyphenols and carotenoids, respectively. The optimization of this processing technology was performed taking into account the modulation of process parameters as well as treatment temperatures by applying a pre-heating step (40, 60 or 80 °C) in order to evaluate the role of thermal and electric field induced cell disintegration effects.

The mechanical grinding during mash preparation was found to reduce the particle size while resulting at the same time in cell disintegration index (CDI) values of 0.36 for carrot and 0.6 for apple. In addition, impedance measurement revealed an increase in CDI after pre-heating as well as after PEF treatment, however samples pre-heated at 40 and 60 °C without any further treatments showed the same values of CDI as control one. Therefore, these two treatment conditions have not been studied for subsequent analysis. The obtained results showed an increase of the CDI in the range of 30 – 40 % for all treated carrot samples compared to the control sample independently from the type of pre-treatment. For apple, an increase of that extent could only be detected for the samples that had reached 80 °C (with or without additional PEF) (Figure 9).

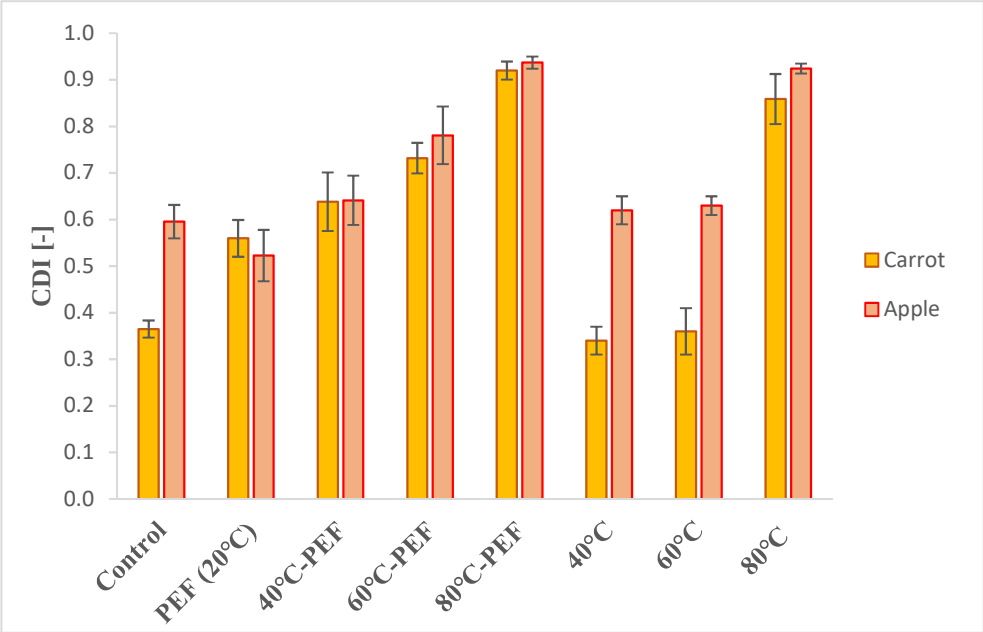


Figure 9. Cell disintegration index-CDI of carrot and apple mashes after grinding (control) and after different pre-treatment (data corresponding to Figure 1 in ***Paper V***).

In general, higher CDI levels detected for pre-treated juice samples at 40, 60 and 80 °C coupled with PEF and for the sample only heated to 80 °C, also corresponded to higher yield of around 10 % for carrot and 5 % for apple compared to the control samples. Hence, similar or even higher juice yield was achieved by lower pre-heating temperatures and additional PEF application compared to more severe thermal conditions. However, combinations of temperature and PEF did not reveal differences in the juice yield for some cases although different levels of cell disintegration have been detected. PEF treatment at room temperature (20 °C) increased the carotenoid extraction yield. All other pre-treatment conditions with temperatures at or above 40 °C led to a decrease in carotenoid content in the final juice.

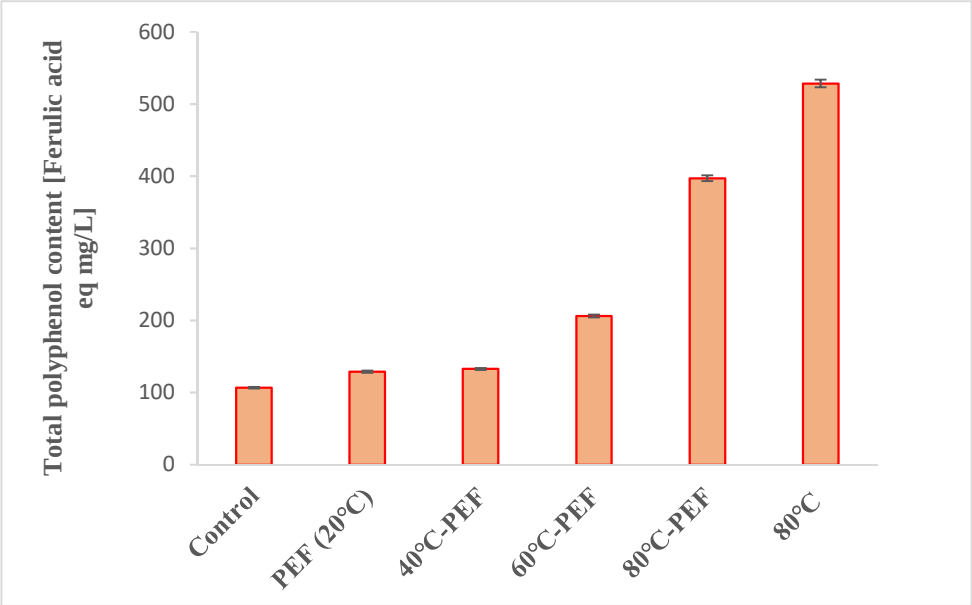


Figure 10. Total polyphenol content in apple juices obtained from pre-treated apple mash (corresponding to Figure 4 in ***Paper V***).

Total polyphenol (TP) content of differently obtained apple juice samples is shown in Figure 10. Significantly higher TP values were obtained for apple juices pre-heated to 80 °C with or without additional PEF treatment.

In ***Paper VI*** the effect of PEF treatment on antioxidant properties, colour and enzyme activity such as peroxidase (POD) for carrot and apple juices and polyphenoloxidase (PPO) for apple juice were investigated. Juices from both raw materials pre-treated at 80 °C with or without PEF showed higher L* and ΔE* values compared to control samples. Larger ΔE values represent a positive deviation from the untreated control sample that showed undesired browning due to enzyme activity and oxidation. However, according to the classification of Cserhalmi et al. (2006), ΔE* changes above 6 indicate great visible changes.

Conversely, the lowest ΔE* values (4.36 - 5.49) were observed for pre-heated juice samples at 40 °C and 60 °C coupled with PEF.

Peroxidase (POD) activity for apple juices is shown in Figure 11. This study revealed that the effect of only PEF treatment at 20°C and 40 °C pre-heating could not reduce the activity of POD in both carrot and apple juice, while for the samples pre-heated at 60°C the reduction of POD activity was observed. This increase on the enzyme activity for both juices could be due to the structural changes of the enzyme that consequently promoted the interactions enzyme-substrate. The highest POD inactivation could be reached by pre-heating to 80 °C with and without additional PEF treatment. POD activity decreased with increasing temperature, and almost no POD activity was detected in juice extracted when the treatment temperature reached 80°C, especially in apple juice samples.

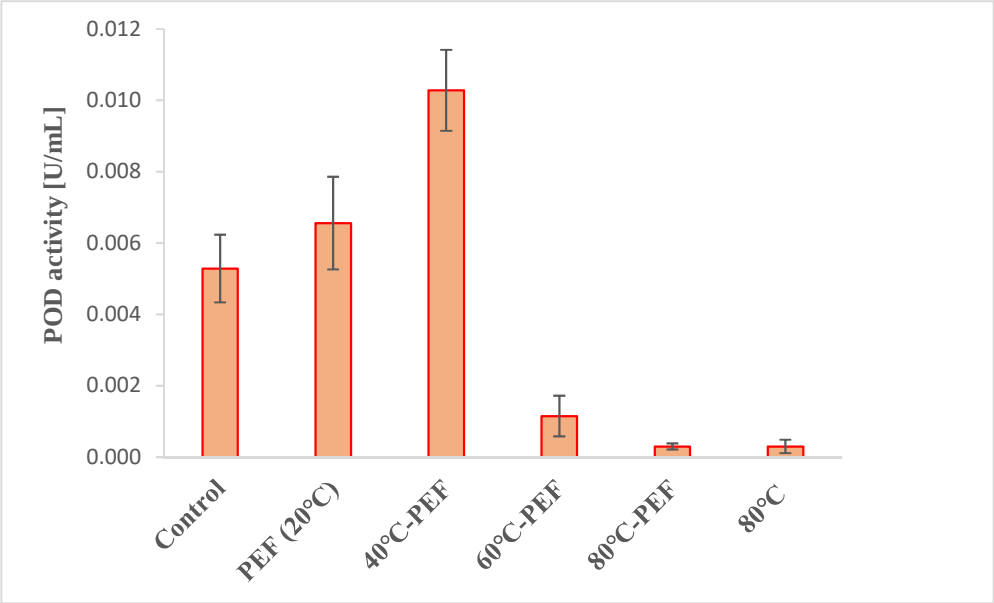


Figure 11. Peroxidase activity in apple juice obtained from pre-treated mash.

Moreover, the variations of colour for carrot and apple juice pre-treated at 80 °C and subsequent PEF application could be explained by the decrease of enzyme activity. PPO activity significantly decrease by PEF application at room temperature compared to the untreated control sample. In addition, a greater inactivation was achieved when the treatment temperature reached 80 °C as well as with just pre-heating.

2. Ohmic heating (OH)

2.1 Introduction

Conventional thermal methods for preservation of food are based on heat transfer, whereby the heat transfer and thermal conductivity are limiting factors for the quick heating of the product. In particular, in the case of viscous and particulate food, the lengthening of the heating time can cause a possible overprocessing of product fractions leading to the loss of quality. Moreover, in the case of indirect heating, heat transfer via hot surfaces can cause possible unwanted temperature peaks in the food product (Goullieux and Pain, 2005).

For this reason, alternative thermal methods have been investigated able to avoid long heating times, overprocessing and unwanted temperature peaks, one of them is the ohmic heating (OH) (Ruan et al., 2001).

2.2 Principles of ohmic heating

During OH there is a conversion of electrical energy into thermal energy. Food with an electrical conductivity in the range of 0.1-10 S/m can be heated by the OH. The treatment can be performed as a batch process or in a continuous flow system (Figure 12).

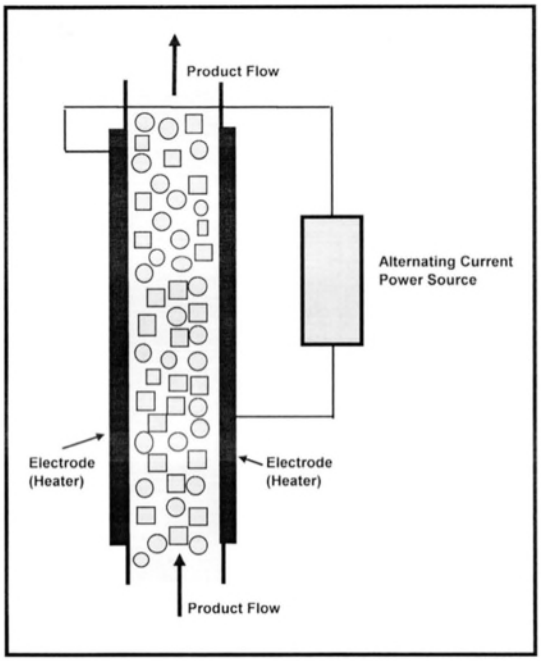


Figure 12. Diagram of heating assembly in an ohmic heating process (www.worldfoodscience.com).

Based on Ohm's law, the current leads to energy generation, which is characterized by complete conversion of the electrical energy into heat. The reached heating rates depend on the design of the treatment chamber and the product properties (conductivity, viscosity and specific heat capacity). OH eliminates variations in temperature throughout the material by a process known as "volumetric heating" which gives a more uniformly processed product than obtained from other heating methods. OH can be achieved with direct or alternating current; however alternating current is mainly used to avoid electrochemical and electrolytic effects. Electrochemical reactions occur less frequently when alternating current is used due to reversed field effect. In the case of OH the focus is on thermal effects, while PEF treatment generates electric effects and minimizing thermal effects. In the case of plant cells, electroporation can occur even at electric field strength lower than 1 kV/cm (Wang and Sastry, 2000; Kulshrestha and Sastry, 2010). Cell lysis through OH can be obtained by thermal permeabilization of the plant cell membrane and as for PEF treatment, it can have an electrical effect (through electroporation of the cell membrane) (Gonzalez and Barrett, 2010). Porous cell walls can allow the cell membrane to build up charges, forming disruptive pores (Cho et al., 1996) (Figure 13). Electroporation occurs because the cell membrane has a specific dielectric strength, which can be exceeded by the electric field. The dielectric strength of a cell membrane is related to the amount of lipids (acting as an insulator) present in the membrane itself. The pores formed can vary in size depending on the strength of the electric field and can reseal after a short period of time. Excessive exposure causes cell death due to the leakage of intracellular components through the pores (Lee and Yoon, 1999).

According to that, OH could also be used for extraction, as an alternative thermal pre-treatment, since it allows heating in a rapid and uniform way, which is especially suitable for high viscous, particulate products such as fruit or vegetable mash (Jaeger et al., 2016). This technique may allow to improve the retention of vitamins, pigments and nutrients due to an optimized heating profile resulting in less thermal damage of heat sensitive substances.

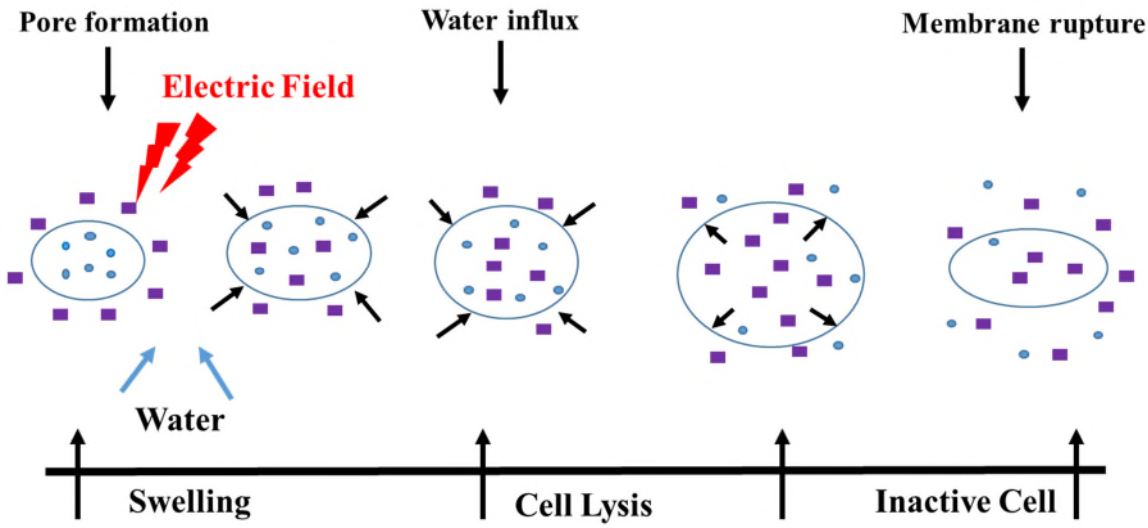


Figure 13. Electroporation process of cell.

To enhance the recovery of the juice and to promote the selectivity of bioactive compounds extraction from plant tissues, ohmic heating has been already investigated (Lima and Sastry, 1999; Wang and Sastry, 2000).

The advantages of OH treatment is the volumetric heating that allows an uniform distribution of the heating (Ruan et al., 2001). In the case of conventional method, there is non-uniformity of heating with the subsequent presence of high temperature peaks called hot spots and low temperatures peaks called cold spots (Jaeger et al., 2016). During OH treatment the cold spots are formed by the fraction with the lowest electrical conductivity and with non-uniform electric field; while on the contrary hot spots are characterized by high electrical conductivity and high electric field strength.

As with conventional heating, the effectiveness of the OH as a thermal process for inactivation of microorganisms depends on the temperature reached in the entire food product and the related holding time.

Combined applications of conventional pre-heating following by OH has been achieving great attention in the last years. The combination of this two treatment offers advantages in terms of product safety. A more uniform temperature distribution is reached through OH, due to the smaller jump in temperature that increases the homogeneity of the treatment compared to the sole OH without pre-heating (Jaeger et al., 2016).

2.3 Exploitation of OH on food processing, components and structure

The electrical conductivity is an important parameter of the food for OH, because it is a requisite for the development of the heat (Wang and Sastry, 1993). Since the conductivity is temperature

dependent, it changes during the heating. Cell structures that have been lysed, as a result of the heat, release ions which lead to a significant change in the food conductivity which affect the OH process. Moreover, OH treatment allows ingredients to have an impact on the structure and conductivity of food. Hydrocolloids such as starch or pectin affect the OH depending on the amount of the substances in the food. Starch solution caused a rise in conductivity as the temperature increased; the conductivity decreased again as the level of gelatinization increased (Jaeger et al., 2016).

For blanching vegetables by using OH it is possible to use larger pieces of vegetables than with conventional heating, where the thermal conductivity is a limiting factor. Thus, larger pieces have a different surface to volume ratio compared to smaller pieces, the loss of soluble compounds is reduced (Mizrahi, 1996). The amount of freely soluble substances increased in white radish under low-frequency conditions, which was shown to be due to increased decomposition of cells and could also explain the faster heating at low frequencies (Imai et al., 1995).

In the case of peach pieces, at low frequency there was a stronger lysis of the cell membranes with a resulting increase in the electrical conductivity and texture degradation. Higher frequencies led to a reduction of these effects, but the required temperature was increased (Shynkaryk et al., 2010). Furthermore, it was possible to increase the carrot and apple juice release by thermal decomposition and electropermeabilisation of the cell membranes (Mannozzi et al., 2018b).

Praporscic et al. (2006) reported the effect of ohmic heating on juice yield from potato and apple slices. A more efficient juice extraction was observed when mild electrically heating (50 °C) was applied especially for potato compared to apple tissues.

The processing of plant tissues compromises the internal compartmentalization that allows the contact between degradative enzymes and their substrates (phenolic compounds), implying the reaction known as enzymatic browning. For this reason, OH treatment has been investigated in order to reduce the enzyme activity because it is considered to be efficient in terms of required energy and in term of avoiding heat induced changes of colour, flavour and nutritional value than the conventional heating (Lasekan et al., 2017).

A study with pea puree indicated that at 50 V/cm for a very short processing time (54 s) the peroxidase activity was reduced if compared to conventional heating (Icier et al., 2006). Whereas for orange juice it was reported that both conventional and ohmic heating (50 Hz and 8 kV) led to a comparable inactivation of pectin methylesterase (by 90-98 %) (Leizerson and Shimoni, 2005).

New findings

Paper V reports the application of OH technology in order to enhance the juice yield and the recovery of bioactive compounds such as polyphenols and carotenoids in apple and carrot mash respectively.

OH treatment was performed taking into account the modulation of process parameters as well as treatment temperatures by applyinga pre-heating step (40, 60 or 80 °C) in order to evaluate at the same time the thermal and electric field effects.

In general, higher cell disintegration index (CDI) levels detected for pre-treated juice samples at 40, 60 and 80 °C coupled with OH and for the sample heated to 80 °C only, corresponded also to higher juice yield, of around 10 % for carrot and 5 % for apple compared to the control samples (Figure 14). Hence, similar or even higher juice yield was achieved by lower pre-heating temperatures and additional OH application compared to more severe thermal conditions.

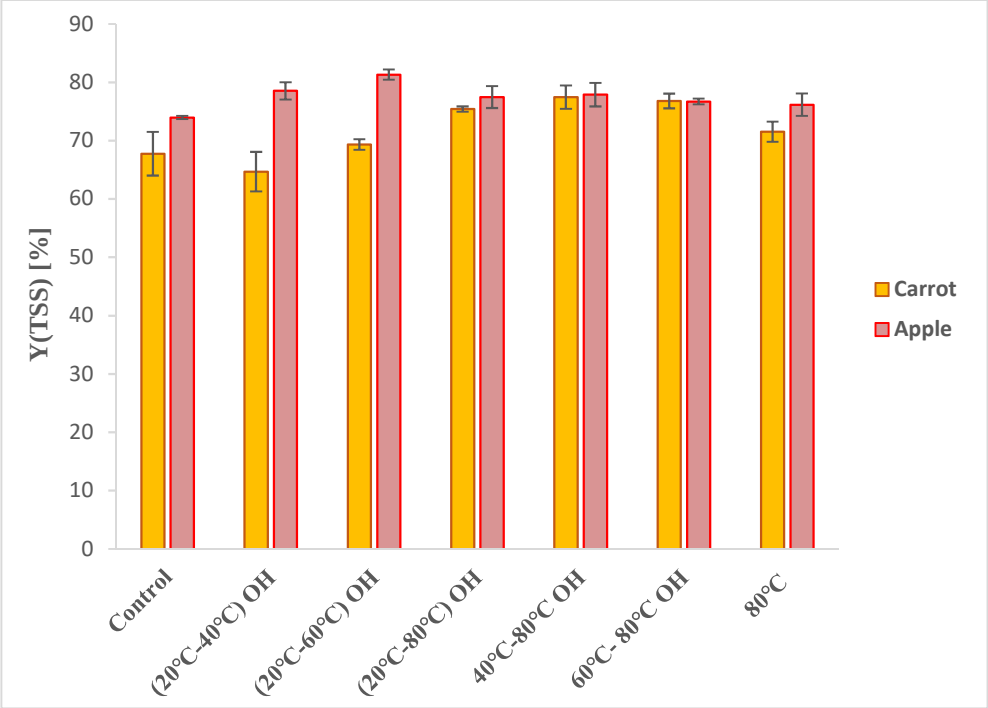


Figure 14. Corrected juice yield (Y_{TSS}) considering the suspended solids in juices obtained from apple and carrot mash after different pre-treatments (data corresponding to Figure 1 in ***Paper V***).

Moreover, in ***Paper VI*** the effects of OH treatment on antioxidant properties, colour and enzyme activity, such as peroxidase (POD) for both juices and polyphenoloxidase (PPO) for apple juice, were investigated. In general, treatment with OH at 80 °C promoted better colour retention (higher L* values) in both juice samples compared to the control one. The detected total colour difference between untreated and treated samples were even more pronounced for carrot compared to apple juice (Figure 15). In general, for both juices, higher L* values promoted also higher total colour differences compared to control one. Larger ΔE* values represent a positive deviation from the untreated control

sample that showed undesired browning due to enzyme activity and oxidation. Therefore, higher ΔE* values indicate a better colour retention of juice and consequently high product quality.

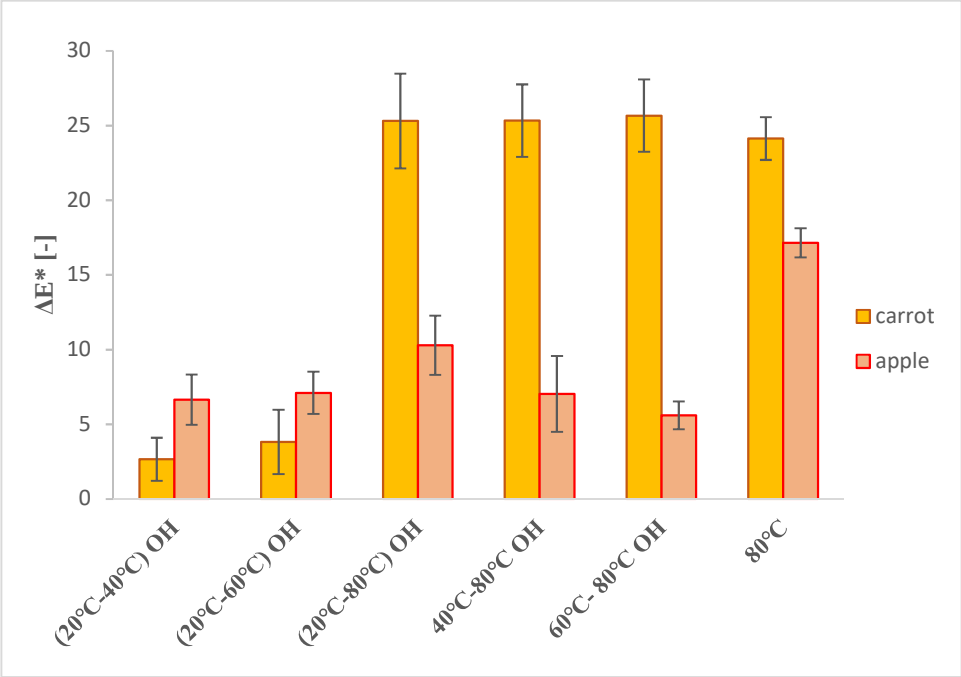


Figure 15. Total colour variation-ΔE* of apple and carrot juices obtained from untreated and treated mash (data corresponding to Figure 1 in ***Paper VI***).

Significantly higher antioxidant activity, detected by both DPPH and ABTS method, were obtained for apple juices pre-heated to 80 °C with or without additional OH treatment (Figure 16). Instead, in samples treated with OH reaching lower temperature (40 °C and 60 °C) the antioxidant activity was lower than in the control juice; this might be due to the activation of degradative enzymes, such as peroxidase and polyphenoloxidase that could have induced bioactive compounds oxidative degradation. The activation of peroxidase and polyphenoloxidase lead to the degradation of phenolic compounds that resulted in subsequent loss of nutritional and sensorial values such as browning and off-flavour (Vámos-Vigyázó, 1995). While higher temperature lead to the inactivation of the oxidative enzymes, thus reducing degradation effects and resulting in higher antioxidant activity in the juice.

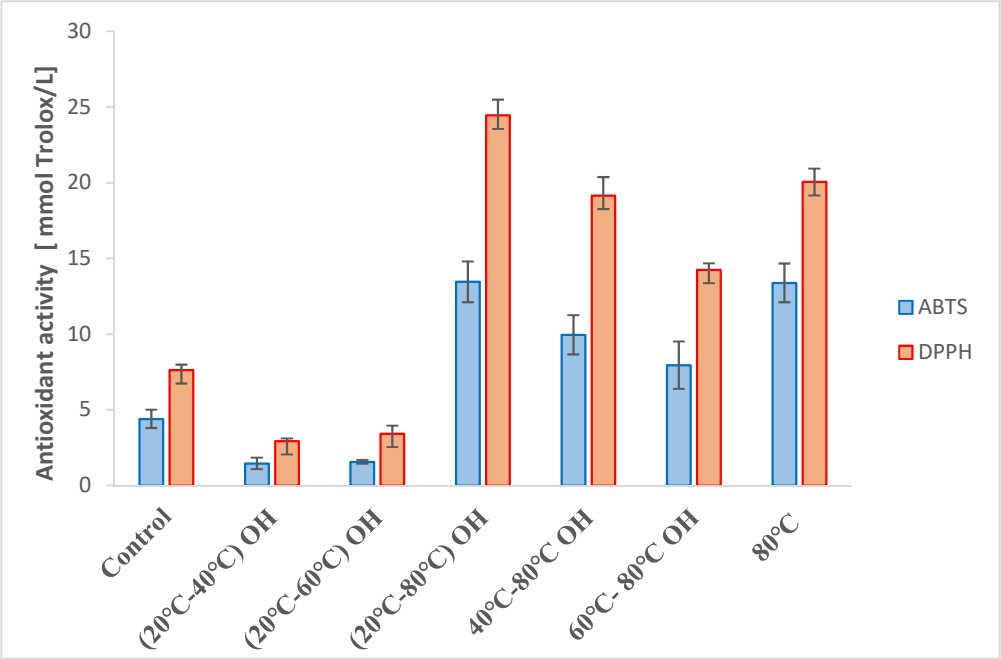


Figure 16. Antioxidant activity (DPPH and ABTS method) of juices of pre-treated apple mash (data corresponding to Figure 4 in ***Paper VI***).

For what concern the enzyme activity, the application of OH treatment reaching 40 and 60 °C increased the POD activity for both juices and PPO activity for apple juice compared to control one. The increase in the enzyme activity can be caused by the pulsating OH treatment that cause biochemical reactions by changing the molecular spacing, thus inducing a better interaction between substrate and enzyme (Castro et al., 2004). All pre-treatment conditions with temperature at or up to 60 °C and 40 °C-80 °C by OH treatment led to a decrease in POD activity in the carrot juice (Figure 17). The POD activity decreased with the application of OH from 20 °C to 80 °C and just pre-heating to 80 °C. This means that for an adequate enzyme inactivation it is required not only the highest temperature but also a suitable total duration of the treatment, which was 237 s and 360 s for (20 °C-80 °C) OH and 80 °C respectively for carrot and apple juice, while for all the other juice samples the heating time was shorter.

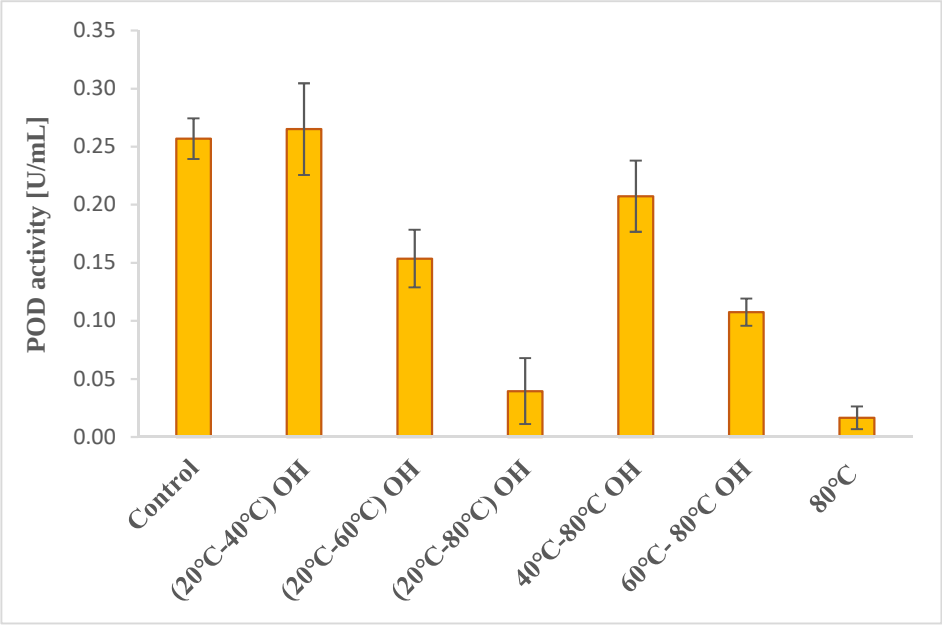


Figure 17. Peroxidase activity in carrot juice obtained from pre-treated mash (data corresponding to Figure 5 in ***Paper VI***).

Moreover, similar behaviour was observed for PPO activity in apple juice (Figure 18). In fact, a greater inactivation of PPO was achieved when the treatment temperature reached 80 °C as well as with OH treatment and just pre-heating.

Similar results were reported in a work of Turk et al. (2012), in which PPO activity was reduced in apple cider mash pre-treated with PEF at 1 kV/cm for100 µs; this result was explained by the inhibition of the enzyme by the oxidised phenolic compounds.

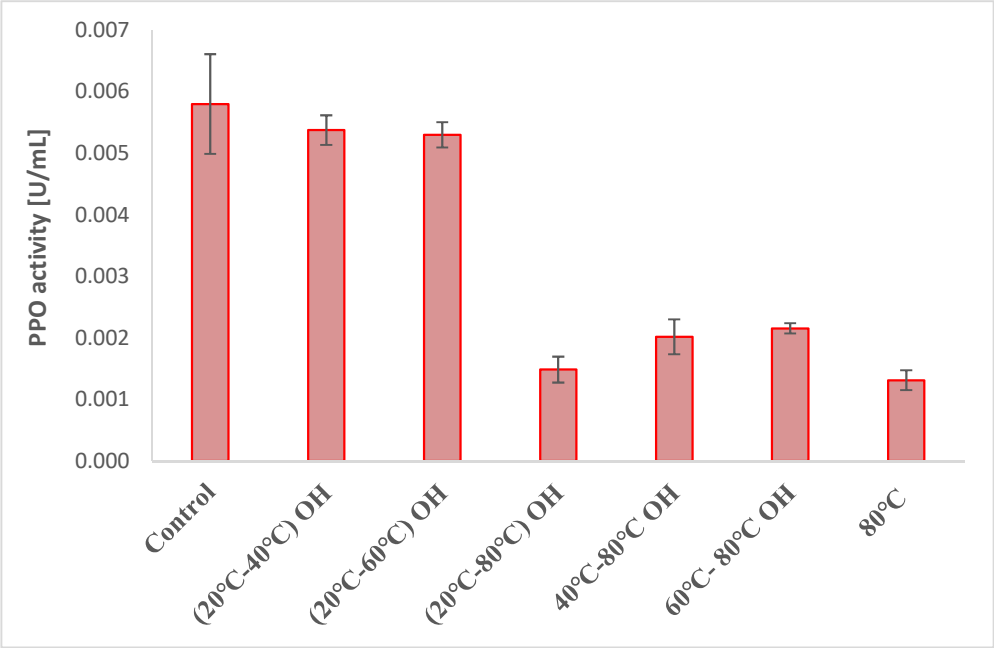


Figure 18. Polyphenoloxidase activity of apple juice obtained from pre-treated mash (data corresponding to Figure 7 in ***Paper VI***).

Moreover, the enzyme inactivation for samples pre-treated at 80 °C with OH treatment provoked an increase of the antioxidant compounds, which lead also to better colour retention compared to the control juice.

Thermal effect promoted an increase in the cell disintegration index and total polyphenol extraction in apple juice; however, this technology caused the degradation of heat or oxygen sensitive compounds such as carotenoids in carrot juice. Thermal and electropermeabilization effects, provided by the OH application, contributing to the colour maintenance, bioactive compounds retention and enzyme inactivation of both carrot and apple juice. OH contributed to a very fast volumetric heating that reduces the overall thermal load of the sample, which are exposed to.

The obtained results concerning the application of OH in order to increase the release of juice, selected compounds and enzyme inactivation have been investigated compared to the PEF treatment, described in a previous chapter.

3. High pressure homogenization principles (HPH)

3.1 Introduction

High pressure homogenization (HPH) is a non-thermal technology applied in the food industry, mainly used to destroy pathogens and spoilage microorganisms, inactivate enzymes and improve the nutritional and technological quality of food products (Basak et al., 2002; Patrignani & Lanciotti, 2016). HPH has been demonstrated, in comparison to thermal treatment, to be less destructive for food components related to nutritional and sensory quality.

Homogenization is a process that involves the application of pressure to liquid foods in order to fragment the solid particles and oil droplets into smaller parts, thus obtaining homogenous dispersions (Figure 19). For this reason, HPH could be used in the citrus industry for increasing the yield of citrus juices (Lortkipanidze et al., 1972), for improving some quality properties of citrus juices, such as viscosity (Patrignani et al., 2009), cloudiness (Baker, 1977), the physical stability of suspended solids (Carle et al., 1998) and colour (Lee and Coates, 2004).

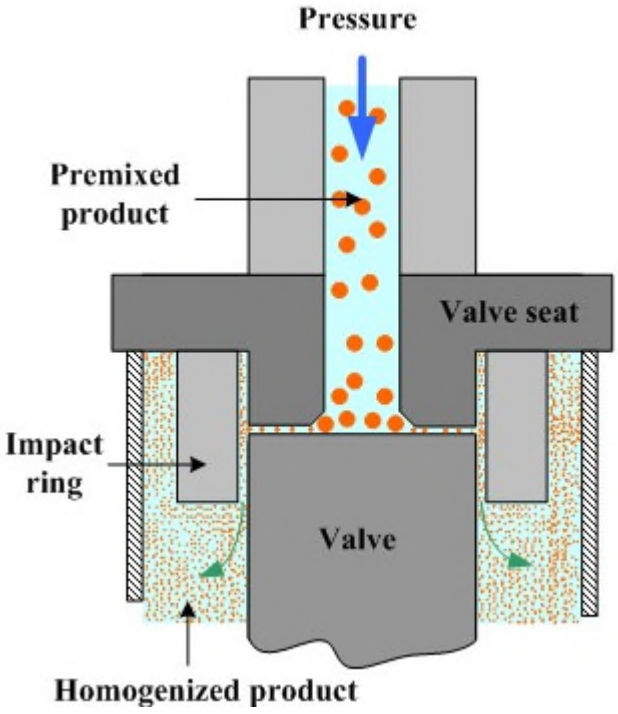


Figure 19. Scheme of HPH working mechanism (www.substech.com).

HP homogenizer is an apparatus that hydrostatically pressurizes a fluid (up to ≈300 MPa). Afterwards, the pressurized food is forced to flow through a minute orifice homogenization chamber. The operating pressure is regulated by adjusting the distance between the valve and the seat. The

passage of a pressurized fluid through this gap causes significant fluid-mechanical stresses such as creation of cavitation, shear and turbulence, which cause the disruption of suspended particles and cells (Figure 20).

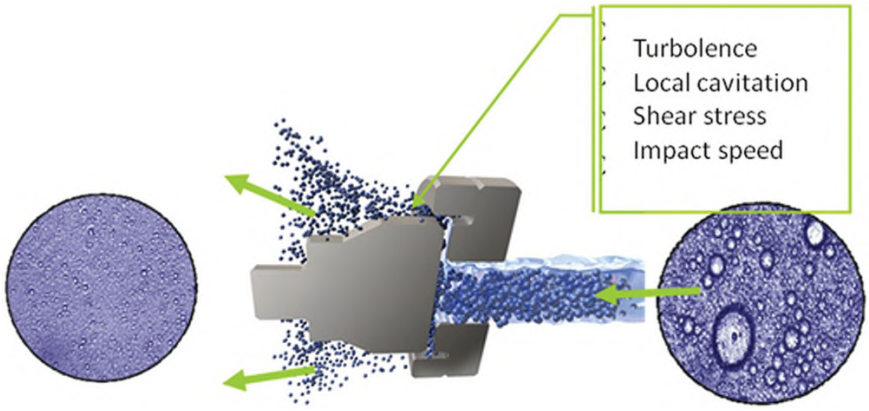


Figure 20. Turbulence (www.substech.com)

During HPH, the temperature in the sample increases due to the heat of compression as well as a homogenization effect. The temperature increase during homogenization (approximately 2.5 °C per 10 MPa) is generally attributed to the viscous stresses caused by the high velocity of the fluid flow leading to the dissipation of pressure energy as heat in the fluid (Patrignani & Lanciotti, 2016). The increased temperature in part depends on the specific geometry of the HPH valve that consequently influences the characteristics of the final processed product. Moreover, the efficiency of HPH depends on the initial fluid temperature, target pressure, and HPH valve design.

3.2 Exploitation of HPH in food processing

HPH treatments have been recently used for the stabilization of liquid food mixtures and the recovery of cell proteins and other biochemicals from microorganisms after cell disintegration. Spatial pressure and velocity gradients, turbulence, impingement, cavitation and viscous shear have been identified as the primary parameters responsible for microbial cell disruption and food constituent modification during HPH treatments (Middelberg, 1995; Kleinig and Middelberg, 1998).

For instance, a 6 log reduction of *Staphylococcus aureus* was observed at 300 MPa and an inlet temperature of 50 °C. With an inlet temperature of 25 °C and 300 MPa, only a 3 log reduction of *Staphylococcus aureu*s was obtained (Diels et al. 2003, Wuytack et al. 2002). Several studies have shown that Gram-negative bacteria are more sensitive to HPH than Gram-positive ones (Vachon et

al., 2002; Wuytack et al., 2002). There is a correlation between cell wall structure and HPH resistance, which suggests that HPH disrupt vegetative bacteria mainly through mechanical destruction of the cell integrity, due to gradients, turbulence and cavitation (Doulah et al., 1975).

Popper and Knorr (1990) reported a reduction of *Escherichia coli*; *Streptococcus lactis* and *Bacillus subtilis* cell populations, respectively, by approximately 3, 1 and 3 log units with HPH treatment at 100 MPa. Whereas, Lanciotti et al. (1994) observed a reduction of *Listeria monocytogenes*, *Yersinia enterocolitica* and *Yarrowia lipolytica* cell population by approximately 2, 3 and 6 log units, respectively when HPH at 100 MPa was applied. Bacterial spores on the other hand seem to be very resistant to HPH, at least at temperatures below 50 °C (Feijoo et al., 1997).

The HPH treatment was reported also to act on food constituents, especially proteins, fat and polysaccharides with consequent modification of their functional properties and susceptibility to enzymatic attack as well as of food microstructure and rheological properties (Sandra and Dalgleish, 2005).

The application of HPH treatment in the extraction process of citrus juices can affect the stability of suspended pulp and hence the functional components suspended in the cloud such as cellular organelles, chromatophores, oil droplets, flavonoid crystals, pectin, cellulose and hemicellulose (Baker and Cameron, 1999). The application of HPH (from 5 to 30 MPa) to mandarin juices has been demonstrated to increase the stability of suspension and therefore improve the availability of bioactive compounds with antioxidant activity (Betoret et al., 2012b).

However, other authors showed that the degradation of bioactive compounds during processing and storage is an important issue. They demonstrated that management of high pressure and high pressure of homogenization process technologies influenced the functional properties of the obtained final products (Donsì et al., 1996; Barba et al., 2012; Barba et al., 2015; Betoret et al., 2015).

Dede et al. (2007) studied the effects of high pressure (HP) treatment (250 MPa, 35 °C for 15 min) and thermal treatments (60 °C, from 5 to 15 min and 80 °C for 1 min) on the antioxidant capacity (DPPH) of carrot and tomato juices during refrigerated storage at 4 °C for 30 days. Both heat treatments showed a significant loss in free radical scavenging activity as compared to untreated samples. However, the HP treated juices showed only a little loss of antioxidant compounds (less than 10 %) during storage. Esteve & Frígola (2008) compared the effect of HP treatment (400 MPa, 42 °C, 5 min) with the effect of heat treatment (90 °C, 20 s) on orange juice stored in refrigerated conditions at 4 and 10 °C. Total antioxidant capacity decreased significantly after orange juice processing with both types of treatment, but the decrease was much smaller in the HP treated juice than in the pasteurized one (4.20 and 38.21 %, respectively). They also observed a reduction in antioxidant capacity in thermally treated and HP treated samples during refrigerated storage at 4 and

10 °C. In this study it was demonstrated that, in comparison with conventional pasteurization, HP treatment led to a higher total antioxidant activity in orange juice immediately after processing, as well as during storage at 4 and 10 °C.

New findings

In ***Paper I*** the effect of high pressure homogenization (HPH) (20 and 100 MPa) and trehalose (10 and 30 %) addition on technological and functional properties of low pulp mandarin juices (LPJ) was investigated.

Generally, an increase in homogenization pressures resulted in a decrease of bioactive compounds degradation during storage. Trehalose addition also contributed to reduce hesperidin degradation, increasing the effect of homogenization pressures (Table 1). This effect could be related with cloudiness stability and trehalose capability to interact and form complexes with bioactive compounds. Smaller particles are able to interact better with LPJ cloud and thus guarantee less availability of bioactive compounds to the degradative reactions.

Table 1. Hesperidin content (mg/L) in low pulp juice-LPJ samples during 0, 3 and 10 days of storage and percentage of degradation calculated from 10 and 0 days of storage. Values expressed as mean ± standard deviation (corresponding to Table 2 in ***Paper I***).

Homogenization pressure (MPa)	Trehalose (%)	0 days	3 days	10 days	Degradation (%)
0	**0**	138.1 ± 0.8^{a}	132.2 ± 0.4^{a}	51.51 ± 17.07^{e}	62.7 ± 12.6
0	**10**	133.5 ± 3.9^{b}	143.4 ± 1.6^{a}	74.7 ± 0.5^{bc}	44 ± 2
0	**30**	145.7 ± 3.2^{a}	145.8 ± 3.7^{a}	76.7 ± 3.8^{bc}	47.3 ± 3.7
20	**0**	88.8 ± 0.4^{d}	83.6 ± 1.9^{be}	72.7 ± 2.2^{c}	18.2 ± 2.9
20	**10**	97.2 ± 0.6^{d}	83.4 ± 2.9^{bf}	84.1 ± 2.2^{ab}	13.5 ± 2.8
20	**30**	106.3 ± 9.6^{c}	93.4 ± 1.7^{bc}	94.7 ± 3.8^{a}	10.7 ± 4.5
100	**0**	69.1 ± 1.9^{f}	79.3 ± 18.5^{cdef}	68.01 ± 1.13^{d}	1.5 ± 4.4
100	**10**	72.1 ± 0.3^{ef}	85.8 ± 0.2^{bd}	69.1 ± 2.9^{d}	4.1 ± 4.6
100	**30**	79.430 ± 3.108^{e}	99.0 ± 7.7^{b}	74.9 ± 1.2^{bc}	5.66 ± 2.13

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Table 2 shows the vitamin C content in mandarin juice and its degradation percentage during storage. It is possible to observe the same behaviour in all analysed samples; those with trehalose both non homogenized and treated at 20 MPa had slightly higher vitamin C content in comparison to those

treated at 100 MPa. This increase of vitamin C content could be due to the food matrix, which has an important influence on bioactive compounds; the juice structure changes, caused by processing or by storage time, could facilitate the extraction of bioactive compounds (Betoret et al., 2015). For all LPJ, a tendency to increase the vitamin C content was reported until third day of storage, while high degradation was observed at the end of the storage (10 days). Moreover, during the storage for trehalose samples the addition of trehalose seems to increase vitamin C degradation by 2-4 %. Hence, the protective effect of trehalose on bioactive compounds (flavonoids and vitamin C) seems to depend specifically on the characteristics of each specific compound.

Table 2. Vitamin C content (mg/L) in low pulp juice-LPJ samples during 0, 3 and 10 days of storage and percentage of degradation calculated from 10 and 0 days of storage (corresponding to Table 3 in ***Paper I***).

Homogenization pressure (MPa)	Trehalose (%)	0 days	3 days	10 days	Degradation (%)
0	**0**	129.2 ± 0.7^{b}	135.63 ± 2.09^{c}	119.3 ± 1.4^{b}	7.7 ± 0.6
0	**10**	134.1 ± 1.4^{a}	139.3 ± 1.6^{ab}	119.6 ± 1.4^{b}	10.8 ± 1.3
0	**30**	133.4 ± 1.4^{a}	137.0 ± 1.6^{bc}	123.1 ± 1.4^{a}	7.7 ± 1.5
20	**0**	128.7 ± 0.4^{b}	135.66 ± 0.15^{c}	118.1 ± 0.5^{b}	8.2 ± 0.41
20	**10**	133.9 ± 1.4^{a}	141.5 ± 0.7^{a}	118.4 ± 0.8^{b}	11.5 ± 1.2
20	**30**	131.6 ± 3.0^{ab}	141.59 ± 3.02^{a}	122.1 ± 2.3^{a}	7.2 ± 0.9
100	**0**	128.6 ± 1.6^{b}	134.9 ± 0.3^{c}	117.7 ± 0.7^{b}	8.44 ± 1.06
100	**10**	122.6 ± 2.6^{c}	131.8 ± 0.6^{d}	109.4 ± 0.7^{d}	10.7 ± 1.4
100	**30**	125.4 ± 1.9^{c}	131.5 ± 0.6^{d}	112.2 ± 1.7^{c}	10.4 ± 0.2

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

H-NMR based approach highlighted four different metabolites mostly influenced by storage time and HPH treatment. The overall observations suggest that HPH solubilization ability together with different microbial activities had a joint effect on the concentration of formic acid, glutamic acid, glucose and alanine molecules. Formic acid concentration in samples at day 0 did not show any correlation with HPH, this was highlighted by its insolubilization after HPH treatment. Nevertheless, its concentration increased with storage time and such increase was not proportional to the applied pressure levels. Contrary, concentrations of glutamic and glucose were proportional to the applied pressure at day 0, suggesting a direct effect of HPH on their solubilisation. Moreover, dissimilarly from formic acid, glutamic acid and glucose together with alanine, decreased proportionally to storage

time in untreated samples and HPH samples at 20 MPa, but increased with the application of 100 MPa. The systematic increase in formic acid with storage, proportional to the pressure levels even when 100 MPa was applied, suggests that this molecule was probably produced as a result of microbial activity and that microbes responsible for its production were not significantly hampered by pressure treatments.

The overall observations suggests HPH solubilisation ability and different microbial activities strongly affect the concentration of these molecules.

Finally, from the obtained results HPH and trehalose addition had a significant effect on functional and technological properties of low pulp mandarin juice. HPH affects the juice cloud structure thus influencing trehalose interactions. H-NMR based approach highlighted the HPH effect on the microbiological aspects of low pulp mandarin juice by the identification of key molecules responsible of the microorganism profile evolution during storage.

In ***Paper VII*** the effects of UHPH (ultra high pressure homogenization) treatments, performed at 200 MPa for 2 and 3 cycles, on quality and safety of organic kiwifruit juice were studied. The samples were evaluated immediately after the treatments and during storage at three different temperatures (5, 15 and 25 °C).

In general, treatments at 200 MPa for both 2 and 3 cycles resulted in a higher viscosity of kiwifruit juice compared to the control one. This result was probably due to the structural modification of kiwifruit juices induced by UHPH treatment, which promotes the disruption of the cell clusters into single cells and/or cell fragments. During the storage at 5 °C a decrease of viscosity was observed in all samples, which was more pronounced for untreated samples and those treated with 200 MPa x 2 cycles. Moreover, while in the control sample (untreated) the separation of the phases was observed in 20 days, the reduction of the macromolecules size in the treated samples induced a delay in separation and sedimentation. Juices stored at higher temperatures maintained a similar viscosity during the whole period, which was 14 days for samples stored at 15 °C and 7 days for those stored at 25 °C.

Table 3 shows lightness (L*) and redness (a*) values of control and treated kiwifruit juices during storage at 5 °C. HPH treatment caused a significant increase of L* parameter in comparison to the untreated samples, which could be attributed to the higher ability of smaller size particles to scatter light, leading to an increase in sample lightness. Concerning a* parameter, both samples treated at 200 MPa showed lower values compared to the control sample. During the storage a slight decrease of L* together with an increase of a* was observed in untreated and 200 MPa *2cycles treated samples.

Table 3. Lightness (L*) and redness (a*) values of organic kiwifruit juices immediately after the treatment and during the storage at 5 °C in relation to the pressure applied (corresponding to Table 4 in ***Paper VII***).

L*						
	5 °C					
	T0	T5	T16	T26	T33	T40
0.1 MPa	33.4±0.7^{b}	34.6±0.6^{b}	30.3±0.4^{b}	30.5±0.2^{b}	-*	-*
200 MPa *2 cycles	38.68±0.08^{a}	38.6±0.6^{a}	36.5±0.2^{a}	35.5±0.8^{a}	35.4±0.3^{a}	35.2±0.4^{b}
200 MPa *3 cycles	38.9±0.2^{a}	38.5±0.5^{a}	36.2±0.1^{a}	36.5±0.8^{a}	36.9±0.4^{a}	37.7±0.2^{a}
a*						
	5 °C					
	T0	T5	T16	T26	T33	T40
0.1 MPa	-2.4±0.3^{a}	-1.8±0.1^{a}	-2.7±0.2^{a}	-2.1±0.2^{a}	-*	-*
200 MPa *2 cycles	-3.7±0.2^{b}	-4.6±0.2^{b}	-3.4±0.1^{b}	-2.9±0.1^{b}	-2.8±0.2^{a}	-2.7±0.1^{a}
200 MPa *3 cycles	-3.4±0.3^{b}	-4.4± 0.2^{b}	-3.7±0.2^{b}	-3.2±0.2^{c}	-3.2±0.2^{b}	-3.15±0.07^{b}

*not performed because the juice spoiled

Means followed by different letters means significant different ($p<0.05$) between samples at each day of storage.

Moreover, during the storage at different temperatures, total polyphenol content and antioxidant activity values decreased in all samples although the highest values were found in kiwifruit juice samples treated with UHPH.

For what concern the microbiological quality of juice samples, the UHPH treatments determined an instantaneous reduction of yeast populations under the detection limits due presumably to the applied pressure, since, to minimize the temperature increase generated during the treatment, a thermal exchanger was applied avoiding to exceed temperature of 45 °C.

The research showed the potential of UHPH in the fruit juice field, since it allowed to significantly maintaining quality characteristics thus increasing the shelf-life of the product.

III. Innovative packaging technologies

The packaging process is one of the most important among all of the food manufacturing processes, as it maintains the quality and the stability of food products for storage and transportation (Kelsey, 1985). Food packaging is designed in order to prevents deterioration in the quality of food and beverages as well as provide required information about the food and make food handling convenient from distribution to consumer's table. The purpose of food packaging has addressed to achieve preservation as well as reducing the volume and/or weight of packaging materials in an effort to minimize resources and costs. Various technologies are used to achieve safety enhancement, extend the shelf-life and retain the nutritional value of food products; among this group particular attention can be given to active packaging, modified atmosphere packaging (MAP) and edible coatings.

In this PhD thesis in particular the application of different coating solutions have been investigated.

1. Edible coating

1.1 Introduction

Edible films and coatings are produced from edible biopolymers and food-grade additives. Film forming biopolymers can be proteins, polysaccharides (carbohydrates and gums), lipids or a mixture of them.

Edible films and coatings enhance the quality of food products, protecting them from physical, chemical and biological deteriorations (Kester & Fennema, 1986). Their application can improve the mechanical resistance of food products, improve visual and tactile characteristics of product surface, reduce moisture migration, gas exchange, microbial growth and oxidative reactions, as well as reduce or even suppress physiological disorders (Rojas-Graü et al. 2009). Most commonly, edible films and coatings act as barriers against oils, gases or vapours and as carriers of active substances such as antioxidants, antimicrobials, colorants, nutrients, structural agents etc. aimed to enhancing the quality of food products, resulting in shelf-life extension and safety improvement (Krochta & De Mulder-Johnston 1997; Mannozzi et al., 2018a). Moreover, edible coatings must be transparent, tasteless and odourless.

Edible films and coatings are biodegradable this is one of the greatest benefits along with their edibility (Debeaufort et al., 1998).

Many functions of edible coatings are similar to those of synthetic packaging films; however, they must be chosen for food packaging purpose according to the specific food applications, the types of food product and the major mechanisms of quality deteriorations. The use of this kind of materials can potentially replace partially or totally the conventional packaging materials by simplifying the

total structure of the packaging. Currently, edible films and coatings are used with several food products, mainly fruits, vegetables, candies and some nuts (Petersen et al., 1999).

1.2 Characterization of edible coatings

In edible coatings, the major film forming ingredients are biopolymers, such as protein, polysaccharides, lipids or a mixture of them. The physico-chemical characteristics of the biopolymers influence the properties of the final edible coatings. Film forming components can be either hydrophilic or hydrophobic, or both; however, in order to maintain the edibility, the solvents used could be only water and ethanol. The minor components usually includes polyols acting as plasticizers, such as glycerol to improve flexibility and elasticity (Vargas et al., 2008).

Polysaccharides are the most widely used components found in edible coatings for fruit (Kester & Fernema, 1986). They show effective gas barrier properties although they are highly hydrophilic and show high water vapour permeability in comparison with plastic films.

Many proteins from plants and animals (e.g. corn, gelatin, casein, whey protein) have been studied for their application in the formulation of edible coatings. Edible coatings obtained from proteins have the potential to decrease moisture and gas permeability, solubility and improve mechanical properties.

The main polysaccharides included in edible coating formulations are starch and starch derivatives, cellulose, alginate, carrageenan, chitosan, pectin and several gums. Based on the molecular weight, conformation, electrical charge and hydrophobicity, variations in physicochemical properties and performance of the coatings formed occurred. Starch is the natural polysaccharide; it is inexpensive, abundant, biodegradable and easy to use. Starch typically contain amylose and amylopectin, different functionality can be achieved by modulating the concentration of them. High amylose content formulation showed excellent oxygen barrier property, lower water solubility, lower retrogradation temperature and more stable mechanical properties compared with those made with normal starch.

Cellulose and cellulose derivatives-based coating are very efficient oxygen barrier and their water-vapour barrier may be improved by the addition of lipids.

Sodium alginate is a natural linear polysaccharide obtained from brown seaweeds and has many important physical and biological properties, such as moisture retention, gel-forming capability, good biocompatibility, low price and high availability (Pei et al., 2008).

Chitosan (poly β-(1,4)N-acetyl-D-glucosamine) polymer is industrially produced from chemical deacetylation of the chitin found in exoskeletons of crustaceans. This biopolymer can also be extracted from the cell wall of mushrooms, being biodegradable, non-toxic and non-allergenic, which contribute to its use in many fields, including food, biomedicine, agriculture and environmental

protection (Shahidi et al., 1999; Kim & Rajapakse, 2005). Moreover, it has been shown to have mechanical and antimicrobial properties, no toxicity, biodegradability and to inhibit the growth of fungi on the surface of different fruits (Rojas-Graü et al., 2008; Treviño-Garza et al., 2015).

Pectin is a complex of acidic polysaccharides that form an interpenetrating network in the plant cell wall; it is one of the most important citrus by-products that are industrially extracted from apple pomace and citrus peels. Generally, it is used to increase viscosity and gel strength of food products (Krochta et al, 1997).

For what concern the lipids used to develop edible coatings beeswax and other kind of wax fatty acids are applied on the food surface. Lipid-based edible coatings have low affinity for water, which explains why they have low water vapour permeability. The increase on carbon number of fatty acids lead to act as moisture barrier due to the increase of the non-polar part of the molecules and thus promotes neither water solubility into the film (Morillon et al., 2002).

1.3 Functions and advantages of edible coatings

The most beneficial characteristics of edible films and coatings are their edibility and inherent biodegradability, therefore all the used ingredients must be food-grade and environmentally safe (Krochta, 2002).

Edible films and coatings provide many benefits in term of handling convenience. In fact, coated fruits and vegetables have much higher resistance against bruising and tissue damage caused by physical impact. They also contribute to maintain and/or enhance the quality of the final food products, delaying surface dehydration, moisture adsorption, oxidation reactions, aroma loss, frying oil adsorption and microbial deterioration of food products. Their application contribute not only to the physical and chemical quality enhancement of the food products, but also lead to improve the visual appearance.

The oxygen-barrier properties of films and coatings layers can prevent oxidation of lipid ingredients in products such as nuts, moreover, it is also useful for retarding the respiration rate of fresh product, thus many climacteric fruit can be coated in order to decelerate their respiration rate and polyphenoloxidase activity, responsible of the enzymatic browning.

Moisture barrier property is important for the protection of fresh fruits and vegetables from dehydration, in fact, the migration of water can occur between food and surrounding environment, food and packaging materials, or among heterogeneous ingredients in the food product itself (Krochta, 1997). All barrier properties of edible films and coatings are affected by film composition and environmental factors (relative humidity and temperature). Plasticizers included in coating formulation can increase the permeability of most migrants. At higher relative humidity conditions,

oxygen permeability increases substantially, therefore, it is very important to maintain low relative humidity environments to maximize the effectiveness of edible coating as gas barriers (Bonilla et al., 2012). Temperature is another important factor that determines the changing permeability, increasing temperature provides more energy to the migrating substances.

Instead, the oil barrier property of some edible coatings is utilized to increase the oil resistance of fried food products, resulting in lower oil intake of the products (Garcia et al., 2002).

Therefore, the maintenance of quality is directly related to the shelf-life extension and safety of the products. For protective function and preserving quality of food, it is important also to consider the enrichment of based coating formulation with active substances carrier such as antimicrobials, antioxidants, flavours, nutraceutical compounds, colorants, etc. There are several categories of antimicrobials that can be potentially incorporated into edible films and coatings, including organic acids (acetic, lactic, benzoic, sorbic) polypeptides (lysozyme, peroxidase, nisin) plant essential oils (cinnamon, oregano, lemongrass), nitrites among others (Franssen & Krochta, 2003). Garcia et al. (2001) observed the reduction of microbial growth below 6 log CFU/g at the maximum storage time assayed (28 days) and extension of storage life of fresh strawberries using a starch-based coating containing potassium sorbate and citric acid. However, in the last years consumers demand for natural products such as essential oils have reached considerable interest. An important characteristic of essential oils is the hydrophobicity, which makes them able to pass through cell membranes and making them more permeable; however, the essential oil application in food is still limited due to their impact on organoleptic food properties and their possible interactions with food components (Burt, 2004). Severino et al. (2014) reported the application of chitosan coating formulation containing 0.05 % nanoemulsion of mandarin essential oils tested in combination with γ-irradiation, UV-C and ozonated water treatment on green beans. The obtained results in terms of antimicrobial activity showed synergistic effect between coating and γ-irradiation with a microbial reduction (3 log CFU/g) over the entire shelf-life (14 days). Raybaudi-Massilia et al. (2008) reported that the addition of cinnamon, clove or lemongrass oils at 0.7 % (v/v) into an alginate-based coating increased the antimicrobial effect, reduced the *E. coli O157:H7* population by more than 4 log CFU / g and extended the microbiological shelf-life of Fuji apples for at least 30 days. The application of antioxidant compounds into edible coatings has been studied by various authors to protect fruit against oxidative reaction, degradation and discoloration (Rojas-Graü et al., 2007; Yang et al., 2014; Mannozzi et al., 2018). Nair et al. (2018) studied the influence of chitosan and alginate coating enriched with pomegranate peel extract (1 % w/w), showing that chitosan coating containing pomegranate peel extract was more efficient than alginate in maintaining the quality of guavas during 20 days at 10 °C. Moreover, few studies reported the effect of the addition of nutraceutical

compounds in the functionality of edible coatings. For instance, Tapia et al. (2007) developed alginate and gellan film forming solution containing viable bifidobacteria on fresh-cut apple and papaya cylinders. Obtained results showed higher than 10^6 CFU / g *Bifidobacterium lactis* Bb- 12 values on both studied fruits in comparison to control during 10 days of refrigerated storage.

New findings

As previously stated, edible coating represents an alternative and/or additional way for fruit preservation during postharvest storage (Tezotto-Uliana et al., 2014).

Paper III and ***Paper IV*** report the effects of different coating formulations: sodium alginate (Al), pectin (Pe), sodium alginate plus pectin (Al + Pe) (***Paper III***), chitosan from mushrooms (C) and chitosan from mushrooms enriched with procyanidins extracted from grape seeds (CP) (***Paper IV***), on fresh blueberry quality maintenance and microbial growth, during 14 days of storage at 4 °C.

In ***Paper III*** coating induced a general lower lightness and a more intense blue hue colour in blueberry samples as compared with the control one, probably due to the glossy effect of coating (Table 4). The observed lower luminosity value of coated samples could be caused by the modifications in the surface reflection properties (Hoagland & Parris, 1996). The visual perception of the intensity of blue colour was always more intense in the coated than in the control samples, as indicated from their highest hue values. Moreover, the blueberry samples showed a general decrease in hue values from 0 to 10 days, these values increased again on 14^{th} day. The h° decrease of blueberries during the first period of storage was probably caused by oxidation or condensation reactions of phenolic compounds resulting in loss of anthocyanins during cold storage (Reque et al., 2014). Moreover, the increase of hue values at the end of storage might be caused by a possible anthocyanins synthesis during ripening.

Table 4.Lightness-L* and Hue angles- h° of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples during 14 days of storage at 4°C (corresponding to Table 3 in ***Paper III***).

	L*					
	T0	T2	T4	T6	T10	T14
Control	21 ± 1^{a}	28.4 ± 0.1^{a}	31.5 ± 0.8^{a}	30.5 ± 0.5^{a}	28.5 ± 0.6^{a}	29 ± 1^{a}
Al	19.33 ± 0.07^{a}	18.9 ± 0.1^{b}	22.74 ± 0.05^{c}	22.2 ± 0.6^{c}	19.4 ± 0.6^{b}	16.48 ± 0.00^{c}
Pe	14 ± 2^{b}	19.5 ± 0.5^{b}	23.2 ± 0.4^{bc}	26.0 ± 0.2^{b}	19.3 ± 0.2^{b}	19.59 ± 0.02^{b}
Al+Pe	15.3 ± 0.6^{b}	15.9 ± 0.8^{c}	24.9 ± 0.2^{b}	25.6 ± 0.5^{b}	17.6 ± 1.4^{b}	19.9 ± 0.4^{b}
	h°					
	T0	T2	T4	T6	T10	T14
Control	100 ± 11^{b}	90 ± 3^{c}	97 ± 5^{c}	93 ± 4^{b}	72 ± 6^{c}	89 ± 6^{c}
Al	140 ± 11^{a}	126 ± 10^{ab}	117 ± 7^{b}	102 ± 9^{b}	75 ± 6^{b}	145 ± 11^{b}
Pe	145 ± 11^{a}	139 ± 7^{a}	128 ± 5^{a}	134 ± 6^{a}	87 ± 6^{a}	151 ± 11^{b}
Al+Pe	154 ± 11^{a}	123 ± 9^{b}	111 ± 7^{b}	126 ± 5^{a}	85 ± 5^{ab}	179 ± 11^{a}

Data are reported as average values and standard deviations obtained from twelve replicates for each treatment-time conditions. Values with different letter within the column are significantly different ($p < 0.05$).

As shown in Figure 21 blueberry coated samples presented a significantly ($p < 0.05$) higher firmness compared to control sample until the first 10 days of storage. After this period, texture of blueberries coated samples decreased, reaching the same value of control one (1.75N). The higher firmness values of coated samples are probably due to the presence of coating that provide a structural rigidity to the surface of fruit (Duan et al., 2011).

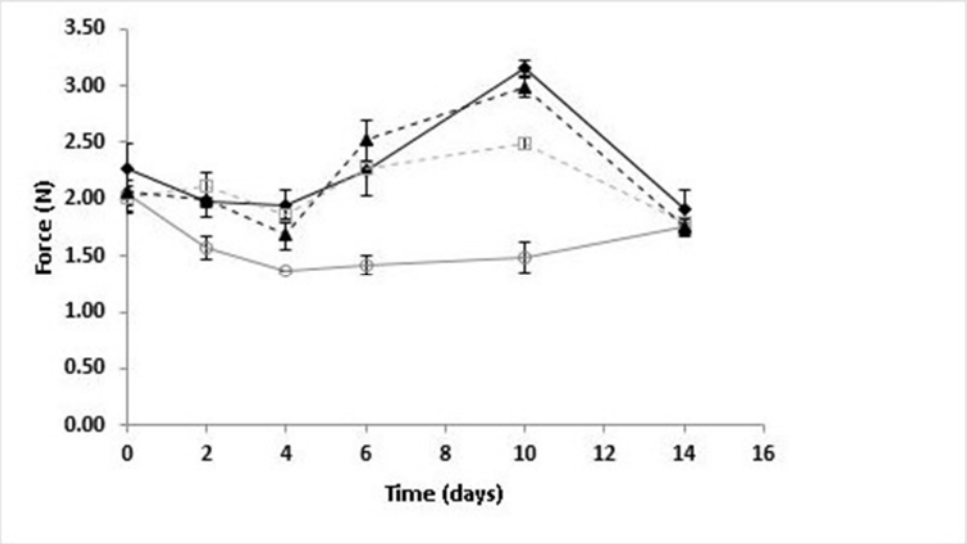
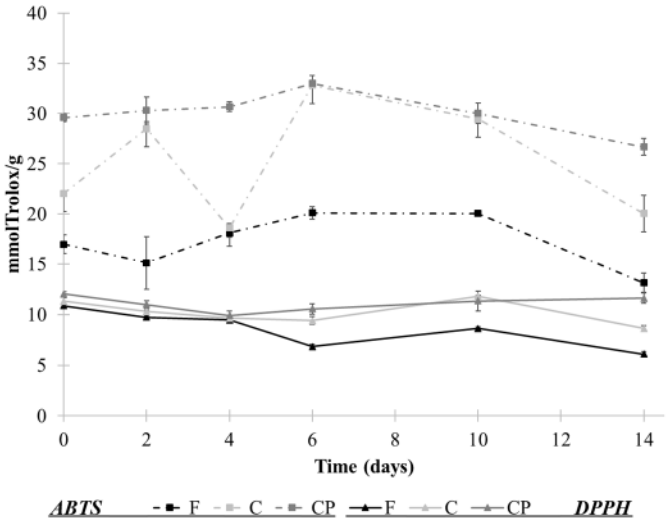


Figure 21. Firmness (N) of control (Control ○) and differently coated blueberry samples (sodium alginate - Al ♦; pectin - Pe □; sodium alginate plus pectin – Al + Pe ▲) during 14 days of storage at 4°C (corresponding to Figure 1 in ***Paper III***).

Similar results, for what concern colour and firmness were obtained in ***Paper IV*** by using an innovative edible coating, based on chitosan from mushrooms enriched with procyanidins extracted from grape seeds. In ***Paper IV*** the antioxidant activity of blueberry was investigated by using two different methods DPPH and ABTS (Figure 22). Under both the analytical methods, the CP (chitosan plus procyanidins) coated blueberries showed a higher antioxidant activity already at 0 day, in comparison to the C (only chitosan) and the F (fresh) samples. Its better retention during the overall storage period is probably due to the presence of chitosan and procyanidins in the coatings that provides the enhancement of antioxidant compounds. The use of procyanidins from grape by-products induced an improvement of the nutritional value of coated blueberry fruit.



ABTS

Time	F	C	CP
0	bc C	d B	abc A
2	cd B	b A	bc A
4	ab B	e B	ab A
6	a B	a A	a A
10	a B	b A	ab A
14	d C	c B	c A

DPPH

Time	F	C	CP
0	a B	b B	a A
2	b C	c B	ab A
4	b A	d A	c A
6	d C	d B	bc A
10	c B	a A	ab A
14	e C	e B	ab A

Figure 22. Antiradical activity with DPPH method (▲) and ABTS method (■) of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4°C (corresponding to Figure 2 in ***Paper IV***).
Means with different lowercase letters means significant difference ($p<0.05$) during time (days, in columns) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in rows).

Moreover, the results of microbial growth showed that the application of coatings reduced the growth kinetics of yeasts and mesophilic aerobic bacteria, in particular with the application of Al, Pe (***Paper III***) and chitosan-based coating samples (***Paper IV***).

The overall results of these studies demonstrated the efficacy of the new type of coating ingredients (chitosan alone and with natural procyanidins) applied to maintain the overall quality of fresh blueberries during storage.

IV. Materials & Methods

In this chapter, the experimental methods and techniques employed in all research works carried out during the PhD period are described.

1. Pulsed electric field (PEF)

1.1 Osmotic dehydration

Organic strawberries (*Fragaria+ananassa)* var ‘‘Alba’’ (10 ± 1 °Brix) were used for the OD/PEF experiments (***Paper II***). Two rectangular pieces (approximately 1.3 g) were placed into a PEF treatment chamber equipped with two stainless steel electrodes with a gap between them of 30 mm and filled with 5 mL of a sodium chloride solution with the same conductivity as the strawberries (1.6 mS/cm). The PEF treatments were applied to the strawberry samples using an in-house developed pulse generator equipment, based on MOSFET technology that delivers near-rectangular shape pulses. PEF pre-treatments were carried out by applying a train of 100 pulses at three different pulsed electric field (E) strength (100, 200 and 400 V/cm), a fixed pulse width of 100 µs and a repetition time of 10 ms (100 Hz). Afterwards the OD treatment was carried out by immersing the strawberry samples in 40 % (w/w) hypertonic solutions. Two different solutions were prepared, one with sucrose (S) and one with trehalose (T) dissolved in distilled water. Calcium lactate (CaLac) at a concentration of 1 % (w/w) was added to both the solutions as a structuring agent. The samples were analyses at different treatment times: 0, 15, 30, 60 and 120 min (Figure 23).

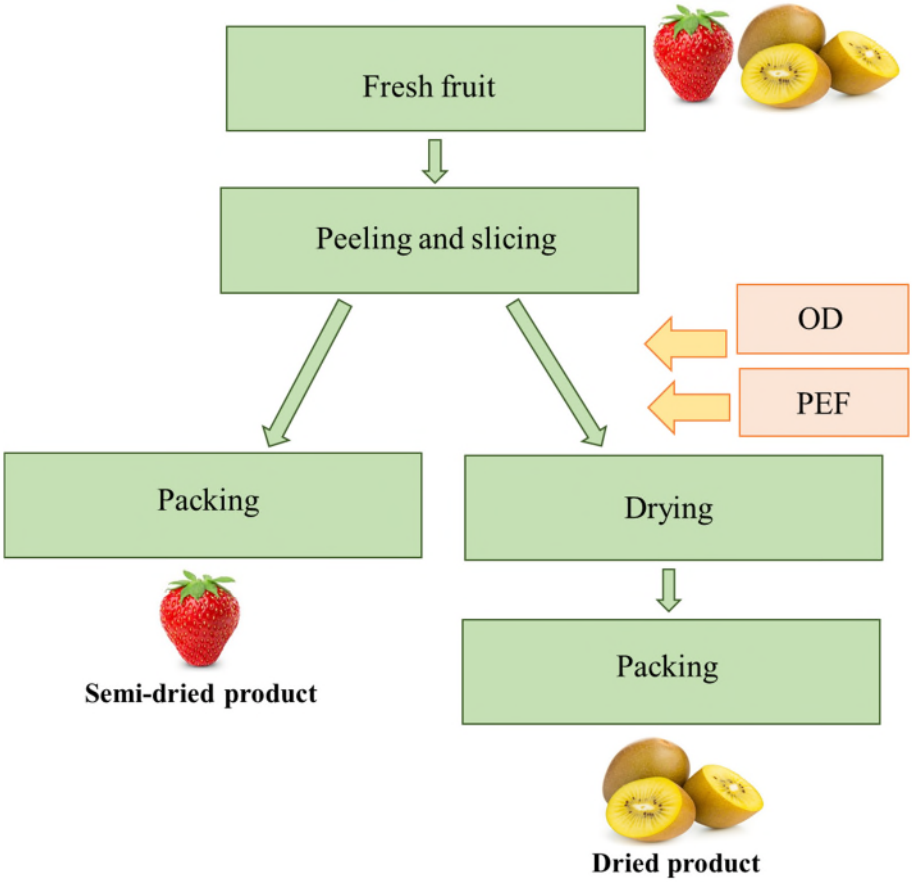


Figure 23. Flow sheet for dehydration of strawberries and drying of kiwifruits.

All obtained samples were summarised with related abbreviations in Table 5.

Table 5. Codification of strawberry samples (corresponding to Table 1 in ***Paper II***).

Sample code	Electric field ($V\ cm^{-1}$)	Type of solution
NoPEF_S	0	Sucrose
PEF_100_S	100	Sucrose
PEF_200_S	200	Sucrose
PEF_400_S	400	Sucrose
NoPEF_T	0	Trehalose
PEF_100_T	100	Trehalose
PEF_200_T	200	Trehalose
PEF_400_T	400	Trehalose

1.2 Drying

Kiwifruit var “Jintao” (13 ± 1 °Brix) were used for the experiment. PEF treatment by using pulse generator S-P7500 60A 8kV (Alintel srl., Bologna) was applied before and/or after OD by using electric field strength of 200 V/cm and 100 near-rectangular shaped pulses, with fixed pulse width of 10 μs and repetition time of 10 ms. Kiwifruits slices (3 mm thick) were subjected to the osmotic dehydration (OD) process (40 % trehalose, 35 °C, 2.5 h). The OD treatment was performed in continuous stirring maintaining a ratio fruit:OD solution of 1:4 in order to avoid concentration changes of the solution during the treatment. The differently treated samples were subjected to air drying at 50, 60 and 70 °C until water activity of 0.2 in a tray drier CLW 750 TOP+ (Pol-Eko-Aparatura SP.J., Poland) with transverse air flow, air velocity 2 m/s, and an air renewal fee of 50 % were obtained (Figure 23).

Table 6 reports the applied treatments and the codification of the samples obtained at each drying temperature.

Table 6. Overview on treatment applied for kiwifruit slices at each drying temperature (50, 60 and 70 °C).

Treatment	Sample
Untreated	Control
OD	OD
PEF	PEF
OD + PEF	OD/PEF
PEF + OD	PEF/OD
PEF + OD + PEF	PEF/OD/PEF

1.3 Extraction

Fresh commercial carrots and apples were purchased from the local market. The fruit and vegetable matrices were washed and pre-cut. The mash was prepared using a mill (Alexanderwerk, Austria) (2 mm for carrot and 5 mm for apples) (***Papers V*** and ***VI***).

For PEF treatment of apple and carrot mash, a batch PEF system (DIL, Germany) equipped with a parallel plate electrode treatment chamber (distance 5 cm) was used. The voltage was set to 4 kV in order to achieve an electric field strength of 0.8 kV/cm in the treatment chamber. A number of 50 exponential decay pulses (discharge capacity 0,5 μF, pulse energy 4 J) with a pulse width of 10 μs was applied to 400 g of mash resulting in a total treatment time of 0.5 ms and a total specific energy input of 0.5 kJ/kg. Different initial pre-heating temperatures (40, 60 and 80 °C) were used before

applying the PEF. In order to provide a fast pre-heating of the mash, a microwave (MT 267, Whirlpool) with a power of 850 W was used. Depending on initial and final mash temperature and based on an average heating rate of 0.2 ± 0.03 K/s, heating times were between 90 – 360 s (Figure 24). After the different pre-treatments, the mash was collected and cooled to room temperature before pressing at 10 bar for 4.45 min using a manual laboratory juice press (Hafico, Germany) with textile cloth.

In Table 7 the applied treatments with the resulting mash temperatures and related samples are reported.

2. Ohmic heating (OH)

For the OH treatment, the treatment chamber with parallel plate electrode (distance 5 cm) was used and connected to a generator (DIL, Germany) providing 572 V at 12 kHz resulting in an electric field strength of 114 V/cm. The resulting electrical current flow reached 1.1 A and therefore a power of 0.6 kW. Different temperature-time profiles were obtained depending on the selected temperatures for the different treated mashes. Ohmic heating was performed in order to reach mash temperatures of 40, 60 and 80 °C. Depending on initial and final mash temperature and based on an average heating rate of 3.7 ± 0.2 K/s, heating times were between 74 - 237 s. Different initial pre-heating temperatures were used before applying the OH (40, 60 and 80 °C) by using a microwave (MT 267, Whirlpool) with a power of 850 W, depending on initial and final mash temperature and based on an average heating rate of 0.2 ± 0.03 K/s, heating times were between 90 – 360 s (Figure 24).

In Table 7 the applied treatments with the resulting mash temperatures and related samples are reported.

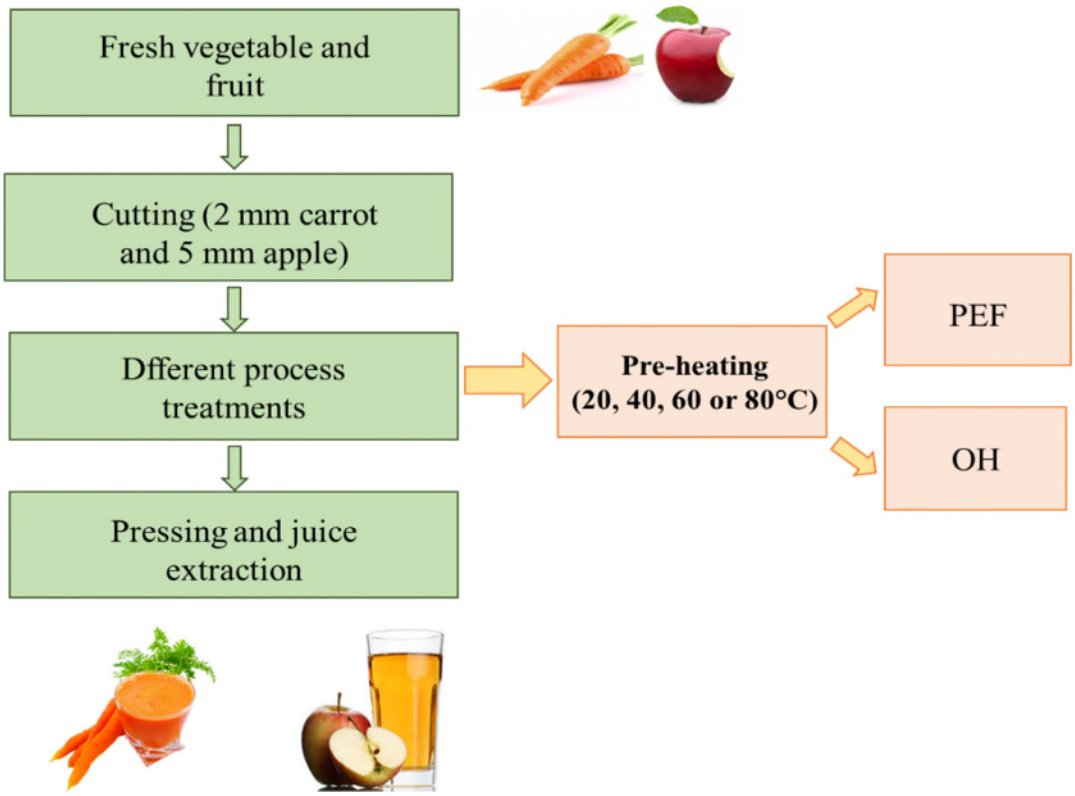


Figure 24. Flow sheet of carrot and apple juice production.

Table 7. Overview on mash treatment (PEF, OH) conditions applied for apple and carrot mash (corresponding to Table 1 in ***Paper V and VI***).

Treatment	Sample	$W_{specific}$ [kJ/kg]
Untreated	Control	0
PEF at 20 °C	PEF (20 °C)	0.5
Pre-heating 40 °C + PEF	40 °C-PEF	192.5
Pre-heating 60 °C + PEF	60 °C-PEF	382.5
Pre-heating 80 °C + PEF	80 °C-PEF	765.5
OH from 20 °C to 40 °C	(20 °C-40 °C) OH	110
OH from 20 °C to 60 °C	(20 °C-60 °C) OH	222
OH from 20 °C to 80 °C	(20 °C-80 °C) OH	355
Pre-heating 40 °C + OH to 80 °C	40 °C-80 °C OH	402.5
Pre-heating 60 °C + OH to 80 °C	60 °C- 80 °C OH	497.5
Pre-heating 40 °C	40 °C	192
Pre-heating 60 °C	60 °C	382
Pre-heating 80 °C	80 °C	765

3. High pressure homogenization (HPH)

Ortanique fruits, a hybrid of tangerine and sweet orange (*Citrus sinensis x Citrus reticulata*) were used for the first case of study (***Paper I***).

The preparation of the juices was carried out according to the patent WO/2007/042593 titled ‘‘Method of obtaining refrigerated pasteurized citrus juices” (Izquierdo et al., 2007).

Raw juice was centrifuged at 3645 g during 5 min at 4 °C (Beckman Coulter AvantiTM J-25, Milan, Italy), homogenized with a Panda Plus pilot homogenizer (Niro Soavi, Parma, Italy) at 20 and 100 MPa. The HPH treated, control and pasteurized at 63 °C for 15 s (Roboqbo, Bologna, Italy) juices were collected in sterile jars and quickly frozen at -18 °C until they were analyzed. In juice samples with trehalose, an amount of 10 and 30 % (w/w) was added before homogenization (Figure 25).

Organic kiwifruits (*Actinidia deliciosa* cultivar ‘‘Hayward’’) (13± 1°Brix) were used in the second case of study (***Paper VII***). Three different UHPH treatments at 0.1 MPa (used as control), 200 MPa for 2 cycles and 200 MPa for 3 cycles were applied by using a Panda Plus pilot homogenizer (Niro Soavi, Parma, Italy).

The inlet temperature of the juice samples was about 4 °C and the increase rate of temperature was about 2 °C every 10 MPa. The untreated and treated kiwifruit samples were collected in 250 ml sterilized bottles, stored at 5, 15 and 25 °C and analysed over time (Figure 25).

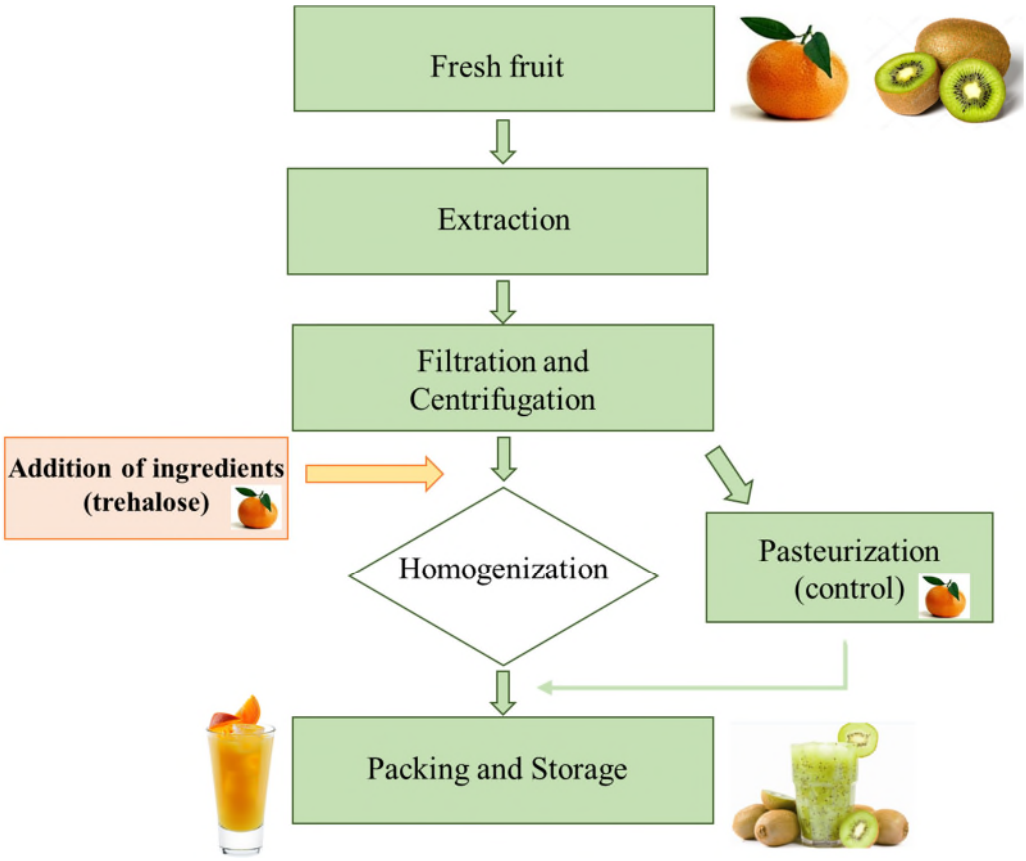


Figure 25. Flow sheet of mandarin and kiwifruit juice production.

4. Edible coatings

Organic blueberries were purchased from local market and were characterized by dry matter of 15.1 ± 0.3 g/100g. Fresh blueberries with the same colour and size and no damages were selected for the experiments (***Papers III, IV***).

Three different coating solutions were prepared, each of them contained 1.5 % (w/w) of glycerol (≥ 99.5 % Sigma-Aldrich, St. Louis, MO USA), 0.20 % (w/w) of Tween® 20 (Sigma-Aldrich, St. Louis, MO USA) and solved in distilled water. In a first solution, sodium alginate (2 % w/w) (Sigma-Aldrich, St. Louis, MO USA) (Al), in the second one pectin from citrus peel (2 % w/w) (Galacturonic acid ≥ 74.0% Sigma, St. Louis, MO USA) (Pe) and in the third one the combination of both (1 +1 % w/w Al+Pe) were added.

In the second part of the work (***Paper IV***) different polysaccharides were used for edible coating solution; one solution contained chitosan from mushroom provided by Agrovin (Alcazar de San Juan, Spain) (1 % w/w) (C) and the second one prepared with chitosan from mushroom (1 % w/w) and procyanidins from grape seeds (Chardonnay berries) (0.8 % w/w) (CP). In both cases of study blueberry dipped in distilled water were used as control (F). Afterwards, all coating solutions were homogenised at 5000 rpm for 2 min in order to remove air bubbles.

In both experiments, all blueberry fruits were dipped in two different steps (each one of 30 s) and were drained at 25 ± 1 °C for 30 min following the first step of dipping, and for 60 min following the second step of dipping. Then they were placed in plastic trays (PET), closed with micro-perforated bags (PLA) and stored at 4 °C for 14 days (Figure 26).

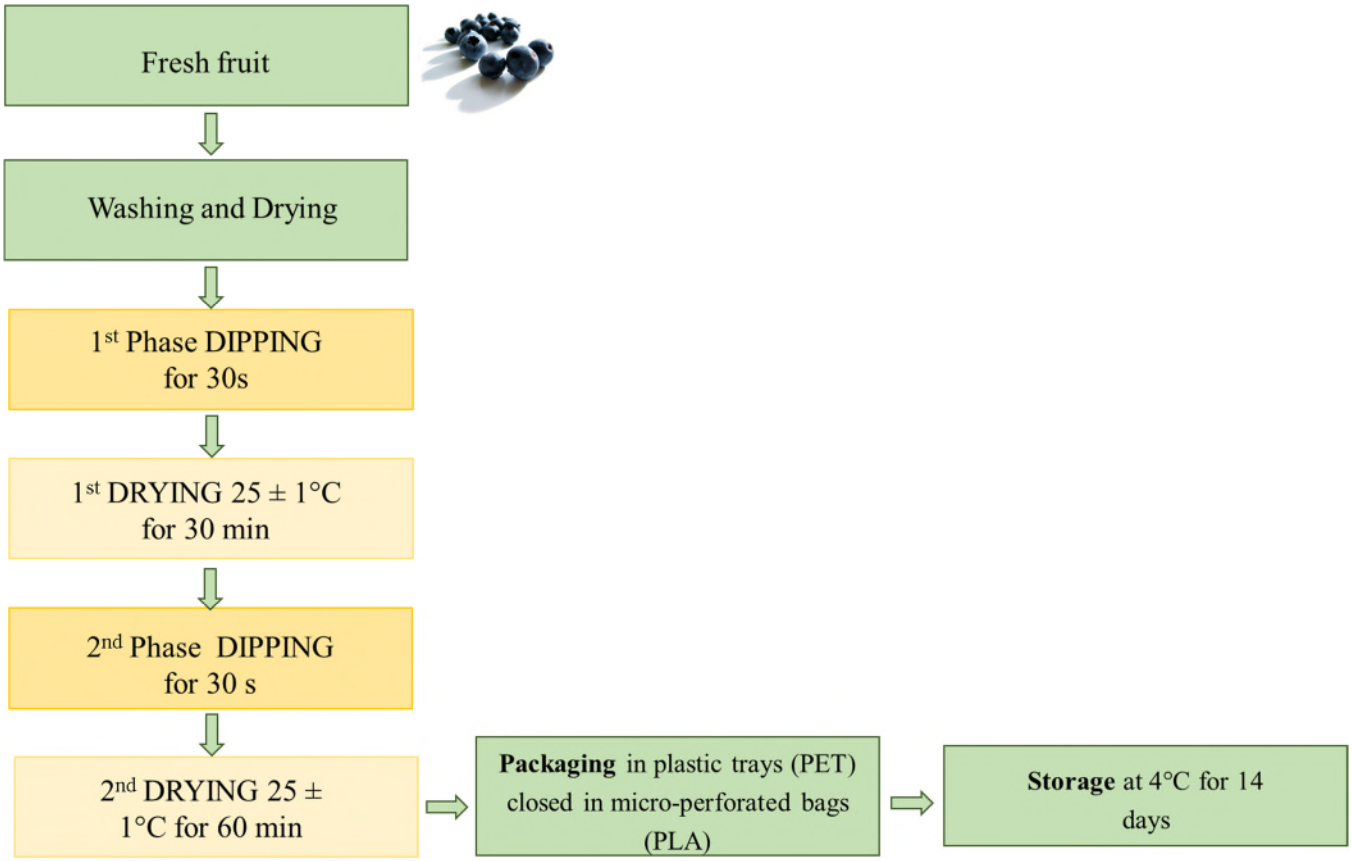


Figure 26. Scheme of the different steps of coating application on fresh blueberries.

5. Analytical determinations

In this PhD thesis, different innovative technologies for the processing of the different vegetable matrixes have been applied in order to maintain and / or increase quality and stability of the obtained products.

The evaluation of qualitative, nutritional and functional characteristics of differently obtained products have been assessed after the processing (OD, PEF, OH and HPH) and application of innovative packaging solutions (edible coatings), as summarized respectively in Figures 27a, 27b and 28.

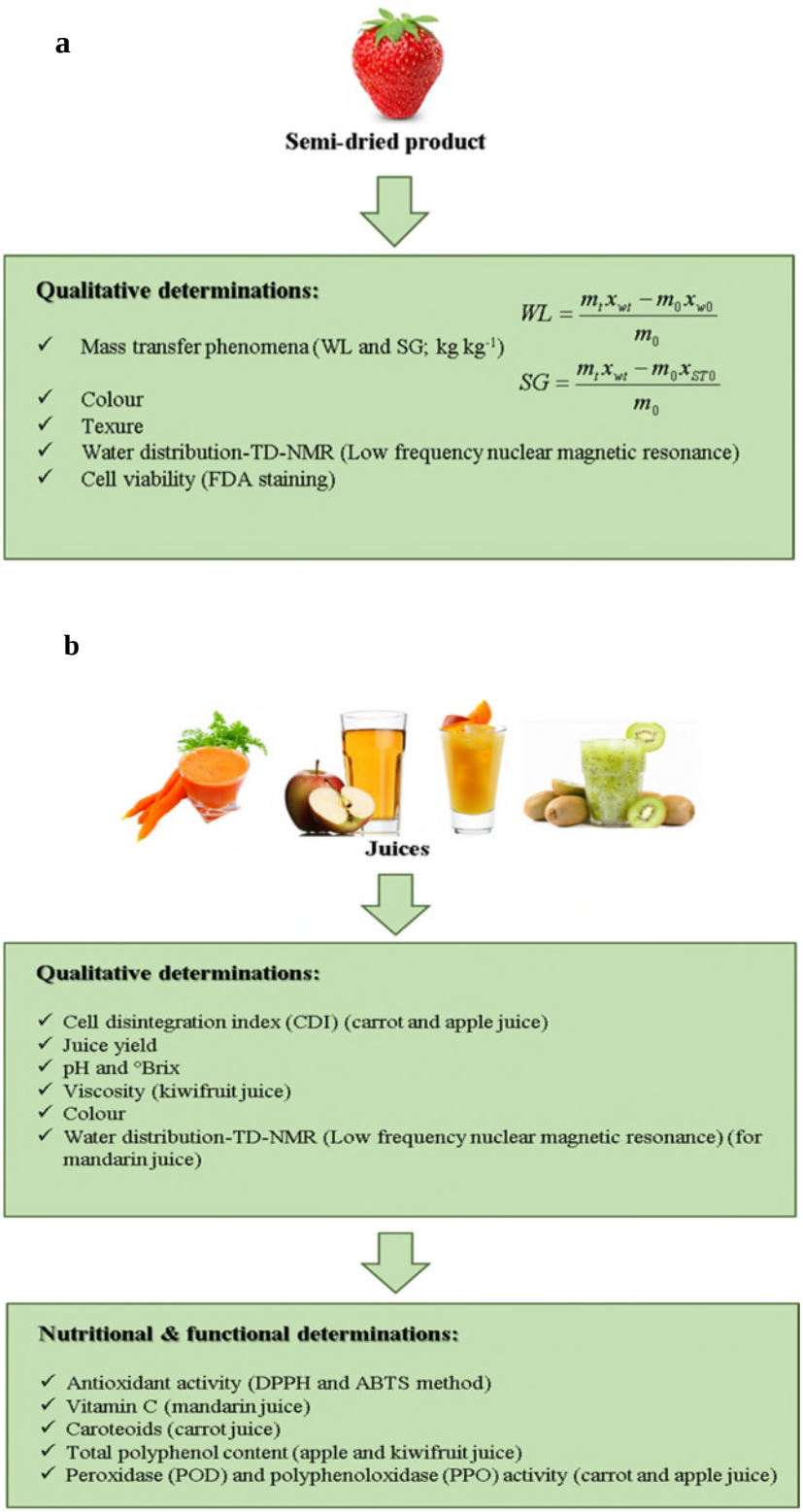


Figure 27. Summary of the analytical determinations carried out on strawberry slices (a) and carrot, apple, mandarin and kiwifruit juices (b).

Concerning the kiwifruit slices subjected to OD, PEF and drying processes the methodologies used for analytical determination of antioxidant activity and texture are reported in ***Paper IV***. The vitamin C content was carried out by a redox titration using iodine solution (0.005 mol/L). As the iodine was added during the titration, the vitamin C (ascorbic acid) was oxidized to dehydroascorbic acid, while the iodine is reduced to iodine ions. Due to that, when all the ascorbic acid has been oxidized, the excess iodine was free to react with the starch indicator by forming the blue starch-iodine complex that represents the endpoint of the titration (Outreach, New Zealand).

Vitamin C content on the air-dried kiwifruit slices was expressed as mg of ascorbic acid per 100 g of dry matter. The values provided are the average of three replicates from each sample at each drying temperature.

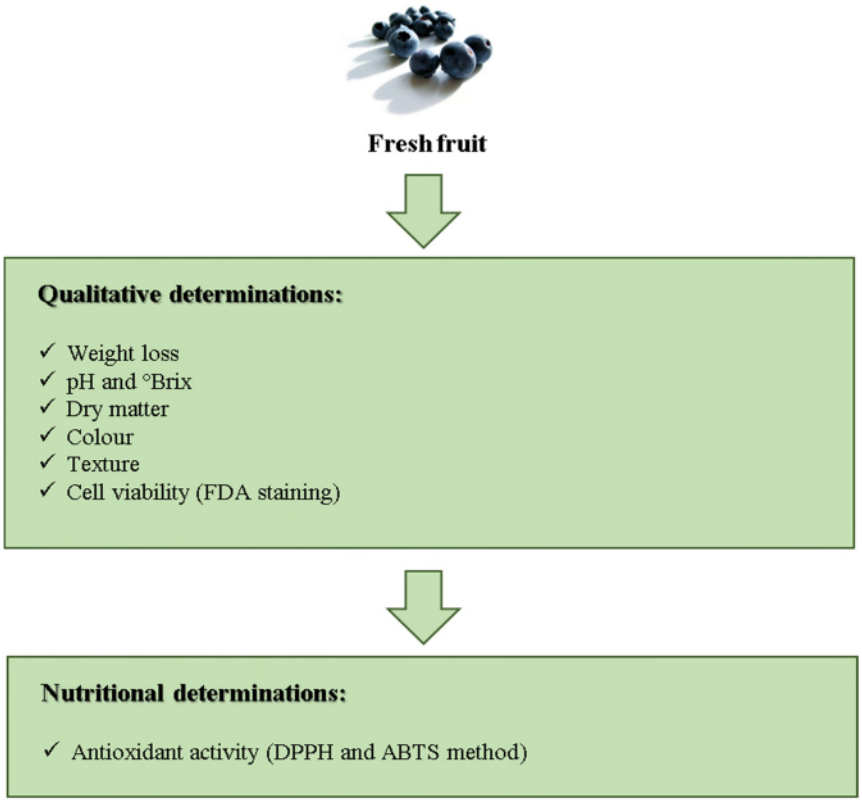


Figure 28. Scheme of analytical determinations of uncoated and coated fresh blueberries.

All methods were reported in detail in the list of papers section (***Paper I*** - ***VII***).

V. Conclusions

Based on the overall results obtained in the present PhD research work, it can be concluded:

- the application of PEF treatment at low field strength and the use of trehalose for the dehydration process seems to be an optimal combination for obtaining a semi-dried strawberry product with quality characteristics similar to the fresh one.
- For what concern the extraction process, PEF lead to an increase of the carrot and apple juice yield. It was demonstrated also that a thermal treatment followed by PEF or conducted by OH can still improve the cell disintegration and juice yield; moreover, the application of high temperature may contribute to the faster inactivation of enzymes, reflected by improved colour values, leading to the release of polyphenols. However, an increase in temperature can promote higher degradation of sensitive bioactive compounds such as carotenoids, which may be triggered due to high temperatures and the presence of oxygen. Hence, raw material dependent processes need to be designed taking into account the textural properties and the degree of cell disintegration for the release of juice and selected compounds.
- The application of HPH has a positive effect on maintaining the functional and technological properties of mandarin and kiwifruit juices. Moreover, the HPH contributes to guarantee the protecting effect of trehalose on mandarin juice structure during storage and to obtain a stable kiwifruit juice for more than 40 days under refrigerated storage by increasing its shelf-life. However, this technology could be further implemented by adopting higher pressure (up to 400 MPa) and aseptic packaging in order to achieve shelf-stable juice products.
- For what concern the innovative packaging solutions, the use of different kind of edible coatings showed positive effect on maintaining and/or increasing firmness, colour retention and antioxidant activity. Results from this study suggest the possibility of using innovative edible coatings to develop high quality ready-to-eat fresh blueberries without compromising their shelf-life, or even increasing it.

The exploitation of the different innovative processing technologies contribute to improve the quality of different processed plant product; in addition, the stability of fresh fruit could be extended by the application of innovative coating formulations. However, in the future, further studies could be performed in order to investigate and understand possible positive effects of combining different emerging technologies.

The results of this PhD research work can greatly contribute to obtain high quality plant processed products in an efficient and environmentally sustainable way, thus determining a benefit not only to the scientific community but also to food companies and consumers.

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Betoret E., **Mannozzi C.**, Dellarosa N., Laghi L., Rocculi P., Dalla Rosa M. (2017)

Metabolomic studies after high pressure homogenization processed low pulp mandarin juice with trehalose addition. Functional and technological properties.

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Abstract

This work aimed to determine the effect of homogenization pressures (HPH) and addition of trehalose on the functional and technological properties of low pulp mandarin juice (LPJ). A set of experiments was designed, combining a non-targeted metabolomic ^{1}H-NMR based approach together with suspended pulp and transmittance, hesperidin, vitamin C and antioxidant activity analysis. Suspended pulp increased with HPH and trehalose addition. Flavonoid hesperidin initially decreased with HPH but trehalose addition resulted in less flavonoid degradation during storage, increasing the effect with the HPH. Vitamin C was not affected by trehalose and pressure treatment but more Vitamin C degradation was observed in trehalose samples during storage. Antiradical activity improvement by trehalose was conditioned by homogenization pressures and specific bioactive compounds. ^{1}H-NMR based approach highlighted the HPH effect on the microbiological aspects of low pulp mandarin juice by the identification of key molecules responsible of the microorganism profile evolution during storage.

Key words: high pressures homogenization, trehalose, vitamin C, flavonoids, NMR.

1. Introduction

High pressure homogenization (HPH) process is a non-thermal technology applied in the food industry, mainly used to disrupt pathogens and spoilage microorganisms, inactivate enzymes and improve the nutritional and technological quality of food products. HPH has been demonstrated, in comparison with other technologies such as thermal treatments, to be less destructive of food compounds when related to sensory and nutritional properties. HPH can be used in the citrus industry for increasing the yield of citrus juices (Lortkipanidze *et al*., 1972) and for improving quality factors such as viscosity (Crandall & Davis, 1991; Patrignani *et al*., 2009), shelf-life (Maresca *et al*., 2011) and colour (Lee & Coates, 2004). The application of HPH to mandarin juices has been demonstrated to increase the stability of suspension and therefore improve the availability of bioactive compounds with antioxidant activity (Betoret *et al*., 2012). However, the degradation of those compounds during processing and storage is important. Previous studies have demonstrated that management of processing technologies can have influence on the functional properties of the final products obtained (Betoret *et al*., 2015; Barba *et al*., 2015a; Barba *et al*., 2015b; Zinoviadou *et al*., 2015; Barba *et al*., 2012). The addition of ingredients able to interact with food matrix can have a significant influence on bioactive compounds activity, degradation or release. Trehalose is a disaccharide able to maintain and preserve a wide group of biologically active molecules. This effect is due to the establishment of interactions that can contribute to the formation of a barrier able to maintain the integrity of the cellular structures and to prevent the decay during processing operations and/or storage (Colaço & Roser, 1995).

Juices are complex mixtures of macro- and micro- components. In most cases, the process treatment can modulate the entire molecular profile of the juices, beyond the few molecules at the center of attention, with possible unexpected consequences on the overall quality and acceptance. This is particularly important when the studied treatment is known to influence simultaneously several quality aspects, such as microbial spoilage, enzymatic activity or bioactivity. When possible unknown consequences of a treatment are looked for, a non-targeted screening exploration, analysing tens of compounds simultaneously, is highly desirable. In this respect, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy has recently gained interest in food and nutritional sectors, due to its ability to give intrinsically quantitative information about the metabolic profile of foodstuff. Being non-destructive and highly reproducible over a wide range of metabolites concentration, ^{1}H-NMR is able to analyze hundreds of compounds simultaneously within minutes and with minimal sample preparation (Laghi *et al*., 2014).

The aim of this work is to study the effect of high pressure homogenization (20 and 100 MPa) and trehalose (10 and 30 %) addition, on technological and functional properties of Ortanique citrus fruit low pulp juices (LPJ). With the goal of obtaining a combination of information both on aspects of known interest and on the overall molecular profile of juices, a set of experiments was designed, combining a non-targeted metabolomic investigation based ^{1}H-NMR together with suspended pulp and transmittance, hesperidin, vitamin C and antioxidant activity analysis.

2. Material and methods

2.1.Sample preparation and processing.

Ortanique fruit, a hybrid of tangerine and sweet orange (*Citrus sinensis* x *Citrus reticulata*) was provided by Rural S. Vicent Ferrer cooperative located in Benaguacil (Valencia), Spain. The preparation of the juices was carried out according to the patent WO/2007/042593 titled ‘‘Method of obtaining refrigerated pasteurized citrus juices” (Izquierdo *et al*., 2007). The fruits were washed by immersing them in tap water, drained, and squeezed in an extractor (“GAM” MOD.SPA 1400 rpm, Cesena, Italy). Raw juice was centrifuged at 3645 g during 5 min at 4 °C (Beckman Coulter AvantiTM J-25, Milan, Italy), homogenized with a Panda Plus pilot homogenizer (Niro Soavi, Parma, Italy) 20 and 100 MPa and no homogenized, pasteurized at 63 °C for 15 s (Roboqbo, Bologna, Italy), collected in sterile jars, and quickly frozen at -18 °C until analyzed. In juice samples with trehalose, an amount of 10 and 30 % (w/w) was added before homogenization.

2.2.Physicochemical characterization

Total soluble solids were measured as Brix with a digital refractometer (Pal-1; Atago Co., Ltd., Tokyo, Japan). Total titratable acidity was assessed by titration with 0.1 N NaOH and expressed as the percentage of citric acid. pH was measured with a potentiometer (micropH Crison GLP21). The values provided are the average of three replicates.

2.3.Suspended pulp and transmittance.

Suspended pulp was evaluated reading the separated pulp (%) by centrifugation at 3500 g during 10 minutes at 27 ºC (FMC FoodTech, 2005). The supernatant was collected and evaluated its transmittance at 650 nm in spectrophotometer (Shimadzu UV-1601). The values provided are the average of six replicates.

2.4.Flavonoid hesperidin.

The content of the main flavonoid hesperidin was determined using HPLC LC-1500 (Jasco, Carpi, MO, Italy) with a diode array detector (DAD) and filled with a C18 reversed-phase column (150 x 4.60 mm, Phenomenex Kinetex® 5U C18 100°) following the method described in Betoret *et al*., 2009. The juice samples were measured after 0, 3 and 10 days of storage. The values provided are the average of three replicates.

2.5.Vitamin C

Vitamin C content was measured by HPLC LC-1500 (Jasco, Carpi, MO, Italy) equipped with thermostat autosampler and diode array detector (DAD).

Fresh juice samples were centrifuged at 15000 g (4 °C, 5 min) and aliquots (1 mL) of supernatant were filtered with nylon filter 0.45 μm and then 10 μL were injected into the HPLC C18 reverse phase column (150 x 4.60 mm, Phenomenex Kinetex® 5U C18 100°). System conditions were established according to Odriozola-Serrano *et al*., (2007).

The juice samples were measured after 0, 3 and 10 days of storage. The values provided are the average of three replicates.

2.6.Antiradical activity

The antiradical activity was determined by ABTS and DPPH tests. The ABTS test was based on the method proposed by Polydera *et al*., (2005). A volume of 15.3 μL juice was added to ABTS solution. The absorbance was measured with a spectrophotometer Beckman Coulter DU 730 Life Science model every 30 s for a total time of 30 min. The results were expressed as TEAC (Trolox Equivalent Antiradical Capacity). The values provided are the average of twelve replicates. The DPPH test was based on the method proposed by Brand-Williams *et al*., (1995). A volume of 30 μL of juice was added to DPPH solution. The absorbance was measured with a spectrophotometer (Beckman Coulter model DU 730 Life Science) at 515 nm every 2 min for a total time of 70 min. The results were expressed as $mmol \cdot L^{-1}$ equivalents of ascorbic acid. The values provided are the average of twelve replicates.

2.7. Untargeted metabolomics approach

Samples were prepared for analysis, and ^{1}H-NMR spectra were registered and processed, according to Dellarosa *et al*., 2016. Spectra were manually integrated giving rise to 89 protons signals in the typical regions of sugars, amino acids, organic acids, alcohols, polyphenols and nucleotides.

At least five replicates were analysed for each sample group. The obtained 102 × 89 (samples × signals) matrix, scaled and centred, underwent signals assignments and multivariate analysis.

NMR signals assignment was performed by comparison with works performed on similar food matrices at comparable pH (Capitani *et al*., 2012; de Oliveira *et al*., 2014; Le Gall *et al*., 2001; Spraul *et al*., 2009), assignment through Chenomx software (Chenomx, Alberta, CA) and comparison with HMDB and Madison public databases. In case of unresolved ambiguity, suitable 2D experiments were performed.

To study the changes occurring during the storage period and upon the tested treatments, sparse Partial Least Square Regression (sPLSR) (Lê Cao *et al*., 2008) and its discriminant analysis counterpart (sPLSDA) (Lê Cao *et al*., 2011), were performed, as implemented in mixOmics package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Train and test sets accounted for 70% and 30 % of the samples respectively. The sPLSR and sPLSDA models were trained by 10-fold validation based on minimal root mean square error (RMSEP) and error rate, respectively. The maximum parsimony of the models was looked for by building and testing 1000 models, and by retaining only the molecules with average VIP value (Variable Importance in Projection) (Eriksson *et al*., 2001) above one and accepted by sparsity algorithm more than 500 times. The key metabolites arisen from the sPLS models were employed for linear regression and linear discriminant analysis (Ripley, 1996), in order to describe changes during storage and upon HPH treatments. This approach led to models where single coefficients were needed to correlate metabolite concentration with each response. Such choice combined user-friendliness to high accuracy and precision.

2.8.Statistical analysis

In order to evaluate whether the average values were significantly different a multi factorial ANOVA and Tukey's multiple comparisons, with 95 % confidence level, were performed in R statistical software (R Foundation for Statistical Computing, Austria). All the experiments were repeated at least three times and results were expressed as mean ± standard deviation of replications.

3. Results and discussion

3.1. Physicochemical characterization of LPJ and evaluation of suspended pulp and cloudiness.

Fresh LPJ samples were characterized by measuring the soluble solids content (13.40 ± 0.02 $g_{soluble\ solids}/g_{liquid\ phase}$), acidity (2.35 ± 0.02 $mg_{citric\ acid}/100g_{juice}$), maturity index (5.7 ± 0.3) and pH (2.83 ± 0.06).

Suspended pulp and supernatant transmittance at 3500 g of all samples homogenized at 20 and 100 MPa and no homogenized with trehalose addition in proportion 0, 10 and 30 (%) (w/w) were determined. The results are shown in table 1. An analysis of variance showed, with a confidence level of 95 %, than both variables pressure homogenization and trehalose addition, as well as their interaction, have a significant effect on suspended pulp and transmittance.

Homogenization is a unit operation that involves pressure application to liquids to fragment the solid particles and oil droplets into smaller particles. Orange cloud particles ranges in size from 400 to 5000 nm are more stable than those smaller than 2000 nm (Buslig & Carter, 1974). As expected, there is a tendency to decrease the suspended pulp with homogenization pressures applied (table 1). The homogenization pressures decrease the particle size of the LPJ cloud making the juice suspension more stable. In the same way, low values of suspended pulp result in low levels of transmittance and high levels of cloudiness.

In the industrial juices processing, trehalose addition varies between 0.4 % of final product to 50 % of sugar replacement. It has been used traditionally in order to improve the aromatic profile, colour, reduce sweetness and stabilize pH in processed juices (Richards & Dexter, 2011). On an equal level of pressure applied, the addition of trehalose results in a decrease of separated pulp and transmittance values (table 1).

Table 1. Suspended pulp and turbidity determination. Separated pulp at 3500 g and transmittance of the supernatant. Values expressed as mean ± standard deviation. The values provided are the average of six replicates.

Homogenization pressure (MPa)	Trehalose (%)	Separated Pulp (%)	Transmittance (%)
0	**0**	9.03 ± 0.02^{a}	22.3 ± 0.8^{a}
0	**10**	5.30 ± 0.05^{d}	19.2 ± 2.6^{b}
0	**30**	2.00 ± 0.05^{f}	14.8 ± 1.6^{c}
20	**0**	8.50 ± 0.02^{b}	7.5 ± 1.5^{e}
20	**10**	5.110 ± 0.012^{e}	7.3 ± 2.2^{e}
20	**30**	1.00 ± 0.03^{g}	4.7 ± 3.3^{f}
100	**0**	7.10 ± 0.02^{c}	8.3 ± 0.9^{d}
100	**10**	5.22 ± 0.03^{e}	8.20 ± 1.02^{d}
100	**30**	1.000 ± 0.012^{g}	4.3 ± 1.6^{f}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

LPJ cloud is formed by different particles types such as cellular organelles and membranes, oil droplets, flavonoids and cell wall fragments such as pectin, cellulose and hemicellulose (Baker & Cameron, 1999). Trehalose is a disaccharide able to interact with various compounds, forming a glassy amorphous matrix around the tertiary structure of the proteins and phospholipids exerting a protective effect on various technological processes (Colaço & Roser, 1994; Crowe *et al.*, 1990; Rudolph & Crowe, 1985). Trehalose could interact with LPJ cloud compounds stabilizing the suspension and maintaining the juice cloudiness. These interactions could be promoted by homogenization pressures as a result of smaller particle size after treatment.

3.2 Functional compounds determination

The main mandarin juice flavonoid hesperidin was determined by HPLC in LPJ samples homogenized at 20 and 100 MPa and no homogenized with trehalose addition in a proportion 0, 10 and 30 % (w/w) after 0, 3 and 10 storage days. The results obtained are shown in table 2.

Table 2. Hesperidin content (mg/L) in LPJ samples during 0, 3 and 10 days of storage and percentage of degradation calculated from 10 and 0 days of storage. Values expressed as mean ± standard deviation. The values provided are the average of three replicates.

Homogenization pressure (MPa)	Trehalose (%)	0 days	3 days	10 days	Degradation (%)
0	**0**	138.1 ± 0.8^{a}	132.2 ± 0.4^{a}	51.51 ± 17.07^{e}	62.7 ± 12.6
0	**10**	133.5 ± 3.9^{b}	143.4 ± 1.6^{a}	74.7 ± 0.5^{bc}	44 ± 2
0	**30**	145.7 ± 3.2^{a}	145.8 ± 3.7^{a}	76.7 ± 3.8^{bc}	47.3 ± 3.7
20	**0**	88.8 ± 0.4^{d}	83.6 ± 1.9^{be}	72.7 ± 2.2^{c}	18.2 ± 2.9
20	**10**	97.2 ± 0.6^{d}	83.4 ± 2.9^{bf}	84.1 ± 2.2^{ab}	13.5 ± 2.8
20	**30**	106.3 ± 9.6^{c}	93.4 ± 1.7^{bc}	94.7 ± 3.8^{a}	10.7 ± 4.5
100	**0**	69.1 ± 1.9^{f}	79.3 ± 18.5^{cdef}	68.01 ± 1.13^{d}	1.5 ± 4.4
100	**10**	72.1 ± 0.3^{ef}	85.8 ± 0.2^{bd}	69.1 ± 2.9^{d}	4.1 ± 4.6
100	**30**	79.430 ± 3.108^{e}	99.0 ± 7.7^{b}	74.9 ± 1.2^{bc}	5.66 ± 2.13

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

In general terms, with a 95 % confidence level, both homogenization pressures and trehalose addition have a significant effect on hesperidin content during storage. The application of homogenization pressures results in a flavonoid content decrease that it is bigger when higher pressures are applied. These results are different to those obtained by Betoret *et al*., 2012 in which the application of 20 MPa pressures resulted in maintaining and even increased flavonoids content.

The biosynthesis of flavonoid depends on genetic, environmental factors (Bae *et al*., 2014) and shows different peaks during fruits development which generally are owed to the formation of protective compounds in early stages on the one side, and the formation of optical signals at the end of fruit ripening on the other side (Griesser *et al*., 2008; Halbwirth *et al*., 2006). Flavonoid content decreases with more advanced ripening stages explaining the differences observed in the flavonoid content determined in Betoret *et al*., 2012. Maturity index together with the forces and temperature stresses created in the homogenization valve as well as the low pulp juice content could lead a degradation of flavonoid during homogenization in this case.

In table 2, it is possible to see a tendency to increase flavonoid content on third storage day, being bigger in those samples homogenized at 100 MPa with trehalose content. This effect could be related to a physical effect on previous flavonoid extraction procedure. At 100 MPa greater particle size reduction could result on bigger interaction capacity between particles, more stable LPJ cloud and therefore difficult flavonoid extraction. On third storage day, the interaction between particles could have been weakened facilitating flavonoid extraction. Food matrix has an important influence on

bioactive compounds, the structure changes caused by processing or by storage, in those cases in which degradation of bioactive compounds has not been occurred yet, can facilitate the extraction of bioactive compounds (Betoret et al., 2015).

To evaluate the effect of storage together with homogenization pressures and trehalose addition, the degradation percentage was calculated. The obtained results are shown in table 2. Generally, an increase in homogenization pressures results in a decrease of bioactive compounds degradation during storage. Trehalose addition also results in less flavonoid degradation, increasing the effect with the homogenization pressures. This effect could be related with cloudiness stability and trehalose capacity interacting and forming complexes with bioactive compounds. Smaller particles are able to interact better with LPJ cloud and thus are less available for degradative reactions.

Vitamin C content was determined by HPLC in LPJ samples homogenized at 20 and 100 MPa and no homogenized with trehalose addition in a proportion 0, 10 and 30 % (w/w) after 0, 3 and 10 storage days. Vitamin C is an important bioactive compound very well known for its beneficial effects but characterized by its easy degradation and low stability. In all analyzed samples, during all storage time, vitamin C content was in range 110-140 mg/L (table 3).

Table 3. Vitamin C content (mg/L) in LPJ samples during 0, 3 and 10 days of storage and percentage of degradation calculated from 10 and 0 days of storage. Values expressed as mean ± standard deviation. The values provided are the average of three replicates.

Homogenization pressure (MPa)	**Trehalose (%)**	**0**	**3**	**10**	**Degradation (%)**
0	**0**	129.2 ± 0.7^{b}	135.63 ± 2.09^{c}	119.3 ± 1.4^{b}	7.7 ± 0.6
0	**10**	134.1 ± 1.4^{a}	139.3 ± 1.6^{ab}	119.6 ± 1.4^{b}	10.8 ± 1.3
0	**30**	133.4 ± 1.4^{a}	137.0 ± 1.6^{bc}	123.1 ± 1.4^{a}	7.7 ± 1.5
20	**0**	128.7 ± 0.4^{b}	135.66 ± 0.15^{c}	118.1 ± 0.5^{b}	8.2 ± 0.4
20	**10**	133.9 ± 1.4^{a}	141.5 ± 0.7^{a}	118.4 ± 0.8^{b}	11.5 ± 1.2
20	**30**	131.56 ± 3.03^{ab}	141.59 ± 3.02^{a}	122.1 ± 2.3^{a}	7.2 ± 0.9
100	**0**	128.6 ± 1.6^{b}	134.9 ± 0.3^{c}	117.7 ± 0.7^{b}	8.44 ± 1.06
100	**10**	122.6 ± 2.6^{c}	131.8 ± 0.6^{d}	109.4 ± 0.7^{d}	10.7 ± 1.4
100	**30**	125.4 ± 1.9^{c}	131.5 ± 0.6^{d}	112.2 ± 1.7^{c}	10.4 ± 0.2

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

The statistical analysis showed, with a 95 % confidence level, that both variables pressure and trehalose have a significant effect of vitamin C content and this effect depends on storage time. On day 0, homogenization pressures together with trehalose addition interaction have a significant effect

on vitamin C content. On days 3 and 10, the analysis of variance indicates that both variables as well as their interaction have a significant effect on the content of vitamin C.

It is possible to observe the same tendency behaviour in all analyzed samples, with non-homogenized and 20 MPa trehalose samples slightly higher vitamin C content and 100 MPa trehalose samples slightly lower content than non trehalose samples. As in the case of flavonoid, it is possible to observe a tendency to increase vitamin C content until the third day of storage. Physical effect network created by trehalose and homogenization pressures can decrease until the third day of storage in which is possible to observe the maximum quantity of Vitamin C. As previously said, food matrix has an important influence on bioactive compounds, the structure changes caused by processing or by storage time, in those cases in which degradation of bioactive compounds has not been occurred yet, can facilitate the extraction of bioactive compounds (Betoret et al., 2015). Higher degradation on vitamin C content has been observed in all samples after 10 days of storage. Table 3 shows the degradation percentage of vitamin C during storage. As it is possible to see, addition of trehalose seems to increase vitamin C degradation in 2-4 %.

The protective effect of trehalose on bioactive compounds seems to depend specifically on each compound to be protected. Literature lacks specific studies on the effect of adding sucrose substitutes on the content of polyphenols in fruit products. There are some reports describing the effect of adding sucrose, maltose, fructose and trehalose on bioactive compounds degradation during storage (Kopjar *et al*., 2012; Kopjar *et al*., 2009). Kopjar *et al*., 2008 reported a positive effect on anthocyanins protection with trehalose having the most positive effect of all investigated sugars. In contrast to most other disaccharides, trehalose has no direct internal hydrogen bonds. All four internal bonds are indirectly connected via the two water molecules, which form part of the native dihydrated structure. This arrangement gives the molecule an unusual flexibility around the disaccharide bond, which may allow it to fit more closely with the irregular surface of macromolecules than other, more rigid disaccharides, in which the rings are directly hydrogen bonded to each other (Colaço & Roser, 1995). According to Bordat *et al*., (2004), trehalose has effect on “destructuring” the network of water and on slowing down its dynamics. This property could play a key role in the understanding of the microscopic mechanisms of bioprotection.

3.3 Antiradical determination

The ABTS and DPPH tests were performed on distilled water containing trehalose 10 and 30 % (w/w) and on homogenized LPJ with and without trehalose addition. The results obtained are shown in table 4.

Table 4. Antiradical activity of low pulp juice samples by ABTS and DPPH methods. Results expressed as TEAC and mmol·L^{-1} ascorbic acid respectively. Values expressed as mean ± standard deviation. The values provided are the average of three replicates.

Sample	Homogenization pressure (MPa)	Trehalose (%)	ABTS	DPPH
Water	**0**	**10**	0.0 ± 0.0	0.0 ± 0.0
Water	**0**	**30**	0.040 ± 0.012	0.0 ± 0.0
Low pulp juice	**0**	**0**	0.70 ± 0.06^{d}	1.6 ± 0.2^{bd}
Low pulp juice	**0**	**10**	0.99 ± 0.08^{a}	0.91 ± 0.12^{f}
Low pulp juice	**0**	**30**	0.92 ± 0.08^{b}	0.80 ± 0.06^{f}
Low pulp juice	**20**	**0**	0.88 ± 0.12^{b}	1.7 ± 0.2^{a}
Low pulp juice	**20**	**10**	0.94 ± 0.02^{ab}	1.69 ± 0.02^{ab}
Low pulp juice	**20**	**30**	0.59 ± 0.04^{e}	1.57 ± 0.08^{acd}
Low pulp juice	**100**	**0**	0.81 ± 0.02^{c}	1.59 ± 0.12^{bc}
Low pulp juice	**100**	**10**	0.87 ± 0.02^{bc}	1.42 ± 0.04^{cde}
Low pulp juice	**100**	**30**	0.70 ± 0.07^{d}	1.3 ± 0.2^{e}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Two analytical methods were used to determine the total antiradical activity of LPJ, since both have some limitations (Shui & Peng, 2004; Prior *et al*., 2005). DPPH method seems to be more sensitive to the flavanones while ABTS method seems to be more sensitive to the radical scavengers such as vitamin C (Del Caro *et al*., 2004). Indeed, these two methods represent a useful tool to evaluate the antiradical scavenging activity of different fruits (Gil *et al*., 2000; Shui & Peng, 2004).

The analysis of variance indicated, with a probability of 95 %, that the homogenization pressures, the trehalose addition as well as their interaction have a significant effect on the activity antiradical determined through both ABTS and DPPH methods.

In the ABTS method, considering the samples without trehalose, the increase in the homogenization pressure causes a slight increase in the antiradical activity. This increment is bigger in samples no homogenized and homogenized at 20 MPa that from no homogenized and homogenized

at 100 MPa without significant differences between the two levels of pressure applied. In not homogenized samples, trehalose addition supposes an increasing in antiradical activity that it is maintained without significant differences between 10 and 30 %. However, for homogenized samples, the antiradical activity increases in the case of samples with 10 % of trehalose and decreases for 30 % trehalose samples. Samples with trehalose addition have less quantity of LPJ thus a lower antiradical activity expected. As shown in the water solutions, trehalose alone appears to not have antiradical activity. Nevertheless, it is possible that trehalose could have an interaction effect with LPJ cloud as observed in above turbidity measures and this could have an effect on those bioactive compounds that are more sensitive to ABTS method.

In DPPH method, the application of homogenization pressures increases the antiradical activity more from no homogenized samples to homogenized at 20 MPa than from no homogenized samples to homogenized at 100 MPa but the differences observed are not significant. Nevertheless, in not homogenized samples the addition of trehalose results in a minor antiradical activity. It seems that the decreasing particle size by homogenization operation affects trehalose interaction capacity with LPJ cloud and this has an influence on those compounds more sensitive to DPPH method.

Kopjar *et al.*, (2009, 2012) showed the addition of trehalose and sucrose might improve the antiradical activity of fruits products. In our obtained results, antiradical activity improvement by trehalose is conditioned by homogenization pressures and specific bioactive compounds. Antiradical activity results correspond with those obtained for suspended pulp and functional compounds determinations.

3.4 Evolution of molecular profile with storage and HPH treatment.

^{1}H-NMR was employed as an unbiased screening tool for HPH treatments by following a non-targeted approach (Trimigno *et al.*, 2015). Due to the reduced mobility of the solutes caused by the high viscosity, NMR analysis gave rise, in samples added with trehalose, to signals excessively broad and superimposed. The desired untargeted approach by ^{1}H-NMR was therefore limited to the study of the effects of HPH treatments and storage.

When considering the storage time, VIP and sparsity criteria highlighted in sPLSR models 4 signals with the highest variation along time, at 8.27, 2.55, 0.79 and 0.81 ppm respectively, that were assigned, through literature comparison, database and 2D-NMR experiments, to formic acid, glutamic acid and two phenolic moieties respectively. The consequent dramatic reduction of the information complexity allowed outlining the correlation between storage time and molecular profile based on a linear model, based on the evidenced 4 molecules only (figure 1a).

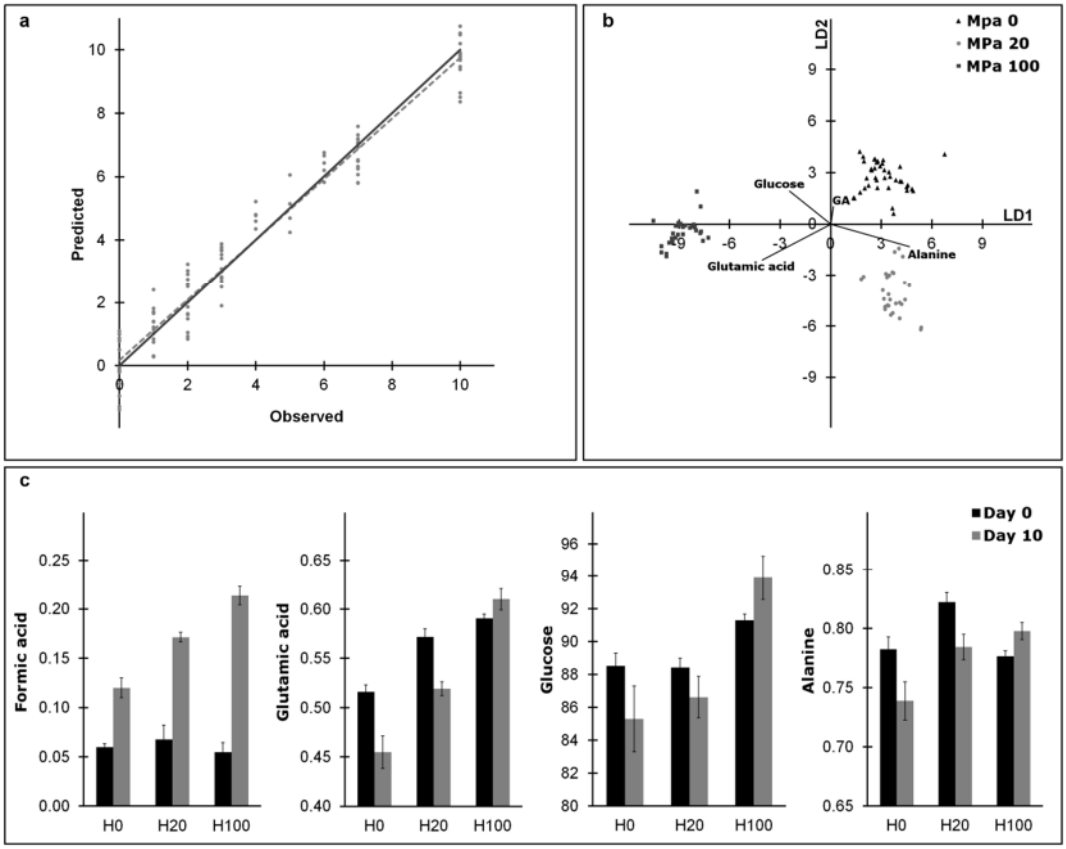

Figure 1. (a) Storage time (days) predicting ability of a linear model based on the concentration of formic acid, glutamic acid and the two phenolic moieties, selected by sPLSR model. **(b)** Consequences of high pressure homogenization treatments (0MPa (H0); 20 MPa (H20); 100 MPa (H100) on the concentration of glucose, glutamic acid, alanine and gallic acid (GA), highlighted by representing the samples treated at 0 (triangles), 20 (circles) and 100 (squares) MPa on the first (LD1) and second (LD2) direction of a linear discriminant model. **(c)** Concentrations (mM), at the beginning (Day 0) and at the end (Day 10) of the storage period, expressed as mean ± standard deviation, of the metabolites mostly influenced by storage time and high pressure homogenization treatment.

Such model was characterized by an uncertainty to 0.7 days, with a coefficient of determination (R2) as high as 0.96.

When considering HPH treatment, there were no biological reasons suggesting a linear relationship between molecular profile and treatment pressures, so that a 3 classes discriminant analysis was setup, by means of sPLSDA algorithm. Among the 89 signals, only four were selected by VIP and sparsity criterion, glucose, gallic acid, alanine and, again, glutamic acid. The 4 molecules were employed to substitute sPLSDA model with its linear counterpart (figure 1b), resulting in an error rate lower than 1%. Interestingly the two dimensions of the linear model ended up representing separately the peculiarities of 100 MPa and of 20 MPa treatments, on LD1 and LD2 respectively.

The molecules showing the greatest variations along storage time and upon high-pressure treatments, highlighted by multivariate analysis, were employed to explore the interactions between the two factors analyzed, a possible key point in order to finely tailor the juice technological treatments. Fig. 1c shows their concentrations at the beginning and the end of the storage period, organized per HPH treatment. Formic acid concentration in samples at day 0 did not show any correlation with HPH, thus highlighting that this treatment had no direct effect on its solubilization. Formic acid concentration, nevertheless, systematically increased with storage time and such increase was now proportional to the applied pressure levels.

Contrary to formic acid, concentrations of glutamic acid and glucose were proportional to the applied pressures at day 0, suggesting a direct effect of HPH on their solubilization. Once more, dissimilarly from formic acid, glutamic acid and glucose, together with alanine, decreased proportionally to storage time in untreated samples and HPH samples at 20 MPa, but increased in HPH samples at 100 MPa.

The overall observation of these trends suggests that HPH solubilization ability and different microbial activities had a joint effect on the concentration of these molecules. The systematic increase of formic acid with storage, proportional to the pressure levels even when 100 MPa were selected, suggests that this molecule was probably the result of microbial activity and that microbes responsible for its production were not significantly hampered by pressure treatments. In this context it is worth underlining that Patrignani *et al*., (2009, 2010) found that a treatment with HPH at 100 MPa inactivates yeasts. Moreover, Maresca *et al*., (2011) showed that a single-pass treatment at 100 MPa led to a significant reduction of yeasts, with no effects on bacteria. Bacteria can be therefore suggested as the main responsible for formic acid production in the observed juices. Glutamic acid and glucose trends show that the concentration of these molecules was reduced by microbes, which growth was hampered by 100 MPa HPH treatments. Following Patrignani and Maresca findings, yeast can therefore be suggested as the main responsible for their concentration changes. Finally, a contribution to the reduced anabolic activity at 100 MPa may be exerted also by formic acid, with known antimicrobial proprieties (Berregi *et al*., 2007).

4. Conclusions

HPH and trehalose addition have a significant effect on functional and technological properties of low pulp mandarin juice. HPH affects the juice cloud structure influencing trehalose interactions. The protecting effect of trehalose during storage it is affected by HPH and it is compound specific. ^{1}H-NMR based approach highlighted the HPH effect on the microbiological aspects of low pulp

mandarin juice by the identification of key molecules responsible of the microorganism profile evolution during storage.

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Effect of pulsed electric field (PEF) pre-treatment coupled with osmotic dehydration on physico-chemical characteristics of organic strawberries

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Effect of pulsed electric field (PEF) pre-treatment coupled with osmotic dehydration on physico-chemical characteristics of organic strawberries

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Abstract

The aim of this work was to evaluate the effect of pulsed electric field (PEF) pre-treatment on mass transfer phenomena, water distribution and some physico-chemical parameters of osmo-dehydrated organic strawberries. For PEF treatments 100 near-rectangular shaped pulses, with fixed pulse width of 100 μs and repetition time of 10 ms were used. Electric fields strength applied were 100, 200 and 400 V cm^{-1}. Afterwards, samples were subjected to OD treatments carried out in two different hypertonic solutions (40% w/w), one with sucrose and the other one with trehalose. The results shown that PEF treatment positively affected the mass transfer during OD even at the lowest electric field strength applied (100 V/cm), partially preserving the cell viability and maintaining at the same time the fresh-like characteristics of strawberries.

Keywords: fruit quality, strawberries, organic, texture, colour, non-thermal treatment

1. Introduction

Increased consumer demand for safety, health and environmental friendly food products make the organic production one of the fastest growing market segments over the last few years. Consumers expect the quality of organic fruits to be higher or at least comparable with the conventionally produced ones, protecting at the same time the nature and reducing the environmental pollution (Barański et al., 2014).

Berries, and in particular strawberries, are very attractive for consumers, because of their unique flavour, texture and red vivid colour, both in a fresh form and in a variety of food products and snacks. They are also highly appreciated by consumers due to their high amount of ascorbic acid and antioxidants (Velickova et al., 2013; Gamboa-Santos et al., 2014). However strawberries are highly susceptible to mechanical injury and also highly perishable (Badawy et al., 2016; Kadivec et al., 2016); these characteristics could be even more pronounced in the organic fruit. Therefore, there is a need to improve the processing of these fruits in order to obtain semi-dried or intermediate moisture products with longer shelf-life. With regards to organic production practices, applied treatments and processes should be aimed at avoiding the chemical additives, while non-thermal processing are used with the aim of maintaining the nutritional and sensorial properties of food products.

Osmotic dehydration (OD) is one of the non-thermal processes used to obtain intermediate moisture products with improved stability over storage. This because, during OD a partial dewatering of plant tissue takes place, reducing both freezable water content and the water activity of the system (Tylewicz et al., 2011; Mauro et al., 2016). The application of OD process on strawberry tissue has been widely studied. Chang et al. (2014) studied the effect of power ultrasound and pulsed vacuum treatments on the dehydration kinetics and the status of water during osmotic dehydration of strawberries, showing that the highest water loss (lower freezable water content) and the highest decrease in firmness occurred using ultrasound treatment, while the highest solid gain and the highest firmness values were achieved by pulsed vacuum treatment. Castelló et al. (2010) observed that OD treatment promoted the structural collapse, however, when calcium was added to the osmotic solution a beneficial effect on the maintenance of the sample texture was observed.

Since OD treatment, especially when applied at room temperature, is a time-requiring process, other pretreatments could be used before OD in order to increase the velocity of mass transfer kinetics.

Pulsed electric filed is a process which promotes the modification of the membrane permeability by application of high voltage short time pulses (Barba et al., 2015). The application of low electric field strength creates pores in the biological membrane which affect the mass transfer in tissues. In fact, several studies of PEF-assisted OD have been carried out on different plant tissues such as apples (Dellarosa et al., 2016a; Dellarosa et al., 2016b; Amami et al., 2006), kiwifruits (Dermesonlouoglou

et al., 2016; Traffano-Schiffo et al., 2016), carrots (Amami et al., 2007), potatoes (Fincan & Dejmek, 2003) etc. While the effect of PEF pre-treatment on enhancing the water loss of OD treated tissues seems to be clearly and well stated, its effect on the solid gain is ambiguous. In fact, some authors reported an increase in solid uptake, for example in mango pieces (Taiwo et al., 2002) and apples (Amani et al., 2006; Dellarosa et al., 2016a), while in PEF pre-treated kiwifruit samples the solutes uptake was lower compared to untreated ones (Traffano-Schiffo et al., 2016). The impact of high-intensity electric field pulses on the mass transfer and on some physical characteristics (leaching of cell constituents, colour and texture) of strawberry halves during osmotic dehydration (OD) has been studied (Taiwo et al., 2003). Higher water loss was obtained in samples treated with a high-intensity electric field before OD. Moreover, the application of PEF before OD minimized changes in product colour and allowed to retain product compactness.

To the best of our knowledge, this is the first work aimed to the evaluation of the effect of PEF+OD low temperature processes on the mass transfer phenomena and water redistribution of strawberry tissue. Moreover, the changes in some quality parameters of treated strawberries from organic production were evaluated.

2. Materials and Methods

2.1. Raw material handling

Organic strawberries (*Fragaria+ananassa)* var ‘‘Alba’’ (10 ± 1 °Brix) were purchased from the local market in Cesena (Italy). The strawberries were stored at 4 ± 1 ºC at high relative humidity until use, for no longer than one week. Before processing, fruits were tempered at 25 ºC, washed, hand stemmed and cut into rectangular shape pieces of the dimension 5 x 10 x 20 mm (height x width x length).

2.2. Pulsed electric field (PEF) treatment

Two rectangular pieces (approximately 1.3 g) were placed into a rectangular treatment chamber equipped with two stainless steel electrodes (20 x 20 mm^2) with a gap between them of 30 mm and filled with 5 mL of a sodium chloride solution with the same conductivity as the strawberries (1.6 mS/cm). The PEF treatments were applied to the strawberry samples at 25°C using an in-house developed pulse generator equipment based on MOSFET technology that delivers near-rectangular shape pulses.

PEF pre-treatments were carried out by applying a train of 100 pulses at three different pulsed electric field (E) strength (100, 200 and 400 V cm^{-1}), a fixed pulse width of 100 µs and a repetition time of 10 ms (100 Hz). The procedure setting was chosen on bases of preliminary experiments.

2.3. Osmotic dehydration (OD) treatment

The OD treatment was carried out by immersing the strawberry samples in 40 % (w/w) hypertonic solutions. Two different solutions were prepared, one with sucrose and one with trehalose dissolved in distilled water. Calcium lactate (CaLac) at a concentration of 1 % (w/w) was added to both the solutions as a structuring agent. The treatment was performed at 25 ºC with continuous stirring maintaining a fruits:OD solution ratio of 1:4 (w/w) that allowed to avoid significant changes in the solution concentration during the whole treatment (data not shown).

The samples were analyses at different treatment times: 0, 15, 30, 60 and 120 min.

Both PEF and OD procedures were repeated twice for each solution.

All obtained samples are summarised with related abbreviations as reported in table 1.

Table 1. Codification of analysed samples

Sample code	Electric field ($V\ cm^{-1}$)	Type of solution
NoPEF_S	0	Sucrose
PEF_100_S	100	Sucrose
PEF_200_S	200	Sucrose
PEF_400_S	400	Sucrose
NoPEF_T	0	Trehalose
PEF_100_T	100	Trehalose
PEF_200_T	200	Trehalose
PEF_400_T	400	Trehalose

2.2. Analytical determinations

2.4.1. Mass transfer phenomena

Mass transfer phenomena during osmotic dehydration of strawberry samples was evaluated by calculating weight reduction (WR, $kg\ kg^{-1}$), water loss (WL, $kg\ kg^{-1}$) and solutes gain (SG, $kg\ kg^{-1}$) adopting the following equations:

$$WR = \frac{m_t - m_0}{m_0} \quad (1)$$

$$WL = \frac{m_t x_{wt} - m_0 x_{w0}}{m_0} \quad (2)$$

$$SG = \frac{m_t x_{STt} - m_0 x_{ST0}}{m_0} \quad (3)$$

where:

m_0 - initial weight before osmotic treatment (kg)

m_t - weight after a time t (kg)

x_{w0}- initial water mass fraction ($kg \cdot kg^{-1}$)

x_{wt} - water mass fraction after a time t ($kg \cdot kg^{-1}$)

x_{ST0} – initial total solids (dry matter) mass fraction ($kg \cdot kg^{-1}$)

x_{STt} – total solids (dry matter) mass fraction after a time t ($kg \cdot kg^{-1}$)

Moisture content was determined gravimetrically by drying the samples at 70°C until a constant weight was achieved (AOAC, 2002).

2.4.2. Water distribution by TD-NMR measurements

In order to measure the proton transverse relaxation time (T_2), strawberry cylinders of about 250 mg (h = 10 mm, d = 8 mm) were cut with a core borer. The samples were placed inside 10 mm outer diameter NMR tubes, in order to not exceed the active region of the radio frequency coil, and analyzed at 25 °C with the CPMG pulse sequence (Meiboom & Gill, 1958) using a 'The Minispec' spectrometer (Bruker Corporation, Germany) operating at 20 MHz. Each measurement comprised 4000 echoes over 16 scans, with an interpulse spacing of 0.3 ms and a recycle delay set at 10 s. The specified parameters, chosen to prevent sample and radio frequency coil overheat, allowed the observation of the protons with T_2 higher than a few milliseconds. According to the protocol set up by Panarese et al. (2012), the CPMG decays were analyzed with the UPEN software (Borgia et al., 1998), which inverts the CPMG signal using a quasi-continuous distribution of exponential curves, and through fittings to the sum of an increasing number of exponential curves. Furthermore, a multi exponential discrete fitting was successively applied to accurately determine T_2 and relative intensities of the water populations (Mauro et al., 2016). The experiment was conducted in triplicate at each treatment condition.

2.4.3. Cell viability test by Fluorescein diacetate (FDA) staining

The cell viability test was performed on 1 mm-thick strawberry slices, cut with a sharp scalpel, using fluorescein diacetate (FDA, Sigma-Aldrich, USA, λ_{ex} = 495 nm, λ_{em} = 518 nm), as described by Tylewicz et al. (2013) with some modifications. Strawberry slices were incubated for 5 min in a solution containing FDA (10^{-4} M) and sucrose in isotonic concentration (10 %, w/w) in the darkness at room temperature. The dye used in the experiment can passively penetrate the protoplast and then it is hydrolysed by cytoplasmic esterases, producing the polar product named fluorescein that only the viable cells are able to accumulate intracellularly, because it is unable to cross cellular membranes

that remain intact (Mauro et al., 2016). Hence, viable cells could be easily identified by a bright fluorescence. Observations were performed under a fluorescent light in a Nikon upright microscope (Eclipse Ti-U, Nikon Co, Japan) equipped with a Nikon digital video camera (digital sight DS-Qi1Mc, Nikon Co, Japan) at a magnification of 4 ×.

2.4.4. Colour

The colour changes of fresh, PEF pre-treated and osmodehydrated samples were investigated using a spectro-photocolorimeter mod. Colorflex (Hunterlab, USA). The measurements were made using CIE $L^*a^*b^*$ scale. The instrument was calibrated with a black and white tile (L^* 93.47, a^* 0.83, b^* 1.33) before the measurements. Moreover, the hue angle (h°) parameter was calculated using the following equation:

$$h° = tan^{-1}\frac{b^*}{a^*} \quad (4)$$

where: a* (red–green) and b* (yellow–blue) are parameters of color measurement (Vega-Gálvez et al., 2012).

The analysis were conducted in twelve repetitions for randomly selected strawberry samples for each PEF pre-treatment and osmotic dehydration condition.

2.4.5. Texture analysis

Firmness (N) was evaluated by performing a penetration test on strawberry rectangular pieces using a TA-HDi500 texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 N load cell. A stainless steel probe of 2 mm diameter was used and rate and depth of penetration were of 1 mm/s and 95 %, respectively. The analysis were performed in twelve replicates.

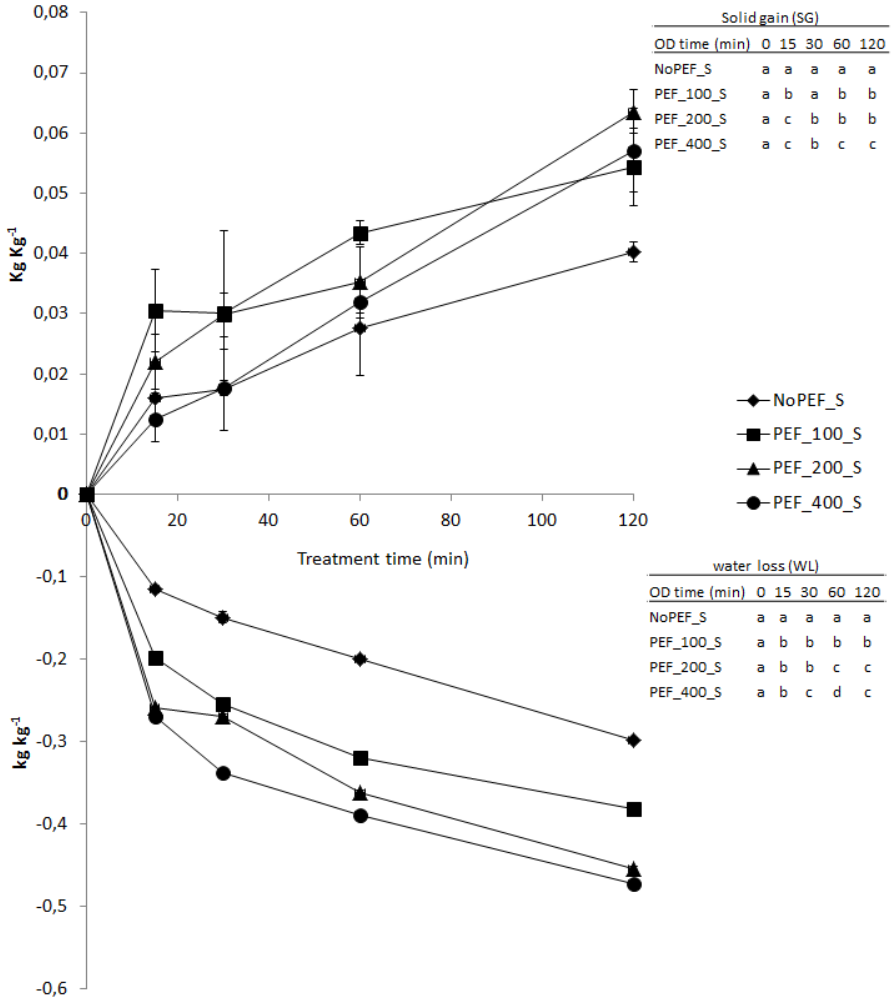
2.5. Statistical Analysis

Significance of the PEF treatment and OD effects was evaluated by one-way analysis of variance (ANOVA, 95% significance level) and comparison of means by Duncan test at a 5% probability level using the software STATISTICA 6.0 (Statsoft Inc., Tulsa, UK).

3. **Results and discussion**

3.1. Mass transfer phenomena

The kinetics of water loss and solid gain during OD are shown in Figure 1 and Figure 2 for sucrose and trehalose solutions, respectively. Figure 1 shows also the effect of the different electric field strength applied on water loss and solid gain during osmotic dehydration of strawberries immersed in sucrose-based solution. Samples subjected to the PEF pre-treatment presented a significantly higher water loss compared to the untreated strawberry samples.



Solid gain (SG)

OD time (min)	0	15	30	60	120
NoPEF_S	a	a	a	a	a
PEF_100_S	a	b	a	b	b
PEF_200_S	a	c	b	b	b
PEF_400_S	a	c	b	c	c

water loss (WL)

OD time (min)	0	15	30	60	120
NoPEF_S	a	a	a	a	a
PEF_100_S	a	b	b	b	b
PEF_200_S	a	b	b	c	c
PEF_400_S	a	b	c	d	c

Figure 1. Solid gain and water loss of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in sucrose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).

An improvement of water loss upon PEF pre-treatment has already been observed by Taiwo et al. (2003) on strawberries (1200 V cm^{-1}) and by Traffano-Schiffo et al. (2016) on kiwifruit (up to

400 V cm^{-1}). The acceleration of the kinetics of water and solids transfer is due to the effect of permeabilization of the cell membranes induced by the PEF treatment (Amani et al., 2006; Barba et al., 2015). In the present study, the application of the lowest electric field intensity (100 V cm^{-1}) resulted already sufficient to increase the water loss by 12 % after one hour of osmotic dehydration. This result is in contrast with those obtained by Dellarosa et al. (2016), who observed that the treatment with 100 V cm^{-1} did not have any effect on mass transfer of apple cylinders during the OD conducted for 60 min. This difference could probably be explained by the different microstructure of strawberries which resulted in a different sensitivity to the electric field strength. In addition, it needs to be mentioned that, due to both the different conductivity of samples/media and the higher number of delivered pulses, the energy input applied to the strawberry samples (123 J kg^{-1}) was much higher compared to the one delivered to the apples (8 J kg^{-1}). The initial mass transfer rate in PEF treated samples was faster compared to the untreated one, proportionally to the PEF intensity. Although at the end of the osmotic treatment the samples treated at 250 and 400 V cm^{-1} did not differ significantly, in agreement with Traffano-Schiffo et al. (2016). As reported by various authors (Ade-Omowaye et al., 2003; Angersbach et al., 2002; Dellarosa et al. 2016a), PEF effects can be considered time-dependent and the formation of pores and their growth in the membrane are not immediate but continue for several minutes after the treatment. This highlights the importance of taking into account the time elapsed from the application of pulsed electric fields before any other treatment in order to optimize PEF application in a combined multi step manufacturing process.

Similarly to water loss, solid gain was favoured by the application of PEF. After 120 min of OD, the solid gain was about 4 % in the strawberry untreated tissue, while PEF pre-treated sample reached a 5–6 % gain, in agreement with the results of Dellarosa et al. (2016a).

The lower enhancement of solid gain compared to the water loss has already been observed by Ade-Omowaye et al., (2003), that attributed this result to the higher molecular size of solutes compared to water and to a selective membrane permeabilization that favour dewatering rather than solute diffusion through the tissue.

The SG and WL behaviours of strawberry samples dehydrated in the trehalose-based solution were similar (Fig.2). However, water loss in trehalose-based solution was characterized by a higher initial rate compared to the treatment in the sucrose solution but by a lower final dehydration level. At the end of the treatment, the samples treated at 200 and 400 V cm^{-1} reached the highest WL of about 50 %.

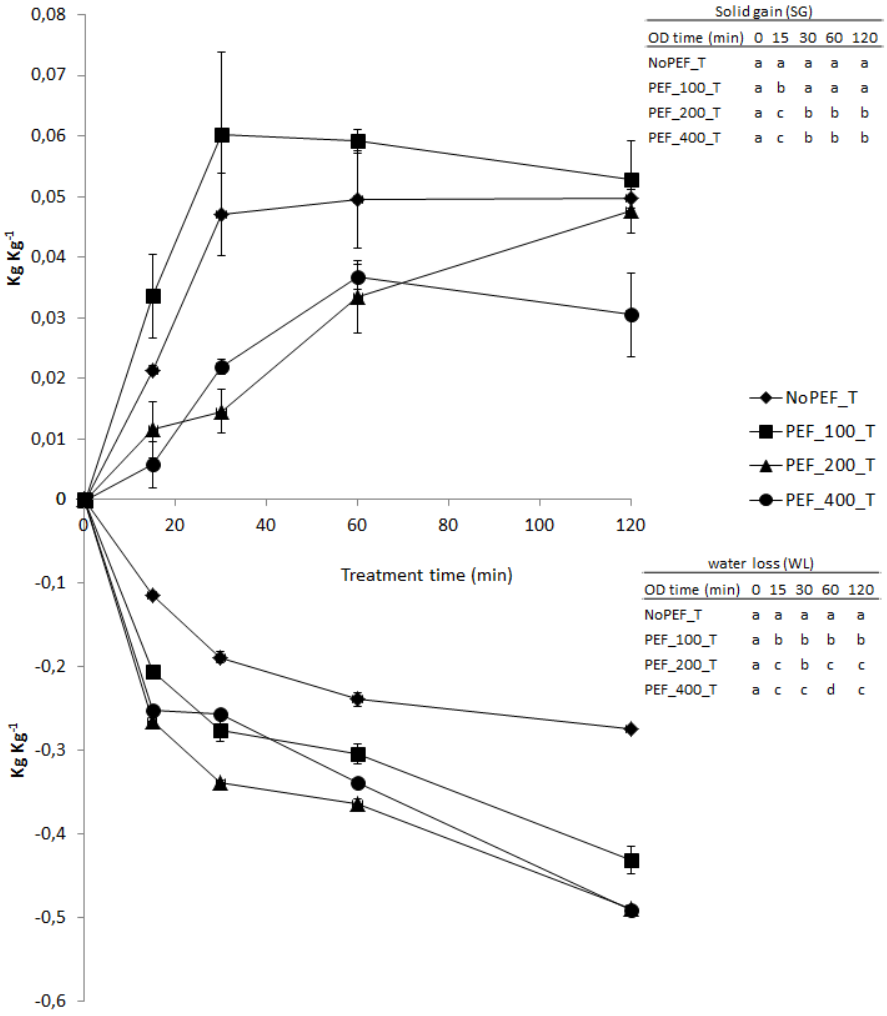


Figure 2. Solid gain and water loss of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in trehalose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).

Interesting results were observed for solid gain. Up to 120 min, only the treatment with the lowest electric field strength caused a higher solid gain compared to the untreated sample, while the treatment at 200 and 400 V cm^{-1} reduced the trehalose uptake due to a lower initial mass transfer rate. Generally thought, samples treated at 400 V cm^{-1} showed a noticeably lower solids impregnation. Trehalose is known to exert a protective effect on cell membranes during drying or freezing (Ferrando & Spiess, 2001; Atarés et al., 2008), thanks to its ability to form hydrogen bonds with the biomolecules that allows to stabilize cells and tissues preserving viability and structures (Vicente et al. 2012). In the present study, the combination of PEF with trehalose allowed to obtain a higher dewatering effect without increasing solute uptake or even reducing it.

This could be considered a positive effect if you want to increase the stability of a perishable organic product while maintaining/considering its nutritional properties.

3.2.Water redistribution upon treatments

Osmotic dehydration itself, generally, promotes important changes in cellular structure of different plan tissues, that can affect the water mobility and its distribution through different parts of the cellular tissue (Tylewicz et al., 2011; Panarese et al., 2012; Mauro et al., 2016). TD-NMR permitted to separately observe two main water populations located in vacuoles and cytoplasm plus extracellular spaces of strawberry tissue that corresponded to the relaxation time (T_2) of 1139.82 ± 129.56 and 251.24 ± 23.51, respectively. During OD treatment it was possible to observe the decrease of the signal intensity related to the water protons located in the vacuole throughout 120 min. As a consequence, the shrinkage of vacuole led to the increase of the intensity of the water protons belonging to the cytoplasm and extracellular space, as shown in the way of example for the sucrose treated samples in figure 3a.

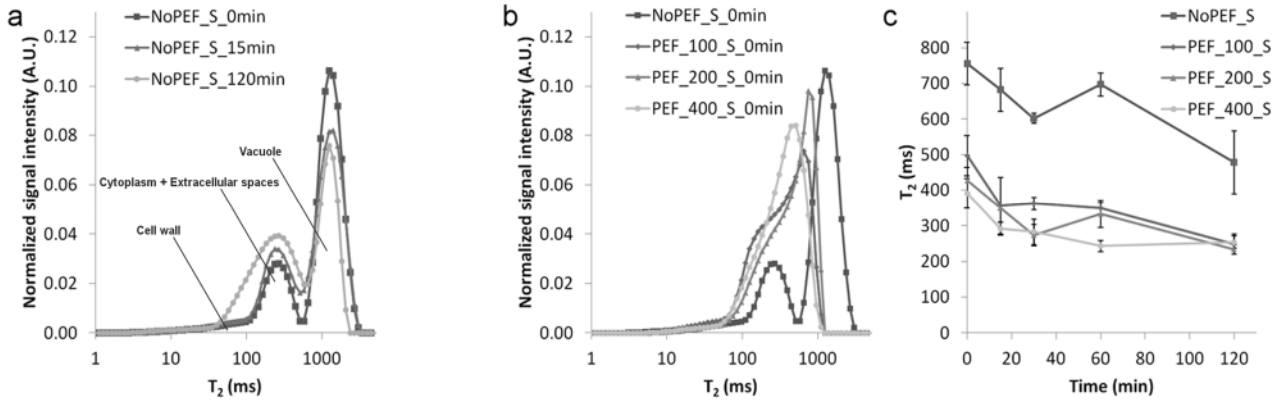


Figure 3. T_2 -weighted signal distribution, normalized to unitary area, of OD samples with sucrose (a) and sample immediately after PEF pre-treatments (b). Mean transverse relaxation time (T_2) values ± standard deviation PEF pre-treated and control strawberries during 120 min from immersion into the sucrose solution (c).

Results are in agreement with those reported by Cheng et al. (2014), who studied the effect of water-osmotic solute exchange on the strawberry cell compartments (vacuole, cytoplasm plus intercellular space, and cell wall) subjected to the ultrasound and vacuum assisted OD treatment in sucrose solution. The authors also observed that, upon OD treatments, the relative space occupied by the vacuole decreased while the one occupied by the cytoplasm and intercellular space increased. In other fruits such as kiwifruit (Tylewicz et al., 2011; Panarese et al., 2012) and apples (Mauro et al., 2016) similar behaviour on water distribution was observed, confirming the migration of water from the inner compartments toward the external ones.

Figure 3b shows the effect on water distribution due to the application of PEF on the strawberry tissue before immersion in the hypertonic solution. The electroporation induced by the treatment led to a

loss of compartmentalization that is highlighted by the merging of the two proton populations into a single one. This effect was more pronounced when applied E was increased from 100 to 400 V cm^{-1}. Dellarosa et al. (2016) studied the water distribution in apple tissue subjected to PEF treatments at similar voltages and determined a no-reversibility threshold at around 150 V cm^{-1} with 60 train pulses. In the present study even the lower voltage applied (100 V cm^{-1}) seemed to promote a collapse of the cellular structures although less markedly compared to the higher voltages. As mentioned above, this discrepancy could be explained by the higher energy input applied in the present experiment and the different sensitivity of strawberry tissue to the field strength in comparison with apples.

Figure 3c illustrates mean T_2 values of the water populations throughout 120 min of the osmotic treatment. As expected, this value decreased during OD due to the water removal and the different water-solutes-biopolymers interaction. Indeed, the water that is leaving the tissue during OD is characterized by high mobility, hence with long T_2. Therefore, a marked decrease of T_2 values, from 755 ± 60 ms to 478 ± 89 ms, for untreated strawberries was observed. Interestingly, each applied electric field strength also showed values spanning in the range 390-500 ms, immediately after PEF treatment. Such results might not be attributed to the different water content, but to the dissimilar water-solutes-biopolymers induced by the loss of compartmentalization within the strawberry tissue. In addition, similarly to control trends, T_2 values continued to decline during the whole duration of the osmotic dehydration process, so when water was also removed. These results, in accordance with mass transfer data, demonstrated that OD efficiency could be highly influenced by PEF pre-treatments which eased the diffusion of inner water by markedly affecting the permeability of membranes.

The samples dehydrated in trehalose-based solution (data not shown) followed a similar trend as the samples dehydrated in sucrose. Probably the marked effect of PEF contributed to hide the effect of different solutes used for dehydration.

3.3.Cell viability test by Fluorescein diacetate (FDA) staining

Figure 4 presents images of strawberry tissue after the PEF treatment followed by staining with FDA in order to investigate the possible loss of cell viability.

Indeed, the creation of pores in the cell membrane, through the phenomenon of electroporation, which is a function of temperature, intensity of the applied electric field, number of pulses, pulse shape, type of tissue etc. (Buckow et al., 2013), may lead to irreversible damages causing loss of cell viability.

In order to determine the threshold of irreversible electroporation, Dellarosa et al. (2016 b) measured the metabolic heat production and the respiration rate of apple cylinders subjected to 100, 250 and 400 V cm^{-1}. The authors found that the medium and the high applied voltages promoted a drastic loss

of cell viability that was attributed to the irreversible damages of the membranes. On the other hand, the tissue treated with 100 V/cm showed metabolic indexes comparable to the fresh tissue indicating that the electroporation was only reversible and did not cause loss of cell viability. In the present experiment, although cell viability was not completely lost, strawberry samples treated with an intensity of the electric field strength of 100 V cm^{-1}, showed residual cell viability, also if much lower than the fresh sample intensity, as shown in figure 4. The increase of the electric field strength induced a greater structural damage, as found in samples treated at 200 and 400 V cm^{-1} where there was a complete loss of cell viability. Consequently, cell viability was maintained even after 120 min of osmotic treatment of untreated samples (data not shown). The preservation of cell viability was observed also by Mauro et al. (2016) after 120 min in 40 % of sucrose solution. In the Mauro's study when 30% sucrose + 3 % of calcium lactate was used the cell viability was also preserved, while increasing quantity of calcium lactate up to 4% in 40% of sucrose compromised the cell viability. However, in the present study, only 1 % of calcium lactate was used, therefore this parameter was not affected by OD process, but just by PEF pre-treatment. Moreover, the PEF treated samples at 100 V cm^{-1} partially preserved their viability also after OD process (data not shown), while samples treated with higher E were not further investigated, due to the viability loss following PEF treatment. Therefore, with the aim of increasing the shelf-life of an organic product, characterized by quality parameters as close as possible to the fresh one, the lowest electric field strength applied in the tested range seems to be the suitable.

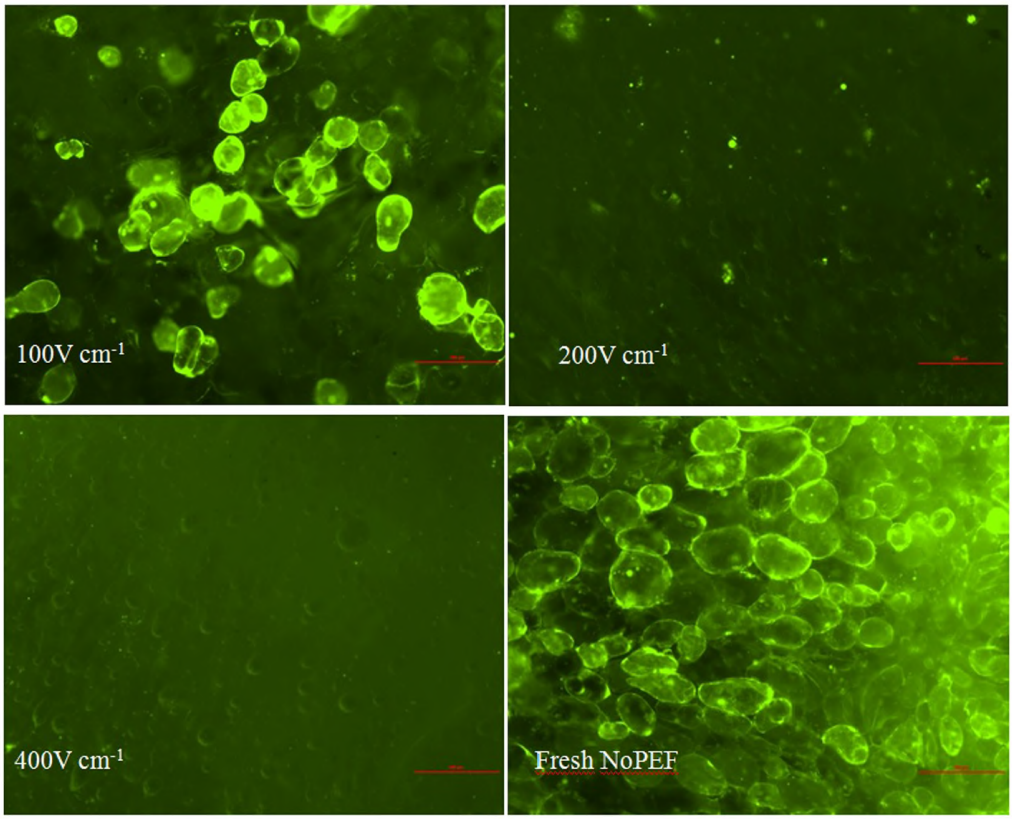


Figure 4. Microscopy images of fresh strawberry tissue and after the PEF treatment followed by staining with FDA.

3.4.Colour

Table 2 shows the L* and hue angle (h°) values of untreated and PEF treated strawberry tissues subjected to osmotic dehydration for 120 min in both solutions. L* parameter of untreated samples did not change during the whole OD treatment. Similar results were obtained by Nuñez-Mancilla et al. (2013) who did not notice any variation of the L* parameter in strawberry samples subjected to the OD process, while this parameter was influenced significantly by the application of high hydrostatic pressure.

The luminosity of the samples resulted to be affected by the electric field intensity. In fact, this parameter increased significantly after the application of PEF at the intensity of 100 V cm^{-1}, while decreased due to the application of PEF at highest field intensity. Also Wiktor et al. (2015) observed that the colour measurement showed unchanged or lower L* value of PEF treated samples at E=1.85 kV cm^{-1} and E=3 or E=5 kV cm^{-1}, respectively, in comparison with the untreated apple tissue. The darkening of the PEF treated samples at 400 V cm^{-1} could be related to the higher release of enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) and their substrates after the electroporation of the strawberry cells membrane. In fact, Chisani et al. (2007) observed that the browning of the strawberry fruit during the storage was related to both oxidase activities. However, after 120 min of

OD treatment the PEF treated samples increased their L* values, which was significantly higher in comparison to untreated ones.

Table 2. Colour parameters (L* - Lightness, h° - hue angle) of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in both sucrose and trehalose solutions.

OD Time	0 min	15 min	30 min	60 min	120 min
			L*		
NoPEF_S	35 ± 4 ^{b}	32 ± 6 ^{b}	40 ± 6 ^{a}	38 ± 3 ^{bc}	37 ± 4 ^{de}
PEF_100_S	42 ± 4 ^{a}	38 ± 5 ^{ab}	38 ± 3 ^{ab}	42 ± 3 ^{a}	45 ± 5 ^{a}
PEF_200_S	35 ± 1 ^{b}	35 ± 2 ^{b}	34 ± 2 ^{bc}	39 ± 2 ^{ab}	42 ± 2 ^{ab}
PEF_400_S	26 ± 2 ^{c}	42 ± 2 ^{a}	34 ± 2 ^{bc}	35 ± 2 ^{cd}	41 ± 2 ^{abc}
NoPEF_T	35 ± 4 ^{b}	37 ± 6 ^{ab}	36 ± 5 ^{abc}	37 ± 5 ^{bc}	34 ± 5 ^{e}
PEF_100_T	41 ± 4 ^{a}	35 ± 6 ^{ab}	33 ± 2 ^{c}	35 ± 3 ^{cd}	35 ± 4 ^{ce}
PEF_200_T	28 ± 3 ^{c}	30 ± 1 ^{c}	34 ± 2 ^{bc}	33 ± 2 ^{d}	39 ± 3 ^{cd}
PEF_400_T	27 ± 2 ^{c}	37 ± 4 ^{ab}	33 ± 3 ^{c}	35 ± 3 ^{cd}	38 ± 2 ^{cde}
			h°		
NoPEF_S	40 ± 2 ^{a}	36 ± 4 ^{a}	36 ± 2 ^{a}	35 ± 1 ^{a}	35 ± 2 ^{a}
PEF_100_S	35 ± 2 ^{c}	29.9 ± 0.9 ^{b}	29 ± 2 ^{cd}	29 ± 2 ^{b}	29 ± 2 ^{c}
PEF_200_S	38 ± 2 ^{ab}	29 ± 1 ^{b}	31 ± 2 ^{bc}	28 ± 1 ^{b}	25 ± 3 ^{de}
PEF_400_S	35 ± 4 ^{bc}	24 ± 1 ^{c}	27 ± 3 ^{de}	24 ± 1 ^{c}	23 ± 2 ^{e}
NoPEF_T	40 ± 1 ^{a}	37 ± 2 ^{a}	38 ± 1 ^{a}	33 ± 1 ^{a}	32.1 ± 0.7 ^{b}
PEF_100_T	35 ± 2 ^{bc}	30 ± 2 ^{b}	24 ± 2 ^{e}	24 ± 5 ^{bc}	26 ± 2 ^{d}
PEF_200_T	34 ± 3 ^{d}	28 ± 1 ^{b}	27 ± 1 ^{d}	25.5 ± 0.8 ^{c}	23 ± 2 ^{e}
PEF_400_T	36 ± 2 ^{bc}	28 ± 2 ^{b}	32 ± 3 ^{b}	28 ± 1 ^{b}	24 ± 2 ^{e}

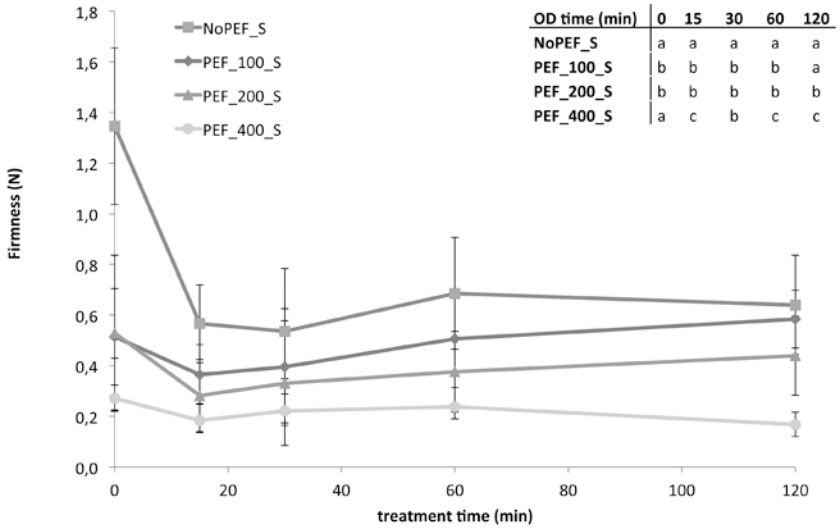
The same letter on the same column means no significant difference by the Duncan test ($p < 0.05$).

Since the colour of strawberries is the mixture of red and yellow, the hue angle (h°) was also calculated and its values are reported in table 2, respectively for strawberries treated in sucrose and trehalose solution. In general OD treatment promoted a decrease of this parameter. The application of PEF promoted a further decrease of hue angle in comparison with untreated samples, which was proportional to the electric field strength applied, at least in samples dehydrated in sucrose solution. Similar results were observed by Osorio et al. (2007). The reduction of h° colorimetric parameter could be due to both solubilisation of pigments in the osmotic solution and degradation of anthocyanin induced by PEF-treatment (Fathi et al., 2011; Odriozola-Serrano et al., 2008). In samples dehydrated in trehalose non significant differences were observed among PEF-treated samples, if not for the samples treated by 100 V cm^{-1} at 30 min after OD that showed a significantly lower h° value compared

to the others. Wiktor et al. (2015) observed that the effect of PEF treatment strongly depends on the raw material properties and the treatment conditions. In fact, the authors noticed the different behaviour of carrot and apple tissue subjected to electric field strength at different intensities. In both cases browning of the tissue was observed, however in carrots it was more pronounced when the low voltage treatment was applied, while in apple with high voltage.

3.5.Texture

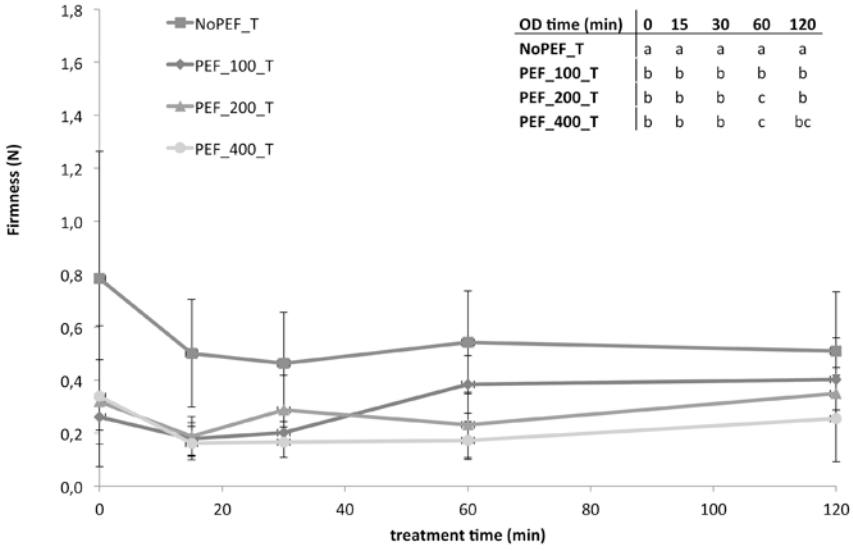
It is well known that OD induces plasmolysis, shrinkage of the vacuole compartment, changes in size and structure of the cell walls of outer pericarp and dissolution of the middle lamella, which could be translated in decreasing of the firmness of the plant tissue (Chiralt & Talens, 2005; Panarese et al. 2012). The changes of firmness of untreated and PEF treated strawberry tissue subjected to OD treatment up to 120 min in sucrose-based solution is shown in Figure 5. OD of untreated samples promoted a decrease of strawberry firmness, already 15 min after the treatment, and increased slightly during the OD treatment. In the present experiment, PEF pre-treatment drastically reduced the hardness of strawberry samples; further, the PEF treated samples remained below the untreated ones during the whole OD process and the effect was proportional to the electric field strength applied. Also Taiwo et al. (2003) observed the decrease in firmness of strawberries halves treated with PEF (1200 V cm^{-1}; 350 μs) and then osmodehydrated for 4 hours in binary (sucrose, NaCl) solution. The reduction of firmness of PEF treated samples could be due to the alteration of the membrane permeability due to the pores creation and the rupture of internal structure, which promotes the softening of the tissue (Fincan & Dejmek, 2002; Wiktor et al., 2016).



OD time (min)	0	15	30	60	120
NoPEF_S	a	a	a	a	a
PEF_100_S	b	b	b	b	a
PEF_200_S	b	b	b	b	b
PEF_400_S	a	c	b	c	c

Figure 5. Firmness (N) of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in sucrose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).



OD time (min)	0	15	30	60	120
NoPEF_T	a	a	a	a	a
PEF_100_T	b	b	b	b	b
PEF_200_T	b	b	b	c	b
PEF_400_T	b	b	b	c	bc

Figure 6. Firmness (N) of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in trehalose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).

The slight increase of the texture observed after longer OD times could be probably due to the penetration of Ca^{2+} into the strawberry tissue. The structural role of calcium ions in the cell wall is

due to their interaction with pectic acid polymers to form cross-bridges that reinforce the cell adhesion, thereby reducing cell separation, which is one of the major causes of plant tissue softening (Van Buggenhout et al., 2008; Mauro et al., 2016). This increase has not been observed in the samples treated at 400 V cm^{-1}, probably because the tissue was already completely disintegrated after the PEF treatment, and did not permit the incorporation of calcium ions in the cell walls.

Similar results were observed in strawberries samples dehydrated in trehalose-based solution (Fig. 6). However, considering that the firmness of the material (0.8 ± 0.1 N) used for the experiment was almost half compared with the value relative to the raw material used in the experiment with sucrose (1.35 ± 0.2 N), the decrease of firmness following the OD process was less marked. In fact, the firmness of samples dehydrated in trehalose decreased only by 36 % in comparison to 57 % of decrease observed in sucrose dehydrated samples already 15 min after the treatment. This behaviour could probably be due to the protective effect of trehalose on the tissue structure, as reported by Phoon et al. (2008). The intensity of the applied electric field strength seems to be not so relevant in comparison to samples dehydrated with sucrose. Shayanfar et al. (2013) observed texture softening and loss of turgor in frozen/thawed potatoes after the PEF treatment. However, when CaCl2 and trehalose were added to the liquid medium used in PEF treatment, the samples maintained their firmness when compared to solely PEF treated samples.

4. Conclusions

PEF treatment prior to osmotic dehydration was found to positively affect the mass transfer, in term of water loss from the strawberry tissue. The application of the lowest electric field intensity (100 V cm^{-1}) resulted already sufficient to increase the water loss by 12 % and 6%, after one hour of osmotic dehydration, respectively for strawberries dehydrated in sucrose and trehalose solution, partially preserving the cell viability and maintaining at the same time the fresh-like characteristics of fruits. Concerning the solid gain results, while the solid gain was favoured by the application of all the PEF intensities in samples dehydrated in sucrose solution, the treatment at 200 and 400 V cm^{-1} reduced the trehalose uptake due to a lower initial mass transfer rate.

In most of the cases, the PEF effect on different strawberry characteristics investigated was proportional to the electric field strength applied.

TD-NMR results showed that the diffusion of inner water was eased by PEF application because of a marked effect on membranes permeability.

Although similar effects on the investigated parameters were observed by using sucrose or trehalose solutions, the combination of PEF with trehalose allowed to obtain a higher dewatering effect without increasing solute uptake or even reducing it.

Definitely, the application of the lower field intensity and the use of trehalose for the dehydration process, seem to be the optimal combination for obtaining a semi-dried strawberry product with quality characteristics similar to the fresh one, that is a fundamental requirement for an organic production.

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Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life

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Abstract

Edible films or coatings could be used as an alternative way of conservation, because of their ability to reduce respiration and transpiration rate, maintain firmness and generally delay fruit senescence. The aim of this research was to evaluate the influence of different types of coating: sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe), on some blueberries quality characteristics, cell viability and microbial growth during 14 days of storage at 4°C.

Blueberry samples differently coated did not show significant differences in weight loss, pH, soluble solid and dry matter content. However, the application of Al, Pe and Al + Pe improved the firmness of blueberry samples as compared to the uncoated one. Changes in the surface reflection properties in the coated blueberries induced a general lower lightness and a more intense blue hue colour than the control sample. The microbiological results indicated that the coating of blueberry, in particular with Al or Pe, significantly reduced the growth kinetics of yeasts and mesophilic aerobic bacteria.

Keywords

Fruit, quality, storage, alginate, pectin

1. Introduction

Blueberries are appreciated for their rich composition in bioactive compounds such as flavonoids, phenolic acids, tannins and anthocyanins giving them nutraceutical properties. However, fresh fruit deteriorate rapidly due to loss of water and juice (product of superficial lesions), mould and/or putrefaction (Yang et al., 2014). The shelf-life of fresh blueberries usually is in the range of 10-40 days depending on different factors such as fruit maturity, cultivar, harvest method and storage conditions (Abugoch et al., 2016). Various technologies are used to reduce spoilage, extend the shelf-life and retain the nutritional value of fruit products; among this group particular attention can be given to refrigeration, UV irradiation, ozonation and modified packaging atmosphere (Duan, Wu, Strik, & Zhao, 2011). The use of edible films or coatings represents an alternative way of preservation because of their ability to reduce moisture, solute migration, respiration and transpiration rate, to maintain firmness and generally delay senescence (Tezotto-Uliana, Fargoni, Geerdink, & Kluge, 2014). The efficiency and stability of edible coatings or films depend on their compositions. Edible films and coatings are generally based on biological materials such as proteins, lipids and polysaccharides, alone or, more often, in combination.

Sodium alginate is a natural linear polysaccharide obtained from brown seaweeds and has many important physical and biological properties, such as moisture retention, gel-forming capability, good biocompatibility, low price and high availability (Pei, Chen, Li, & Zhou, 2008).

Pectin is a complex of acidic polysaccharides that form an interpenetrating network in the plant cell wall; it is one of the most important citrus by-products that are industrially extracted from apple pomace and citrus peels. Generally it is used to increase viscosity and gel strength of food products (Krochta & Mulder-Johnston, 1997).

Some studies confirm that the application of edible coatings on fruit surface can increase the shelf-life of different fruits, for example raspberries (Tezotto-Uliana et al., 2014) and tropical fruits (Cerqueira, Lima, Teixeira, Moreira, & Vicente, 2009). However, there are few works about coatings effects on blueberries (Duan et al., 2011; Chiabrando & Giacalone, 2015). In both papers, the authors showed that the use of alginate coating on berries had a positive effect on firmness, titratable acidity and maintained surface lightness of coated fruit products. However, to the best of our knowledge there are no papers presented in the literature on the effect of pectin-based coating on blueberries.

Although edible films are not intended to completely replace conventional packages, the efficiency of food protection can be improved by combining both actions. The objectives of this study were to investigate the effectiveness of sodium alginate, pectin and both of these polysaccharides based coatings in improving some qualitative characteristics of blueberry fruits during shelf-life.

2. Material and methods

2.1. Fruit material

Organic blueberries were purchased once from local market. Berry fruits were kept at 0 ± 1°C until they were used, for no longer than one week, as suggested by Perkins-Veazie, Clark, Collins, & Magee, 1995 and Jackson, Sanford, Lawrence, McRae, & Stark, 1999. Fresh blueberries with the same colour and size and no damages were selected for the experiments.

2.2. Preparation of coating solutions

Three different coating solutions were prepared, each of them contained 15 g/kg of glycerol (≥ 99.5% Sigma-Aldrich, St. Louis, MO USA) and 2 g/kg of Tween® 20 (Sigma-Aldrich, St. Louis, MO USA) and solved in distilled water. In a first solution, sodium alginate (Al) (Sigma-Aldrich, St. Louis, MO USA) was added in the quantity of 20 g/kg. The second one was enriched by 20 g/kg of pectin (Pe) from citrus peel (Galacturonic acid ≥ 74.0% Sigma, St. Louis, MO USA), and the third one was prepared by combination of Sodium Alginate and Pectin (Al + Pe) in equals amounts of 10 g/kg + 10 g/kg. Afterwards, all coating solutions were homogenised at 5000 rpm for 2 min in order to remove air bubbles.

2.3. Sample preparation

Blueberry fruits were sanitized with sodium hypochlorite water solution (0.2 g/kg), rinsed in distilled water and dried with absorbing paper. Whole fruits were dipped in coating solutions, in two process steps, each one of 30 sec duration. The berry samples were drained in a ventilated oven at 25 ± 1 °C for 30 min following the first step dipping, and for 60 min following the second step dipping. Blueberries dipped in distilled water with the same procedures were used as control. Coated berry samples were then placed in plastic trays (PET) closed in micro-perforated bags (PLA) and stored at 4 °C for 14 days. Coated samples and control ones were analysed at 0, 2, 4, 6, 10 and 14 days of storage. Totally 4 samples were obtained: 3 differently coated blueberry samples (Al, Pe, Al+Pe) and 1 not coated control sample. For each sample 540 blueberries were used. Three trays for every sampling time were made, containing 30 blueberries each, which were taken randomly from the three trays and used for analytical determinations.

2.4. Quality determinations

2.4.1. Weight loss, Dry matter, pH and Soluble solid content

Weight loss (WL) of blueberry samples during storage was measured by weighting fruits in the trays before storage and at every day of analysis, following the standard method of AOAC (1994).

Dry matter content was determined gravimetrically by difference in weight before and after drying at 70 °C, until a constant weight was reached (AOAC International, 2002).
pH was determined at 20 °C with a pH meter CRISON GLP21 (Shinghai Shilu-Instruments, China). Soluble solid content (SSC) analysis were performed at 20 °C by measuring the refractive index of blueberry juice with digital hand refraktometer mod. DR301-95 (Kruess, Germany).
For each treatment-time condition, dry matter was determined in triplicate from 8 blueberries from each tray; pH and SSC were determined also in triplicate on three different juice samples obtained from 30 berries from each tray, after filtering through Whatman #1 filter paper.

2.4.2. Colour and Texture

Surface colour of blueberry was measured using spectrophotocolorimeter HUNTERLAB ColorFlexTM, mod. A60-1010-615 (Reston, Virginia). For each sample L*, a* and b* parameters from CIELAB scale were measured and Hue angles (h°) index was calculated.
Penetration test was performed with a Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Godalming, UK) equipped with a 50 N load cell and a 2 mm diameter stainless steel probe. Penetration test speed was 0.5 mm s^{-1}, the test ended when a maximum deformation of 80% was reached. Results were expressed as average of 12 measurements carried out on 12 blueberries for each treatment-time condition.

2.4.3. Cell viability

The cell viability test was performed on blueberries slices obtained from 9 different blueberries using fluorescein diacetate (FDA, Sigma-Aldrich, USA, λ_{ex} = 495 nm, λ_{em} = 518 nm), as described by Tylewicz, Romani, Widell, & Galindo, (2013). Viable cells could be easily identified by a bright fluorescence. Observations were performed under a fluorescent light in a Nikon upright microscope (Eclipse Ti-U, Nikon Co, Tokyo, Japan) equipped with a Nikon digital video camera (digital sight DS-Qi1Mc, Nikon Co, Tokyo, Japan) at a magnification of 4 ×.

2.4.4. Microbial growth

The total loads of mesophylic aerobic bacteria, lactic acid bacteria, yeasts, moulds and total coliforms were evaluated according to the methods reported by Siroli et al., (2015). Briefly, 10 g portion of each sample were used (around 6 berries), suspended in 90 ml of sterile saline solution (9 g/l NaCl, w/w) and homogenized using a Stomacher for 2 min at room temperature; serial dilutions were made. The microbiological analyses were performed in triplicate immediately after treatments and during storage.

2.4.5. Data analyses

Analysis of variance (ANOVA) and the test of mean comparison, according to Fisher's least significant difference (LSD) were applied on all obtained data. Level of significance was $p < 0.05$. The statistical software used was STATISTICA, v 8.0 (StatSoft, Tulsa, Okhlaoma).

3. Results and discussion

3.1 Weight loss, Dry matter, pH and Soluble solid content

The fruits weight loss during storage usually is caused by the migration of the water from the fruit to the surrounding environment. As reported in Table 1, all samples underwent a slight loss of weight during 14 days of storage.

Table 1. Weight loss (%) of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples during 14 days of storage at 4°C.

	Weight loss (%)				
	T2	T4	T6	T10	T14
Control	-1.1 ± 0.1[a]	-1.2 ± 0.3[a]	-2.3 ± 0.3[a]	-3.9 ± 0.2[a]	-5.9 ± 0.8[a]
Al	-1.05 ± 0.05[a]	-1.8 ± 0.4[a]	-2.34 ± 0.05[a]	-4.2 ± 0.2[a]	-6 ± 1[a]
Pe	-0.83 ± 0.07[a]	-1.5 ± 0.2[a]	-2.2 ± 0.3[a]	-4.0 ± 0.5[a]	-5.5 ± 0.2[a]
Al+Pe	-2 ± 1[a]	-2.2 ± 0.1[a]	-2.3 ± 0.5[a]	-4.1 ± 0.6[a]	-5.6 ± 0.3[a]

Data are reported as average values and standard deviations obtained from three replicates for each treatment-time conditions.

Values with different letter within the column are significantly different ($p < 0.05$).

Coated samples did not show any significant differences in weight loss as compared to the control. These results are probably due to a slight loss of water undergone by samples. The moisture loss of fresh fruit and vegetables is due to the gradient of water vapor pressure that occurs from different locations in the cell tissues (Yaman & Bayoındırlı, 2002). The cold storage conditions (temperature and relative humidity) could have an effect on the difference of vapor pressure between blueberries and the environment resulting in non-significant weight losses. In fact, as reported by Nunes (2015) the weight loss up to 4-5% does not significantly influence the freshness of the fruit.

As reported in Table 2, no significant differences ($p < 0.05$) on dry matter and pH were detected, among control and differently coated samples at each considered storage time. Concerning the SSC, significant differences ($p < 0.05$), even if slight, were observed only at 10 days of storage; in particular Al and Al + Pe presented higher SSC values as compared to the control and Pe coated blueberry fruits. As a general trend dry matter, pH and SSC tended to increase during storage in both control and coated fruit samples. pH and SSC showed the same behaviour increasing with longer storage time, similar results have been provided by Duan et al (2011). The increase of pH and SSC is probably due to metabolic processes and reactions during post-harvest storage, which continue to converting starch and acids into the sugar.

Table 2. Dry matter (g/kg) pH and soluble solid content (°Bx) of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (A l+ Pe) coated blueberry samples during 14 days of storage at 4°C.

Dry Matter (g/kg)						
	T0	T2	T4	T6	T10	T14
Control	178.2 ± 0.4[a]	193.0 ± 0.6[a]	183 ± 2[a]	204.5 ± 0.1[a]	199 ± 1[a]	198 ± 1[a]
Al	177.8 ± 0.8[a]	180 ± 2[a]	194.8 ± 0.7[a]	194.30 ± 0.02[a]	183.0 ± 0.1[a]	202 ± 1[a]
Pe	185.8 ± 0.6[a]	179.9 ± 0.7[a]	195.9 ± 0.2[a]	204.9 ± 0.4[a]	196.2 ± 0.2[a]	194.3 ± 0.6[a]
Al+Pe	185.4 ± 0.2[a]	186.5 ± 0.8[a]	190.7 ± 0.5[a]	184.32 ± 0.06[a]	193.5 ± 0.4[a]	188.0 ± 0.6[a]
pH						
	T0	T2	T4	T6	T10	T14
Control	3.49 ± 0.00[a]	4.09 ± 0.03[a]	3.5 ± 0.2[a]	3.7 ± 0.5[a]	3.7 ± 0.2[a]	4.1 ± 0.1[a]
Al	3.47 ± 0.07[a]	3.9 ± 0.2[a]	3.8 ± 0.8[a]	3.35 ± 0.08[a]	3.4 ± 0.2[a]	4.03 ± 0.05[a]
Pe	3.28 ± 0.04[a]	3.8 ± 0.1[a]	3.4 ± 0.2[a]	3.52 ± 0.00[a]	3.38 ± 0.07[a]	4.0 ± 0.1[a]
Al+Pe	3.55 ± 0.04[a]	3.8 ± 0.2[a]	3.5 ± 0.2[a]	3.31 ± 0.02[a]	3.6 ± 0.3[a]	3.58 ± 0.02[a]
SSC						
	T0	T2	T4	T6	T10	T14
Control	13.4 ± 0.7[a]	13 ± 2[a]	15.0 ± 0.2[a]	15.2 ± 0.2[a]	12.7 ± 0.9[b]	15 ± 2[a]
Al	12.6 ± 0.7[a]	15 ± 2[a]	15 ± 3[a]	14.6 ± 0.1[a]	15.1 ± 0.9[a]	15 ± 2[a]
Pe	13 ± 2[a]	15 ± 1[a]	13 ± 1[a]	13.1 ± 0.5[a]	11.6 ± 0.4[b]	18 ± 1[a]
Al+Pe	13 ± 2[a]	14 ± 1[a]	14 ± 1[a]	15.6 ± 0.2[a]	15.0 ± 0.4[a]	17 ± 1[a]

Data are reported as average values and standard deviations obtained from three replicates for each treatment-time conditions.

Values with different letter within the column are significantly different ($p < 0.05$).

3.2 Colour and Texture

In Table 3 colour data (lightness - L* and Hue angles - h°) of blueberry samples during 14 days of storage at 4 °C are reported.

Table 3.Lightness-L* and Hue angles- h° of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples during 14 days of storage at 4°C.

	L*					
	T0	T2	T4	T6	T10	T14
Control	21 ± 1^{a}	28.4 ± 0.1^{a}	31.5 ± 0.8^{a}	30.5 ± 0.5^{a}	28.5 ± 0.6^{a}	29 ± 1^{a}
Al	19.33 ± 0.07^{a}	18.9 ± 0.1^{b}	22.74 ± 0.05^{c}	22.2 ± 0.6^{c}	19.4 ± 0.6^{b}	16.48 ± 0.00^{c}
Pe	14 ± 2^{b}	19.5 ± 0.5^{b}	23.2 ± 0.4^{bc}	26.0 ± 0.2^{b}	19.3 ± 0.2^{b}	19.59 ± 0.02^{b}
Al+Pe	15.3 ± 0.6^{b}	15.9 ± 0.8^{c}	24.9 ± 0.2^{b}	25.6 ± 0.5^{b}	17.6 ± 1.4^{b}	19.9 ± 0.4^{b}
	h°					
	T0	T2	T4	T6	T10	T14
Control	100 ± 11^{b}	90 ± 3^{c}	97 ± 5^{c}	93 ± 4^{b}	72 ± 6^{c}	89 ± 6^{c}
Al	140 ± 11^{a}	126 ± 10^{ab}	117 ± 7^{b}	102 ± 9^{b}	75 ± 6^{b}	145 ± 11^{b}
Pe	145 ± 11^{a}	139 ± 7^{a}	128 ± 5^{a}	134 ± 6^{a}	87 ± 6^{a}	151 ± 11^{b}
Al+Pe	154 ± 11^{a}	123 ± 9^{b}	111 ± 7^{b}	126 ± 5^{a}	85 ± 5^{ab}	179 ± 11^{a}

Data are reported as average values and standard deviations obtained from twelve replicates for each treatment-time conditions.

Values with different letter within the column are significantly different ($p < 0.05$).

Coating induced a general lower lightness and a more intense blue hue colour in blueberry samples as compared with the control one ($p < 0.05$), probably due to the glossy effect of coating. The observed lower luminosity value of coated samples could be caused by the modifications in the surface reflection properties (Hoagland & Parris, 1996). L* values of control and coated samples tended to increase during the first days of storage, then remained relatively stable and decreased after the sixth storage day.

The visual perception of the intensity of blue colour was always more intense in the coated than in the control samples, as indicated from their highest hue values. Moreover, the blueberry samples

showed a general decrease in hue values from 0 to 10 days that tended to increase on 14th day. The h° decrease of blueberries during the first period of storage is probably caused by oxidation or condensation reactions of phenolic compounds resulting in loss of anthocyanins during cold storage (Reque et al., 2014). Moreover, the increase of hue values at the end of storage might be caused by a possible anthocyanins synthesis during ripening as also observed by the higher pH and SSC values (Table 2).

As shown in Fig. 1 blueberry coated samples presented a significantly ($p < 0.05$) higher firmness compared to control sample until the first 10 days of storage.

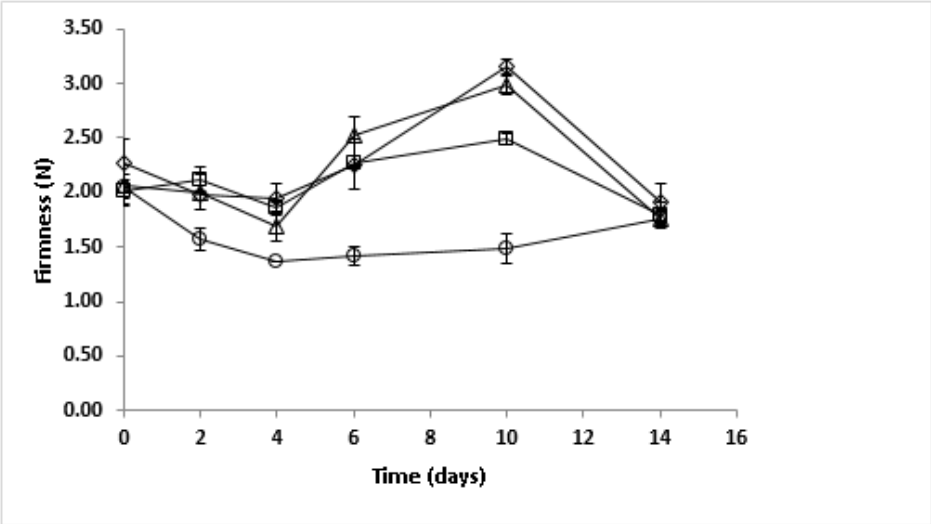

Fig. 1 Firmness (N) of control (Control ○) and differently coated blueberry samples (sodium alginate - Al ◊; pectin - Pe □; sodium alginate plus pectin – Al + Pe Δ) during 14 days of storage at 4°C.

After this period, texture of blueberries coated samples decreased, reaching the same value of control one (1.75N). The higher firmness values of coated samples are probably due to the presence of coating that provide a structural rigidity to the surface of fruit (Duan et al., 2011). Pe and Pe + Al showed the same behaviour of the Al based coating. This result of Al coating was in agreement with Rojas-Graü, Tapia, & Martín-Belloso, (2008) on fresh-cut apple and Fan et al., (2009) on strawberry fruits. Moreover, the retention of firmness could be explained by the delay of pectin and proto-pectin degradation, involved in maintaining the structural integrity of the fruits (Thompson, 1996).

3.3 Cell viability

Figure 2 shows the micrographs resulted from microscopic observations of control (a) and Al + Pe coated blueberry samples (b) after 14 days of storage.

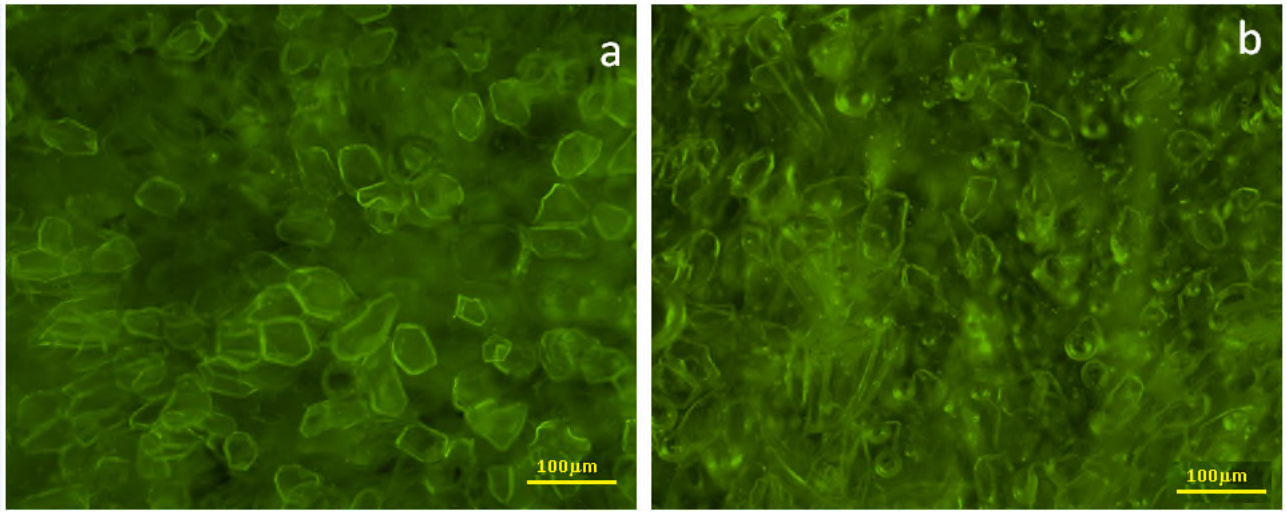

Fig. 2 Cell viability for (a) control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe), coated blueberry samples (b) at 14 days of storage after treatment using fluorescein diacetate (FDA) marker. Bar = 100 µm

The pictures demonstrate that cell viability in all tissues is preserved until 14 days of storage both in case of control and coated samples. The results provide evidence that cell viability (viable cells could be identify by a bright fluorescence on the Figure) can be preserved in blueberries also after the application of coating. If the protoplasts of the cells did not retain the FDA, this means disruption of the plasma membrane (cell lysis) or loss of membrane semi permeability (Halperin & Koster, 2006). These results provide versatile tool to conduct study of the metabolism of blueberry tissues that was maintained despite storage and the application of different types of coatings.

3.4 Microbial growth

As reported in Table 4, yeasts were detected only in control sample after 2 and 4 days of storage while in all the coated samples the yeast cell loads were below the detection limit.

Table 4. Yeast count of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples.

	T0	T2	T4	T6	T10	T14
Control	nd*	2.2 ± 0.3^{a}	3.2 ± 0.2^{a}	3.5 ± 0.3^{a}	3.6 ± 0.3^{a}	3.3 ± 0.3^{a}
Al	nd*	nd*	nd*	nd*	2.1 ± 0.2^{b}	2.0 ± 0.2^{b}
Pe	nd*	nd*	nd*	nd*	1.8 ± 0.3^{b}	1.9 ± 0.2^{b}
Al+Pe	nd*	nd*	nd*	2.2 ± 0.2^{b}	2.2 ± 0.2^{b}	2.2 ± 0.2^{b}

Counts are expressed in log10 cfu/g (± standard deviation). Means followed by different letters are significantly different ($p<0.05$) and are obtained from three replicates for each treatment-time conditions.

* under the detection limit (1 log10 cfu/g)

In samples coated with Al or Pe yeasts were detected only after 10 days of storage. However, at the end of storage the yeast loads of coated samples were 1.09-1.38 logarithmic cycles lower than control samples.

Significant differences were also evidenced in the total aerobic mesophilic cell loads among the samples during the storage period (Table 5).

Table 5. Mesophylic aerobic bacteria of control (Control) and sodium alginate (Al), pectin (Pe) and sodium alginate plus pectin (Al + Pe) coated blueberry samples.

	T0	T2	T4	T6	T10	T14
Control	nd*	2.3 ± 0.3^{a}	2.9 ± 0.3^{a}	3.1 ± 0.3^{a}	4.1 ± 0.3^{a}	4.5 ± 0.3^{a}
Al	nd*	nd*	1.5 ± 0.3^{b}	1.9 ± 0.3^{b}	2.0 ± 0.2^{b}	2.6 ± 0.3^{b}
Pe	nd*	nd*	nd*	2.1 ± 0.3^{b}	2.2 ± 0.3^{b}	2.7 ± 0.2^{b}
Al+Pe	nd*	nd*	1.5 ± 0.3^{b}	3.0 ± 0.3^{a}	3.6 ± 0.4^{a}	4.2 ± 0.4^{a}

Counts are expressed in log10 cfu/g (± standard deviation). Means followed by different letters are significantly different ($p<0.05$) and are obtained from three replicates for each treatment-time conditions.

* under the detection limit (1 log10 cfu/g)

In fact, in this case only the control sample showed mesophilic cell loads above the detection limit after 2 days of storage. Samples coated with Al or Pe showed mesophilic cell loads from the sixth days of storage, significantly lower than the controls and samples coated with Al + Pe. Finally, no significant differences were found for lactic acid bacteria and total coliform cell loads in relation to the coating adopted, whose loads resulted below 2.0 log CFU/g, during the whole period of storage.

The microbiological results indicate that the coating of blueberry, in particular with Al or Pe, significantly reduce the growth kinetics of yeasts and mesophilic aerobic bacteria that play a dominant role in the spoilage of minimally processed fruits (Siroli et al., 2014) .

4. Conclusions

The use of coating showed a positive effect mainly on firmness and microbial growth of treated blueberries samples. The firmness was maintained until 10 storage days also for the Pe and Al + Pe coated blueberries. Furthermore, the application of coatings reduced the growth kinetics of yeasts and mesophilic aerobic bacteria, in particular with the application of Al and Pe based coatings. Results from this study indicate the possibility of using edible coatings to develop ready-to-eat fresh blueberries with no reduction in their shelf-life. Further researches will focus on the effect of these edible coatings on blueberry bioactive compounds and sensorial properties.

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Mannozzi C., Tylewicz U., Chinnici F., Siroli L., Rocculi P., Dalla Rosa M., & Romani S. (2018)

Effects of chitosan based coatings enriched with procyanidin by-product on quality of fresh blueberries during storage

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Abstract

The aim of this work was to evaluate the efficacy of an innovative edible coating, based on chitosan from mushrooms enriched with procyanidins extracted from grape seeds, on fresh blueberry quality maintenance, (weight loss, pH, dry matter, colour, firmness and antioxidant activity) and microbial growth, during 14 days of storage at 4° C.

For weight loss, pH and dry matter no relevant differences were detected among the control and the differently coated samples at each considered storage time. Chitosan and chitosan + procyanidins coatings promoted a slight decrease of luminosity and an increase of blue hue colour of blueberry samples during the whole storage period. The use of coating promoted an increase in the antiradical activity that was the highest in blueberries coated with chitosan + procyanidins. Microbiological analysis results indicated that the chitosan-based coated samples had a significantly higher yeast and mould growth inhibition compared to the uncoated sample.

Keywords Edible coating, chitosan, procyanidins, blueberries, antioxidant activity

1. Introduction

Blueberries are increasingly appreciated for their rich composition in flavonoids, phenolic acids, tannins and anthocyanins giving them a great nutritional value. Anthocyanins are natural pigments, largely distributed in nature and generally present in many fruit and vegetables. In particular, berries demonstrated to have a great antioxidant activity, due to their high content in phenolic acids and flavonoids, which can cause a strong antioxidant capacity in different products (Pellegrini et al., 2003). In addition, phenolic compounds may exert beneficial effects on human health associated with the consumption of fruit and vegetables (Cheynier, 2012).

However, fresh fruits deteriorate rapidly due to loss of water and cellular juice (product of superficial lesions), senescence, mould growth and/or putrefaction phenomena (Yang et al., 2014). Moreover, bioactive compounds are prone to alterative oxidative reactions, which can negatively affect phenolic levels and antioxidant capacity in berry fruits during post-harvest storage (Connor, Luby, Hancock, Berkheimer, & Hanson, 2002). Physical deteriorations that occur during postharvest storage of blueberries are mainly due to loss of firmness and microbial decay (Li, Luo, & MacLean, 2011).

Different technologies have been used in order to delay the fruit deterioration and to extend their shelf-life such as refrigeration, modified atmosphere packaging and UV irradiation (Chiabrando & Giacalone, 2011; Yang, et al., 2014).

The use of edible films or coatings represents an alternative and/or additional way for fruit preservation, because of their ability to reduce moisture, solute migration, respiration and transpiration rate, to maintain firmness and generally delay senescence (Tezotto-Uliana, Fargoni, Geerdink & Kluge, 2014).

In order to improve the efficiency and stability of edible coatings it is essential to find adequate composition of their formulations. The basic coating ingredients are polysaccharides, proteins and lipids, either as pure substances or in combination. Edible coatings have high potential to carry active and functional ingredients such as antimicrobial, antioxidant and antibrowning agents, colorants, nutrients that can enhance the nutritional values and the stability of products during their shelf-life (Rojas-Graü, Tapia, & Martín-Belloso, 2008).

Chitosan (poly β-(1,4)N-acetyl-D-glucosamine) polymer is industrially produced from chemical deacetylation of the chitin found in exoskeletons of crustaceans. This biopolymer can also be extracted from the cell wall of mushrooms, being biodegradable, non-toxic and non-allergenic, which contribute to its use in many fields, including food, biomedicine, agriculture and environmental protection (Shahidi, Arachchi, & Jeon, 1999; Kim & Rajapakse, 2005). Moreover, it has been shown to have mechanical and antimicrobial properties, no toxicity, biodegradability and to inhibit the growth of fungi on the surface of different fruits (Rojas-Graü et al., 2008; Treviño- Garza, García, del Socorro Flores- González, & Arévalo- Niño, 2015).

Procyanidins are one of the most abundant flavonoids present in grape seeds and skin. They are mainly proanthocyanidins (condensed tannins) mostly constituted of oligomeric flavonoids as catechin, epicatechin, epicatechin gallate and epigallocatechin (Souquet, Cheynier, Brossaud, & Moutounet, 1996). During food processing and storage, plant phenolic compounds are converted to a variety of reaction products that could contribute to the quality of plant-based foods, along with the genuine plant components (Cheynier, 2012). Moreover, these bioactive compounds can be used to add value and to improve the nutritional functions of

numerous foodstuffs (dos Reis, de Oliveira, Hagen, Jablonski, Flôres, & de Oliveira Rios, 2015; Rodriguez-Amaya, 2016; Martin & Ferreira, 2017). A lot of by-products from food processing could be a good source for the recovery of polyphenols, protein and pectin, that can be used as natural ingredients and or additive in food production (Kammerer, Kammerer, Valet, & Carle, 2014; Martins et al., 2017).

Nair, Saxena & Kaur (2018) investigated the effect of chitosan and alginate based coatings enriched with pomegranate peel extract, showing that chitosan based coatings was more effective than alginate in maintaining the postharvest quality of guava (*Psidium Guajava L.*). However, to the best of our knowledge, investigations on the influence of coatings based on chitosan from mushrooms alone or enriched with procyanidins, extracted from grape by-product, on fruit or vegetables quality have not been reported yet Thus, the main aim of this research work was to evaluate the effect of the application of specific innovative coatings on some quality characteristics (weight loss, pH, dry matter, colour and firmness), antioxidant activity (ABTS and DPPH assays) and microbial growth of blueberry samples during storage at 4°C for 14 days.

2. Material and methods

2.1 Fruit material

Organic blueberries were purchased from local market. Berry fruits were kept for one day at 0 ± 1°C until they were used. Fresh blueberries with similar colour and size and no damages were selected and these berries were characterized by dry matter of 15.1 ± 0.3 g/100g.

2.2 Preparation of coating solutions

Two different coating solutions were prepared, each of them contained 1.5 % (w/w) of glycerol (≥ 99.5% Sigma-Aldrich, Germany) and 0.20 % (w/w) of Tween® 20 (Sigma-Aldrich, France) and solved in citric acid solution 1% (Sigma- Aldrich, Germany). In a first solution, chitosan from mushrooms (C) provided by Agrovin (Alcazar de San Juan, Spain) was added in the quantity of 1 % (w/w). The second coating solution was prepared by combining chitosan from mushroom (1% w/w) and procyanidins extracted from grape seeds (Chardonnay berries) (0.8 % w/w) (CP). The extraction of procyanidins was performed as follows: briefly, 200 g of dehydrated seeds were extracted with water-ethanol (1:1 w/w) for 2 hours under stirring at 200 rpm. Extracts were rotary evaporated under vacuum at 35°C to remove ethanol. The resulting extracts were washed with hexane to remove lipid-soluble substances, and then rotary evaporated to remove the residual hexane. The aqueous fraction (about 75 mL) was applied to a Diaion HP-20 column (70×500 mm) previously equilibrated with water, and rinsed with 10% ethanol. Procyanidins were eluted using 100 mL water-ethanol 30:70 w/w, spray dried and stored at -30°C before their use.

The final concentration of procyanidins used for coating solution was chosen based on the higher antioxidant activity and unchanged sensorial properties of fruit tested in preliminary trials by trained panel (data not showed). Afterwards, all coating solutions were homogenised at 5000 rpm for 2 min in order to remove air bubbles.

2.3 Sample preparation

Blueberry fruits were surface disinfected by immersion in 200 ppm sodium hypochlorite water solution; successively they were washed in distilled water and dried on the surface with absorbing paper. Whole blueberry fruits were dipped in the coating solutions in two different steps (each one of 30 s), the first dipping was followed by drying step for 60 min at 25 ± 1 °C and the second one for 30 min at the same temperature. Blueberries dipped in distilled water with the same procedure were used as control. Afterwards, coated berry samples were placed in plastic trays (PET), closed in micro-perforated bags (PLA) to maintain aerobic conditions limiting fruit dehydration, and stored at 4°C for 14 days. All blueberries samples were analysed at 0, 2, 4, 6, 10 and 14 days of storage. Three samples were obtained as a total: 2 differently coated blueberry samples (C and CP) and one uncoated sample (F). For each sample, 720 berries were used. For every sampling time 3 trays were prepared, containing 40 blueberries randomly categorized and used for analytical determinations.

2.4 Quality determinations

2.4.1 Weight loss, dry matter and pH

Weight loss (WL) of blueberry samples during 14 days of storage was measured by weighting fruits in all trays per sample at the beginning of the storage and at every day of analysis; the results were calculated as percentage loss of initial weight, following the standard AOAC method (1994).

Dry matter (DM) was determined gravimetrically by difference in weight before and after drying at 70 °C, until constant weight was reached (AOAC International, 2002).

pH was measured at 20 °C with a pH meter CRISON GLP21 (Shinghai Shilu-Instruments, China).

For all treatment times and for each sample, DM was determined in triplicate from 9 blueberries and pH was measured also in triplicate on the three different juice sub-samples obtained from 15 berries (fruit:water 1:1).

2.4.2 Colour

Surface colour of blueberry fruits, were measured using a spectrophotocolorimeter HUNTERLAB ColorFlexTM, mod. A60-1010-615 (Reston, Virginia). For each sample, L*, a* and b* parameters from CIELAB scale were measured. Hue angle (h°), which is the hue in the CIELAB colour wheel, was calculated by the following equation:

$$h° = \tan^{-1}\frac{b^*}{a^*} \quad (1)$$

where: a* (red–green) and b* (yellow–blue) are parameters of colour measurement (Vega-Gálvez et al., 2012). The analyses were carried out in twelve repetitions from randomly selected blueberries from each sample at each storage day.

2.4.3 Texture

Firmness evaluation was conducted with penetration test by means of Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, Godalming, UK), equipped with a 50 N load cell and a 2 mm diameter stainless steel probe. Test speed was 0.5 mm s^{-1} and ended when a maximum deformation of 80% was reached. Results were expressed as average of twelve measurements performed on twelve blueberries from each sample at each storage day.

2.4.4 Antiradical activity (DPPH, ABTS assays)

The extraction was performed by mixing 0.5 g of freeze-dried sample whit 10 mL of methanol 60% (w/w) in centrifuge tube. The mixture was vortexed for 2 min, agitated for 10 min and centrifuged for 10 min at 18600 rpm in a centrifuge (Beckman) set at 4°C. The supernatants were collected and used to evaluate the antiradical activity by DPPH and ABTS assays.

The DPPH scavenging activity was based on the method proposed by Amarowicz, Naczk, & Shahidi (2000). Briefly, 0.1 mL of extract was added to 2 mL of methanol and 0.25 mL of DPPH (Sigma-Aldrich, USA), shaken with a vortex for 1 min and kept to the dark for 30 min. The absorbance was measured with a spectrophotometer (Beckman Coulter DU 730 Life Science model) at 517 nm. Antioxidant activity was quantified by plotting a Trolox calibration curve. Trolox concentration range was 0.001-1.500 mM (r^2 = 0.9980). The results were expressed as mmol Trolox/g of fruit.

The $ABTS^{+\bullet}$ scavenging activity was carried out following the method proposed by Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans (1999). 30 µL of extract were added to 3 mL of diluted $ABTS^{+\bullet}$ solution (Sigma-Aldrich, USA) after mixing and the absorbance was measured with a spectrophotometer (Beckman Coulter DU 730 Life Science model) at 734 nm every 30 s for a total time of 6 min; the results were expressed as mmol Trolox/g of fruit. Antioxidant activity was quantified by plotting a Trolox calibration curve. Trolox concentration range was 0.001-1.500 mM (r^2 = 0.9853).

The values provided are the average of three replicates from each sample at each day of storage.

2.4.5 Microbiological analysis

The cell loads of mesophylic aerobic bacteria, lactic acid bacteria, yeasts, moulds and total coliforms were monitored in all samples over the storage, according to the method reported by Mannozzi et al. (2016). The values obtained are the average of three independent sub-samples for each sample.

2.5 Data analyses

Analysis of variance (ANOVA) and the test of mean comparison, according to Fisher's least significant difference (LSD) were carried out on analytical replicates for F, C and CP blueberry samples. Level of significance was $p < 0.05$. The statistical software used was STATISTICA v 8.0 (StatSoft, Tulsa, Okhlaoma).

3. Results and discussion

3.1 Weight loss, dry matter and pH

The weight loss, dry matter and pH values of F and differently coated samples during 14 days of storage are reported in Table 1.

Table 1. Weight loss (%), dry matter (%) and pH of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4°C.

			Weight loss (%)			
	T2		**T4**	**T6**	**T10**	**T14**
F	-0.89 ± 0.03^{aA}		-1.23 ± 0.06^{aA}	-2.1 ± 0.4^{bA}	-3.80 ± 0.06^{cA}	-4.5 ± 0.3^{dA}
C	-0.87 ± 0.06^{aA}		-1.42 ± 0.05^{bA}	-2.1 ± 0.2^{cA}	-3.5 ± 0.1^{dA}	-4.5 ± 0.1^{eA}
CP	-0.8 ± 0.2^{aA}		-1.2 ± 0.3^{aA}	-2.37 ± 0.04^{bA}	-3.2 ± 0.4^{cA}	-4.4 ± 0.4^{dA}
			Dry matter (%)			
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	15.1 ± 0.1^{aA}	15.1 ± 0.1^{aA}	14.42 ± 0.09^{cB}	15.50 ± 0.02^{aA}	15.1 ± 0.3^{aA}	14.5 ± 0.2^{bB}
C	14.8 ± 0.7^{bcB}	14.8 ± 0.7^{bcA}	15.8 ± 0.2^{abA}	15.7 ± 0.1^{bA}	15.9 ± 0.2^{aA}	14.6 ± 0.1^{cB}
CP	15.0 ± 0.7^{aA}	15.04 ± 0.04^{aA}	15.5 ± 0.9^{aA}	15.0 ± 0.6^{aA}	15.46 ± 0.05^{aA}	15.34 ± 0.03^{aA}
			pH			
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	3.43 ± 0.09^{aA}	3.16 ± 0.05^{cB}	3.22 ± 0.05^{bcB}	3.26 ± 0.04^{bA}	3.19 ± 0.03^{bcA}	3.29 ± 0.08^{aA}
C	3.33 ± 0.11^{aA}	3.35 ± 0.07^{aA}	3.36 ± 0.02^{aA}	3.32 ± 0.05^{aA}	3.34 ± 0.09^{aA}	3.40 ± 0.18^{aA}
CP	3.39 ± 0.23^{aA}	3.29 ± 0.15^{aAB}	3.42 ± 0.09^{aB}	3.24 ± 0.08^{aA}	3.30 ± 0.09^{aA}	3.27 ± 0.10^{aA}

Data are reported as average values and standard deviations.

Means followed by different lowercase letters means significant different ($p<0.05$) during time (days, in rows) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in columns).

All the samples underwent a similar decrease of the weight during cold storage (around 4.5%); this could be due to the migration of water from the fruit to the environment. The weight loss of fruit and vegetables is due to the water vapour pressure gradient that exists from different compartments in the cell tissues (Yaman & Bayoındırlı, 2002). This result was in agreement with Carvalho et al. (2016), who observed that the use of chitosan based coating with trans-cinnamaldehyde was not able to reduce the weight loss of fresh-cut melon during 20 days of storage. Moreover, Mannozzi et al. (2016) observed a progressive decrease of weight loss, without seeing any significant differences between uncoated and differently coated (polysaccharide-based coating) blueberry samples during storage.

For what concern the dry matter (Table 1), no relevant differences ($p < 0.05$) were found between C and CP coated samples during the overall storage. In particular, only F sample underwent a slight decrease of dry

matter during 14 days of storage. The tendency to an increase of dry matter showed by CP sample during storage could be due to the solutes gain caused by the presence of coatings (Carvalho, et al., 2016).
As reported in Table 1, F samples showed, in general, a decrease in pH already after 2 days of storage in comparison to C and CP samples. However, all the blueberry samples showed a slight decreasing trend, even though not significant, of the pH during the overall storage. This is probably due to the greater loss of water and also it is possible that the loss of weight (up to 4 %) that occurred during the postharvest period influenced these values (Hernández-Muñoz, Almenar, Del Valle, Velez, & Gavara, 2008; Chiabrando et al., 2011)

3.2 Colour

Anthocyanins and other pigments derived from phenolic compounds are responsible for the colour of red fruit and wines (Cheynier, 2012). Table 2 reported the lightness (L*), a*, b*and hue angle (h°) values of control and coated blueberry samples during 14 days of storage at 4 °C.

Table 2. Lightness (L*), a*, b* and hue angle (h°) values of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4 °C.

			L*			
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	24.4 ± 0.3^{bcA}	25 ± 1^{bA}	24.8 ± 0.3^{bA}	23.6 ± 0.8^{cA}	24.5 ± 0.6^{cA}	26.1 ± 0.5^{aA}
C	17.80 ± 0.03^{dC}	19.2 ± 0.2^{cC}	16.67 ± 0.5^{eC}	17.5 ± 0.1^{dC}	20.1 ± 0.1^{bC}	20.9 ± 0.4^{aB}
CP	23 ± 1^{bB}	23.9 ± 0.2^{bB}	20.6 ± 0.3^{dB}	21.7 ± 0.2^{cB}	21 ± 1^{cB}	26.2 ± 0.6^{aA}
			a*			
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	-0.2 ± 0.1^{aB}	-0.6 ± 0.1^{bB}	-0.87 ± 0.04^{cA}	-0.7 ± 0.2^{cA}	-0.72 ± 0.04^{bcB}	-0.70 ± 0.09^{bcA}
C	0.46 ± 0.07^{aA}	-0.45 ± 0.07^{cB}	-1.0 ± 0.1^{dA}	-0.9 ± 0.4^{dAB}	-0.1 ± 0.2^{bA}	-0.5 ± 0.1^{bcA}
CP	-0.06 ± 0.06^{aB}	-0.27 ± 0.05^{bA}	-0.97 ± 0.06^{dA}	-1.1 ± 0.1^{dB}	-0.2 ± 0.1^{abA}	-0.5 ± 0.1^{cA}
			b*			
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	-4.28 ± 0.06^{cB}	-5.11 ± 0.09^{dC}	-4.2 ± 0.2^{bcC}	-3.2 ± 0.3^{aC}	-3.9 ± 0.1^{bC}	-4.1 ± 0.2^{bcC}
C	-2.7 ± 0.2^{cA}	-1.8 ± 0.6^{bA}	-1.9 ± 0.1^{bA}	-0.7 ± 0.4^{aA}	-1.5 ± 0.2^{bA}	-1.6 ± 0.1^{bA}
CP	-2.8 ± 0.2^{bcA}	-3.11 ± 0.08^{cB}	-3.2 ± 0.2^{cB}	-2.6 ± 0.4^{abB}	-2.5 ± 0.1^{aB}	-2.6 ± 0.4^{abB}
			h°			
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	88 ± 6^{aB}	83 ± 4^{bAB}	78 ± 4^{cdA}	76 ± 11^{dAB}	80 ± 7^{cB}	80 ± 5^{cA}
C	102 ± 15^{bA}	78 ± 10^{bB}	66 ± 9^{aC}	79 ± 12^{bA}	82 ± 14^{bB}	81 ± 14^{bA}
CP	89 ± 14^{aB}	87 ± 23^{aA}	73 ± 7^{cB}	71 ± 9^{cB}	86 ± 9^{abA}	80 ± 8^{bA}

Data are reported as average values and standard deviations.

Means followed by different lowercase letters means significant different ($p<0.05$) during time (days, in rows) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in columns).

Immediately after coating (T0) C blueberry samples displayed lower L* values than the F and CP ones. The observed lower lightness of chitosan coated blueberry is probably due to the presence of coating that caused changes in the surface properties (Hoagland & Parris, 1996). However, this behaviour has not been observed in CP coated blueberries probably due to the presence of procyanidins.

In C and CP coated blueberry samples a significant decrease of a* values ($p<0.05$) until the 6th day of storage was observed, then the values increased again. For the b* values, both coated blueberry samples exhibited higher values compared to the F one during the overall storage. C blueberry coated sample displayed significantly higher b* values ($p<0.05$) in comparison to CP sample starting from the 2nd day of storage.

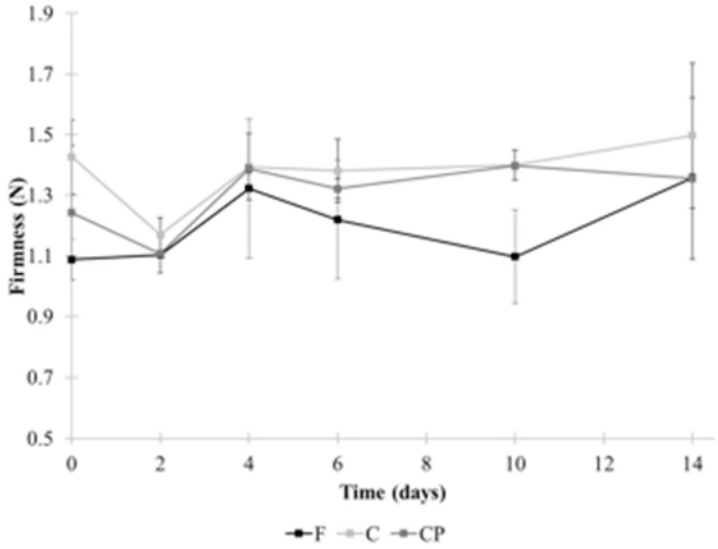
The h° values for all blueberry samples tended to decrease significantly ($p<0.05$) mostly during the first six days of storage, after this time the values raised again. The reduction of hue colour could be due to the oxidation reactions between polyphenol compounds that can cause loss of anthocyanins during cold storage of blueberry (Reque, Steffens, Jablonski, Flôres, Rios, & de Jong, 2014). Castañeda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal (2009) reported that the increased of the polymeric colour is probably due to the co-pigmentation phenomenon which promotes the formation of polymers occurred from the condensation of anthocyanins and other phenolic compounds and also the increase of hue values at the end of storage might be caused by a possible anthocyanins synthesis during ripening.

The h° results are in agreement with those observed by Mannozzi, et al. (2016) who studied the effects of different polysaccharide based coatings such as alginate, pectin and the combination of them on blueberry fruits. In fact, also in their work h° values are highest for all coated blueberry samples compared to control one. However, h° values were in the range from 140 to 179 for all coated blueberry samples, this discrepancy could be explained by the different biopolymer used into the coatings and also strongly depends on the raw materials properties.

3.3 Texture

Firmness is one of the most important critical quality parameter that influences the consumer acceptability of fresh products. As shown in Fig. 1, in general, C and CP coated blueberry samples exhibited a higher ($p < 0.05$) firmness in comparison to F sample, immediately after coating at 0 day of storage, which can be explained by the presence of coatings that provide rigidity to the skin of fruit (Duan, Wu, Strik, & Zhao, 2011). Generally, during the overall storage all the blueberry samples maintained similar texture values. However, coated samples showed significantly ($p< 0.05$) higher values immediately after coating (T0) and 10^{th} day of storage, compared to the uncoated ones. Moreover, the higher firmness of coated blueberry samples could be explained by the thickness of the two different coating formulations. In fact, thickness of C and CP coated blueberries measured in preliminary trials, ranged from 84 to 130 μm respectively.

The added procyanidins induced an increase in thickness and thus created more compact structure of enriched coating formulation compared to chitosan one. In fact the procyanidins that might create a bridge between chitosan and their free functional groups in the molecular structure (Zhang, Yang, Tang, Hu & Zou, 2008).



Time	F	C	CP
0	d C	b A	bc B
2	cd A	c A	c A
4	a A	b A	ab A
6	bc A	b A	b A
10	cd B	b A	a A
14	b A	a A	b A

Fig. 1. Firmness (N) of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4°C.

Means with different lowercase letters means significant difference ($p<0.05$) during time (days, in columns) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in rows).

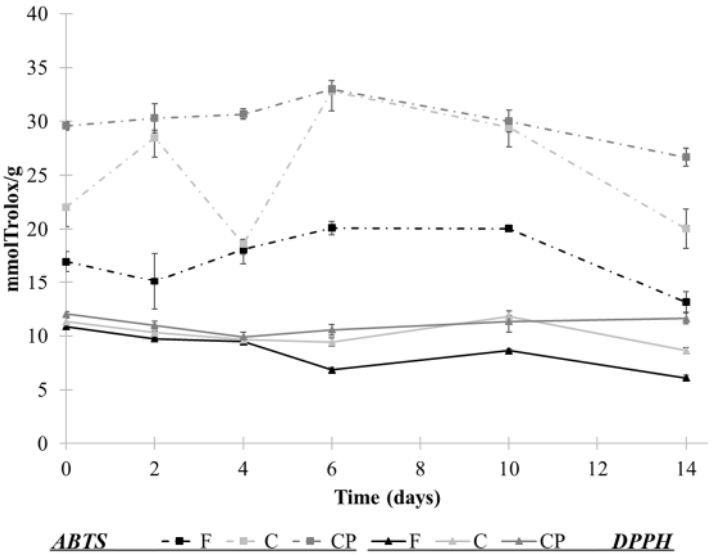
Blueberries are usually subjected to loss of firmness during postharvest, which subsequently tends to decrease fruit quality and shelf life (Li et al., 2011). Previous works showed that edible coatings were able to increase/improve firmness maintenance of blueberries (Duan et al., 2011; Mannozzi, et al., 2016). In general, it is expected that water loss leads to raise firmness during postharvest storage (Chiabrando et al., 2011). It has been well established that the loss of firmness is due to enzymatic hydrolysis of the cell wall and also due to the cell turgor loss promoted by transpiration, that cause softening of the fresh fruit tissues. Moreover, Yaman et al. (2002) reported that coated cherries better retain the firmness values when stored at cold storage temperature, as obviously expected.

3.4 Antiradical activity (DPPH, ABTS assays)

Blueberry fruits have a high antioxidant activity, especially due to their natural phenolic compounds and anthocyanin content, and for this reason could be one of the uppermost antioxidant resources among fruits and vegetables (Cheynier, 2012).

DPPH method seems to be more prone to detect flavanones, while ABTS method seems to be more suitable to detect the radical scavengers such as vitamin C (Del Caro, Piga, Vacca, & Agabbio, 2004). Nevertheless, these two methods are a useful tool to determine the antiradical scavenging activity of different fruits (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000).

In Figure 2, the results of antioxidant activity, obtained with DPPH and ABTS antiradical activity methods, of uncoated and differently coated blueberries during storage are showed.



ABTS

Time	F	C	CP
0	bc C	d B	abc A
2	cd B	b A	bc A
4	ab B	e B	ab A
6	a B	a A	a A
10	a B	b A	ab A
14	d C	c B	c A

DPPH

Time	F	C	CP
0	a B	b B	a A
2	b C	c B	ab A
4	b A	d A	c A
6	d C	d B	bc A
10	c B	a A	ab A
14	e C	e B	ab A

Fig. 2. Antiradical activity with DPPH method (▲) and ABTS method (■) of uncoated (F) and coated blueberry samples (C and CP) during 14 days of storage at 4°C.

Means with different lowercase letters means significant difference ($p<0.05$) during time (days, in columns) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in rows).

The antioxidant activity of blueberry fruits detected by using DPPH method was lower compared to that obtained with the radical ABTS. Despite DPPH scavenging activity is recommended as accurate and simple method for the detection of antioxidant activity of fruit and vegetable, it is less sensitive to the activity of hydrophilic antioxidant compounds (Gil et al., 2000).

Under both the analytical methods, the CP coated blueberries showed a higher antioxidant activity already at 0 day, in comparison to the C and the fresh ones. Its better retention during the overall storage period is probably due to the presence of chitosan and procyanidins in the coatings that provide the enhancement of antioxidant compounds. The use of procyanidins from grape by-products induced an improvement of the nutritional value of coated blueberry fruit. Moreover, all blueberry samples showed similar behaviour, with DPPH and ABTS antiradical activity method. It was possible to observe significant increase in antioxidant activity in C coated sample at 6^{th} and 10^{th} day with respectively ABTS and DPPH methods. This is probably due to the anthocyanins synthesis that occurs during ripening stage (Kalt, Forney, Martin, & Prior, 1999); these results are in accordance with h° colour data. For both analytical methods, studied C and CP based coatings were able to delay the loss of antioxidant compounds. Chiabrando & Giacalone (2015) reported similar results whit the application of chitosan on blueberries during 45 days of storage at 0 °C.

3.5 Microbiological analysis

In Table 3, the cell loads of total mesophilic aerobic bacteria, mould and yeasts during the storage at 4 °C are reported.

Table 3. Mesophylic aerobic bacteria, yeast and mould count of uncoated (F) and coated blueberry samples (C and CP) during 14 days of refrigerated storage at 4 °C

	Mesophylic aerobic bacteria					
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	3.31 ± 0.18^{aA}	2.79 ± 0.19^{bA}	2.11 ± 0.31^{cA}	2.12 ± 0.18^{cA}	2.18 ± 0.33^{cA}	2.97 ± 0.24^{abA}
C	2.70 ± 0.22^{abB}	2.49 ± 0.13^{bcA}	2.41 ± 0.25^{bcdAB}	2.04 ± 0.23^{dA}	2.12 ± 0.14^{cdA}	2.96 ± 0.26^{aA}
CP	3.34 ± 0.21^{aA}	2.75 ± 0.24^{bA}	2.50 ± 0.15^{bB}	2.57 ± 0.17^{bB}	2.70 ± 0.24^{bB}	2.89 ± 0.31^{bA}
	Yeast					
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	3.61 ± 0.33^{aA}	2.97 ± 0.26^{bA}	2.65 ± 0.31^{bA}	1.68 ± 0.33^{cA}	nd*	1.57 ± 0.25^{cA}
C	2.85 ± 0.21^{aB}	2.27 ± 0.31^{bB}	2.06 ± 0.24^{bB}	nd*	nd*	nd*
CP	3.12 ± 0.18^{aB}	2.53 ± 0.24^{bAB}	2.18 ± 0.12^{bcAB}	1.29 ± 0.26^{dA}	nd*	1.87 ± 0.14^{cA}
	Mould					
	T0	**T2**	**T4**	**T6**	**T10**	**T14**
F	2.39 ± 0.38^{aAB}	1.73 ± 0.26^{bA}	nd*	1.47 ± 0.19^{bA}	1.30 ± 0.22^{bB}	nd*
C	2.03 ± 0.17^{aB}	1.53 ± 0.15^{bA}	nd*	nd*	1.16 ± 0.27^{bB}	nd*
CP	2.82 ± 0.25^{aA}	1.81 ± 0.22^{bcA}	1.18 ± 0.24^{d}	1.64 ± 0.23^{cA}	2.07 ± 0.17^{bcA}	1.18 ± 0.23^{d}

Counts are expressed in Log cfu/g (± standard deviation).
Means followed by different lowercase letters means significant different ($p<0.05$) during time (days, in rows) and with capital letters means significant difference ($p<0.05$) between samples at each day of storage (in columns).

* under the detection limit (1 Log cfu/g)

The chitosan coated samples (C) showed a significant lower cell load of mesophilic bacteria at the 1st day of storage compared to the other samples. However, at the 4th day of storage a decrease of mesophilic aerobic bacteria was detected in all the considered samples and without significant differences between them. At the end of storage (T14), an increase of the mesophilic bacteria was detected for all the considered conditions without significant differences. However, the detected cell loads, except for samples F and CP immediately after treatments never exceeded a cell load of 3.0 log cfu/g.

As shown in Table 3, yeasts resulted significantly lower in samples C and CP immediately after treatments. During storage, CP samples showed yeast loads not significantly different in comparison to the samples F. Contrarily, yeast loads in samples C resulted significantly lower than control samples during the whole period of refrigerated storage, and after six days resulted under the detection limit. A similar trend was evidenced for mould cell loads (Table 3).

Lactic acid bacteria and total coliform cell loads resulted under the detection limit, independently from the coating adopted, during the whole storage period (data not shown).

The microbiological results obtained showed that all the considered samples did not reach a significant microbial spoilage during 14 days of storage at 4 °C (FSA of Ireland, 2016). On the other hand, it is widely reported that berries are rich in phenolic compounds that can have an antimicrobial activity (Lacombe, Wu, Tyler, & Edwards, 2010). In particular, Lacombe, Wu, White, Tadepalli, & Andre (2012) showed a strong

antimicrobial activity of phenolic compounds from North American lowbush blueberries against the growth of *E. coli O157:H7*. Moreover, Shen et al. (2014) showed a significant growth inhibition of *Listeria monocytogenes* to blueberry extracts from 4 different cultivars, indicating the potential of blueberry as natural antimicrobials in food products.

In addition, the obtained results showed, even if the microbial spoilage threshold ($>10^6$ cfu/g for yeast, and $>10^7/10^8$ cfu/g mesophylic aerobic bacteria) (FSA of Ireland, 2016) was not reached in all the considered samples, that in samples C there was a significant higher yeast and moulds inhibition compared to the other samples. These results are in agreement with other studies that evidenced the antimicrobial and antifungal activity of pectin, alginate and chitosan coatings on blueberry (Duan et al., 2011; Jiang, Sun, Jia, Wang, & Huang, 2016; Mannozzi et al., 2016).

4. Conclusions

The used innovative coatings (chitosan and chitosan+procyanidin) showed a positive effect mainly on maintaining the firmness and increasing the antioxidant activity (DPPH and ABTS methods) of blueberry samples. The use of procyanidins from grape by-product contributed to add value of coated organic blueberry fruit. In addition, the obtained results showed, even if the microbial spoilage threshold was not reached in all the considered samples, that the chitosan-based coated samples had a significant higher yeast and moulds inhibition compared to the uncoated ones. In general results from this study demonstrated the efficacy of the new type of coating ingredients (chitosan alone and with natural procyanidins) to maintain the overall quality of fresh blueberries during storage. Up to now, the use of chitosan is not allowed by the European regulation for organic production. However, obtained results could help to develop a new regulation that could consider the use of chitosan extracted from mushrooms as a valid opportunity for its application on organic fruits, since it is not a potential allergenic compound as happen for the one extracted from crustaceans (Vo & Kim, 2014).

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Role of thermal and electric field effects during the pre-treatment of fruit and vegetable mash by pulsed electric fields (PEF) and ohmic heating (OH)

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Role of thermal and electric field effects during the pre-treatment of fruit and vegetable mash by pulsed electric fields (PEF) and ohmic heating (OH)

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Abstract

The aim of this work was to quantify the recovery of juice and bioactive compounds of apple and carrot mashes treated by the electrotechnologies (PEF or OH) at different pre-heating temperatures (40, 60 or 80 °C), considering thermal and electric field based cell disintegration.

In general, a higher cell disintegration resulting from the applied pre-treatments also resulted in higher juice yield (around 10 % increase for carrot and 5 % for apple compared to untreated). Regarding the carotenoid content of obtained carrot juices, only the PEF pre-treatment at 20 °C resulted in an increased extractability. A release of total polyphenols from apples into the juice increased in all pre-treated samples compared to the control. This study suggests that a combined pre-treatment can further improve the cell disintegration and juice yield and may have the potential to increase the recovery of bioactive compounds depending on the treatment parameters.

Keywords Thermal effect; juice yield; bioactive compounds; PEF; OH; cell disintegration.

1. Introduction

Cell disintegration of fruit and vegetable mashes is an important step in order to improve the juice yield and facilitate the recovery of bioactive ingredients (Knorr & Angersbach, 1998). Novel technologies such as the application of pulsed electric fields or ohmic heating have already been introduced in order to replace or complement existing processing concepts.

Low intensity pulsed electric field (PEF) could alter the cell permeability (Knorr, Angersbach, Eshtiaghi, Heinz, & Lee, 2001; Dellarosa, Ragni, Laghi, Tylewicz, Rocculi, & Dalla Rosa, 2016; Tylewicz et al., 2017) by inducing reversible or irreversible electroporation on the cell membrane; thus, PEF treatments can increase the release of intracellular compounds from plant tissues (Toepfl, Mathys, Heinz, & Knorr, 2006).

At the same time, ohmic heating (OH) could also be used for extraction pre-treatments, as an alternative thermal method, since it allows heating in a rapid and uniform way is especially suitable for high viscous, particulate products such as fruit or vegetable mash (Jaeger et al., 2016). This technique may allow to improve the retention of vitamins, pigments and nutrients due to an optimized heating profile resulting in less thermal damage of heat sensitive substances.

To enhance the recovery of the juice and to promote the selectivity of bioactive compounds extraction from plant tissues, pulsed electric field (Jemai & Vorobiev, 2006; Guderjan, Elez-Martinez & Knorr, 2007; Schilling et al., 2007) and ohmic heating (Lima & Sastry, 1999; Wang & Sastry, 2000) have been already investigated.

Several aspects such as type of raw material, mash structure and particle size as well a de-juicing system were found to have a considerable impact on the benefits that can derive from a pre-treatment using electrotechnologies (Jaeger, Schulz, Lu, & Knorr, 2012). In addition, simultaneously occurring phenomena during the cell disintegration treatment such as thermal effects and electroporation phenomena need to be taken into account.

Fruit and vegetable raw materials differ in their textural properties, composition, cell size and structure as well as in the presence of secondary plant metabolites which need to be taken into account when developing targeted and tailored cell disintegration concepts.

In carrots, the majority of carotenoids are accumulated within the chromoplasts of the cell in a solid crystalline state surrounded by a membrane of bipolar lipids and proteins (Schweiggert and Carle, 2017). During carrot juice production, high levels of carotenoids are usually retained in the pomace. Many studies have already shown the possible positive effect of short blanching as a pretreatment to juice production on the carotenoid yield in carrot juice (Sharma, Kaur, Sarkar, Singh, & Singh, 2009; Ferrario, Guerrero, & Char, 2017). Higher yields are usually associated to a softening of cell structures, resulting in increasing release of carotenoid crystals from the matrix. Roohinejad, Everett, & Oey (2014) showed that an increasing electric field strength (0.1, 0.3, 0.6, 0.8 and 1 kV/cm) significantly increased the extractability of carotenoids from carrot pomace when organic solvents were used, while PEF treatment had, in general, a negative effect on the total carotenoid content when this has been measured directly in the juice. Hence,

the optimization of other extraction parameters such as the selection of suitable solvents depending on the target compound needs to be performed in order to convert the membrane disintegration achieved by PEF into improved diffusion and extraction properties.

Apples instead, are a rich source of different phenolic compounds that are mainly located in the plant cell vacuoles that can be divided into several classes: procyanidins (the most abundant), hydroxycinnamic acids, dihydrochalcones, flavonols, anthocyanins, and flavan- 3-ols (Oleszek, Lee, Jaworski, & Price, 1988; Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999; Alonso-Salces, Barranco, Abad, Berrueta, Gallo, & Vicente, 2004). Polyphenols are secondary metabolites usually with higher concentrations being present in the peel rather than in the flesh of fruits. They are generally recognized for their health benefits promoting antioxidant properties and contributing to the colour and sensory quality of fresh and processed products (Khanizadeh, Tsao, Rekika, Yang, Charles & Rupasinghe, 2008; Pandey & Rizvi, 2009; Cheynier, 2012). Polyphenol compounds in apple could be affected by the variety, post-harvest factors, ripening stage and storage conditions (Burda, Oleszek, & Lee, 1990; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999).

Jaeger et al. (2012) applied PEF treatment ($W_{specific}$ = 2 kJ/kg and 12 kJ/kg) to apple and carrot mashes and compared the total juice yield, carotenoid content and total polyphenols after applying four different de-juicing systems. The total polyphenol content increased on apple juice pretreated with PEF for all de-juicing systems and carotenoid contents increased as compared to the untreated samples in three of the four de-juicing systems. Praporscic, Lebovka, Ghnimi, & Vorobiev (2006) reported the effect of ohmic heating on juice yield from potato and apple slices. A more efficient juice extraction was observed when mild electrically heating (50 °C) was applied especially for potato compared to apple tissues.

In addition to the optimization of the PEF and OH process parameters to reduce the energy requirement and process time, more information is required on specific effects that may result either from thermal or from electric field cell disintegration. Subsequently, their impact on the recovery of juice as well as selected compounds from the raw material need to be studied.

Therefore, the aim of this work was to investigate the application of PEF and OH treatments for apple and carrot mash in order to enhance the juice yield and the recovery of bioactive compounds such as polyphenols and carotenoids, respectively. The optimization of the two processing technologies was performed taking into account the modulation of process parameters as well as treatment temperatures by applying a pre-heating step (40, 60 or 80 °C) in order to evaluate the role of thermal and electric field induced cell disintegration effects.

2. Material and methods

2.1 Plant raw material and mash preparation

Fresh commercial carrots and apples were purchased from the local market. The fruit and vegetable matrices were washed and pre-cut. The mash was prepared using a mill (Alexanderwerk, Austria) with replaceable stainless-steel screens providing a grinding level of 2 mm for carrots and 5 mm for apples. The

electrical conductivity of the apple mash was 1.3 mS/cm, the carrot mash had an electrical conductivity of 2.3 mS/cm.

2.2 Mash pre-treatment and juice production

For PEF treatment of apple and carrot mash, a batch PEF system (DIL, Germany) equipped with a parallel plate electrode treatment chamber (distance 5 cm) was used. The voltage was set to 4 kV in order to achieve an electric field strength of 0.8 kV/cm in the treatment chamber, reported to be sufficient for an irreversible electroporation of plant cells (Angersbach, Heinz, & Knorr, 2000). A number of 50 exponential decay pulses (discharge capacity 0,5 µF, pulse energy 4 J) with a pulse width of 10 µs was applied to 400 g of mash resulting in a total treatment time of 0.5 ms and a total specific energy input of 0.5 kJ/kg.

For the OH treatment, the same treatment chamber was used and connected to a generator (DIL, Germany) providing 572 V at 12 kHz resulting in an electric field strength of 114 V/cm. The resulting electrical current flow reached 1.1 A and therefore a power of 0.6 kW. Different temperature-time profiles were obtained depending on the selected temperatures for the different treated mashes. Ohmic heating was performed in order to reach mash temperatures of 40, 60 and 80 °C. Depending on initial and final mash temperature and based on an average heating rate of 3.7 ± 0.2 K/s, heating times were between 74 - 237 s.

Different initial pre-heating temperatures were used before applying the PEF and OH treatment. In order to provide a fast pre-heating of the mash, a microwave (MT 267, Whirlpool) with a power of 850 W was used. Depending on initial and final mash temperature and based on an average heating rate of 0.2 ± 0.03 K/s, heating times were between 90 – 360 s.

Table 1 summarizes the applied treatments and the resulting mash temperatures. Temperatures were measured with a PT100 thermocouple and the specific energy consumption was calculated based on power consumption and heating time.

Table 1. Overview on mash treatment conditions applied for apple and carrot mash

Treatment	Sample	$W_{specific}$ [kJ/kg]
Untreated	Control	0
PEF at 20 °C	PEF (20 °C)	0.5
Pre-heating 40 °C + PEF	40 °C-PEF	192.5
Pre-heating 60 °C + PEF	60 °C-PEF	382.5
Pre-heating 80 °C + PEF	80 °C-PEF	765.5
OH from 20 °C to 40 °C	(20 °C-40 °C) OH	110
OH from 20 °C to 60 °C	(20 °C-60 °C) OH	222
OH from 20 °C to 80 °C	(20 °C-80 °C) OH	355
Pre-heating 40 °C + OH to 80 °C	40 °C-80 °C OH	402.5
Pre-heating 60 °C + OH to 80 °C	60 °C- 80 °C OH	497.5
Pre-heating 40 °C	40 °C	192
Pre-heating 60 °C	60 °C	382
Pre-heating 80 °C	80 °C	765

After the different pre-treatments, the mash was collected and cooled to room temperature before pressing at 10 bar for 4.45 min using a manual laboratory juice press (Hafico, Germany) with textile cloth. All treatments were performed in three replicates.

Juice yield, total suspended solids, total dissolved solids and pH were determined directly in the fresh juices. For the determination of the carotenoid and total polyphenol content, juice samples were frozen and stored at -30 °C until analysis.

2.3 Determination of cell disintegration index (CDI)

The degree of cell disintegration of vegetable mashes was determined based on impedance measurement in order to calculate the cell disruption by mechanical grinding as well as by the applied pre-treatments according to Angersbach, Heinz and Knorr (1999). The impedance analyzer (Sigma check, Germany) was working in the range of 5.50 kHz-1.4 MHz and measurements took place at a mash temperature of 20 °C. The measuring cell consisted of two stainless steel cylindrical electrodes (diameter 1 cm) separated to a distance of 1 cm by a polyethylene tube containing the carrot or apple mash respectively. The CDI was quantified between 0 (intact tissue) and 1 (complete cell disintegration) achieved by multiple freezing and thawing cycles.

2.4 Calculation of juice yield and juice quality characterization

The juice yield Y [%] obtained from fruit and vegetable mashes was calculated according to Eq. (1):

$$Y = \frac{m_j}{m_r} * 100 \quad (1)$$

with

m_j = mass of obtained juice

m_r = mass of mash

In order to consider the different content of total suspended solids TSS [%], a corrected juice yield was calculated according to Eq. (2). In this way, it is possible to compare the real juice yield of different applied pre-treatments.

$$Y_{TSScorr} = Y \times \left(1 - \frac{TSS}{100}\right) \quad (2)$$

The TSS content was obtained by gravimetric determination of suspended particles removed from a juice sample of 6 g by centrifugation at 15,000 rcf for 60 min. TSS analysis was performed in duplicate for each obtained juice sample.

In addition, pH was measured with a pH-meter (Portamess, Germany) and total dissolved solids content (TDS expressed in °Brix) was determined with a refractometer (Pal-3 Atago, Japan) in each fresh juice sample immediately after pressing.

2.5 Carotenoid content

For the carotenoid extraction, 1 mL of the juice sample was mixed with 5 mL extraction solvent (50 % Acetone, 50 % (v/v) ethanol containing 50 mg/L butylated hydroxytoluene) and 5 mL hexane in a centrifuge tube (protected from light). The mixture was manually shaken for 2 minutes and 1 mL 10 % NaCl was added to help phase separation. The upper hexane phase was transferred into a 10 mL volumetric flask. A second extraction was done on the residue using 3 mL of hexane. The hexane fractions were combined and filled up to a total volume of 10 mL using hexane.

An aliquot of the hexane fraction was transferred into a quartz glass cuvette and the absorption at λmax (448 nm) was measured using a UV-Vis spectrophotometer (HITACHI U-1100, Japan). The total carotenoid concentration was calculated using Eq. (3) based on Lambert-Beer theory.

$$C\left[\frac{mg}{mL}\right] = \frac{A \times V \times 10^3}{A_{1\,cm}^{1\%} \times 100 \times Vs} \quad (3)$$

with

A - absorption at λmax (448 nm),

$A_{1\,cm}^{1\%}$ - average of the specific absorption coefficient for carotenoids of 2500 100 mL g^{-1} cm^{-1},

V - final volume of the hexane fraction (10 mL) and

Vs - sample size (1 mL).

2.6 Total polyphenols

In apple juice, the total polyphenol content (TP) was determined according to the Folin-Ciocalteu method (Singleton & Rossi, 1965). Juice samples were centrifuged at 10,000 rcf for 15 min. 100 µL of juice sample was added to 0.5 mL of Folin-Ciocalteu Reagent. The mixture was allowed to equilibrate for 5 min and then mixed with 2 mL of sodium carbonate solution (15 %). After incubation in the dark at room temperature for 2 hours, the absorbance of the obtained mixture was read at 750 nm (HITACHI U-1100, Japan). Water was used instead of juice for preparation of the blank sample in the assay. The TP content was expressed as mg of ferulic acid equivalents (FAE) per L of sample based on the standard curve obtained with ferulic acid. The coefficient of determination for the calibration curve was $R^2 = 0.9879$ which indicates a strong linear correlation.

2.7 Data analyses

The obtained data were analyzed using Shapiro-Wilk and Levene's tests to verify the normality of the distribution and the homogeneity of the variances. Whether those conditions were satisfied, parametric analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test were applied to compare the means at the level of confidence of 95 % ($p<0.05$). Conversely, non-parametric ANOVA (Kruskal-Wallis) along with Holm's post-hoc tests were carried out at the same level of significance ($p<0.05$). R statistical software (R foundation for statistical computing, Vienna, Austria) was employed to perform all the tests.

3. Results and discussion

3.1 Cell disintegration index

The mechanical grinding during mash preparation was found to reduce the particle size while resulting in cell disintegration at the same time reaching CDI values of 0.36 for carrot and 0.6 for apple. Due to a larger cell size for apple compared to carrot (average of 200 µm instead of 70 µm), mechanical grinding results in a higher CDI level for apple. In addition, impedance measurement revealed an increase in cell disintegration after pre-heating as well as after PEF and OH treatment, however samples pre-heated at 40 and 60 °C without any further treatments showed the same values of CDI as control one. Therefore these two treatment conditions have not been studied for subsequent analysis. The obtained results showed an increase of the CDI in the range of 30 – 40 % for all treated carrot samples compared to the control sample independently from the type of pre-treatment. For apple, an increase of that extent could only be detected for the samples that had reached 80 °C (with or without additional PEF or OH treatment) (Fig. 1).

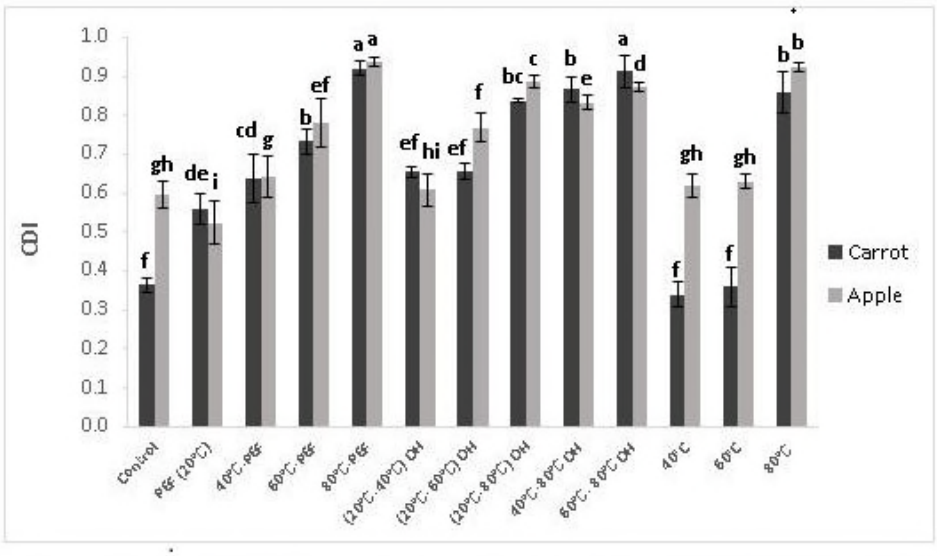

Fig. 1 Cell disintegration index – CDI of carrot and apple mashes after grinding (control) and after different pre-treatments.

Different letters indicate significant differences ($p<0.05$) between samples.

The highest level of cell disintegration (> 0.91) for apple and carrot could be reached by a pre-heating to 80 °C and additional PEF treatment. This finding indicates that a pre-heating to 80 °C did not fully disintegrate the cell membrane leaving intact cells behind that are still sensitive to electroporation by PEF.

Thermal-only treatment to a final temperature of 80 °C either performed by OH or pre-heating using microwave resulted in a similar level of cell disintegration. The thermal cell disintegration leads to the cell membrane denaturation as well as the destruction of the cell due to the cell liquid expansion (Moussa-Ayoub et al., 2016). For the OH with treatment times in the range of 74 – 237 s performed at a field strength of 114 V/cm, cell disintegration based on electropermeabilization may occur to small extent (Praporscic et al., 2006; Lebovka, Praporscic, & Vorobiev, 2004a). However, thermal cell disintegration was found to be more pronounced at increased temperature. For PEF application, the cell disintegration is clearly based on the electroporation of the cell membrane. The effect of electroporation was more pronounced for carrot instead of apple mash. This is mainly due to the larger fraction of cells that is still intact after mechanical grinding of carrot compared to apple.

Jaeger et al. (2012) reported similar results with a disintegration index of around 0.84 for both carrot and apple mash at different grinding levels, after the application of a PEF treatment at 3 kV/cm. Application of PEF at elevated temperature was found to increase the cell disintegration effect compared to PEF applied at 20 °C. Furthermore, pre-heating and subsequent application of OH at elevated temperature was found to be more effective compared to reaching the same final temperature by OH only. Praporscic et al. (2006) showed an increase of CDI with the rise of electric field strength and the temperature (maximum 50 °C) on potato and apple tissues treated by OH for 20 s. Also, Lebovka et al. (2004a) demonstrated that the

application of mild thermal treatment in combination with PEF allows to enhance the cell damage on apple, carrot and potato tissues.

3.2 Juice yield and quality parameters

3.2.1 Juice yield

The corrected juice yield $Y_{TSScorr}$ values for carrot and apple mashes are shown in Fig. 2.

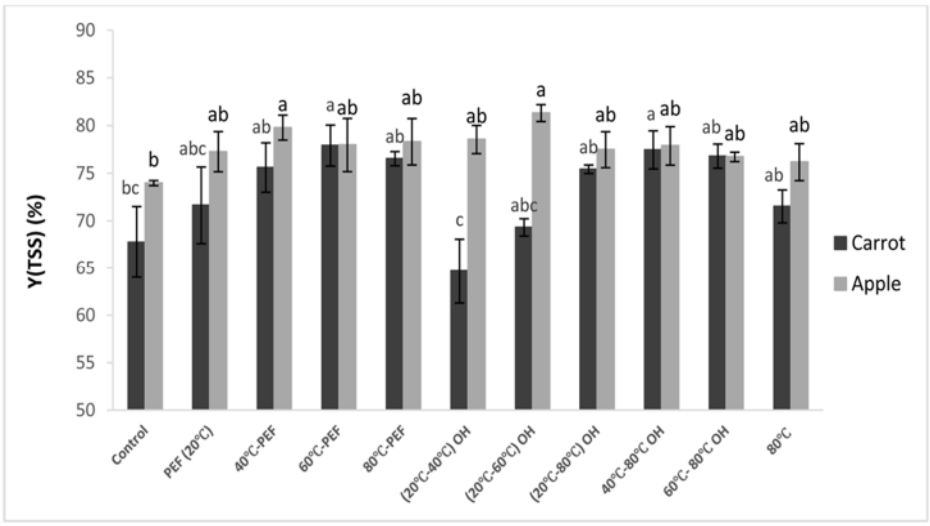

Fig.2 Corrected juice yield considering the suspended solids in juices obtained from apple and carrot mash after different pre-treatments.

Different letters indicate significant differences ($p<0.05$) between samples.

In general, higher CDI levels detected for pre-treated juice samples at 40, 60 and 80 °C coupled with PEF and OH and for the sample heated to 80 °C only, also corresponded to higher Y of around 10 % for carrot and 5 % for apple compared to the control samples. Higher increase of juice yield of around 11 % was also found for carrot tissue by Jaeger et al. (2012) whereas a PEF treatment of apple mash resulted in a juice yield increase of around 6 % only. Schilling et al. (2007) reported an increase of apple juice yield in the range of 1.7 % to 7.7 % by the application of PEF at different field strength intensities (1, 3 and 5 kV/cm, n=30 pulses).

Lower pre-heating temperatures seem to be favorable in order to avoid softening of the mash which may limit the juice yield. Hence, similar or even higher juice yield was achieved by lower pre-heating temperatures and additional PEF and OH application compared to more severe thermal conditions. However, combinations of temperature and PEF or OH did not reveal differences in the juice yield for some cases although different levels of cell disintegration had been detected. Whereas the application of mild heat was found to be beneficial also in other studies, severe heating seems to negatively affect the

mash structure. This effect is also more pronounced for apple compared to carrot with a firmer tissue structure.

Many studies reported the positive effect of the application of PEF and mild heat for the improvement of the juice release from fruit or vegetable tissues (Lebovka, Praporscic, & Vorobiev, 2004b; Praporscic et al., 2006; Jaeger et al., 2012; Moussa-Ayoub et al., 2016). Previous works by Lebovka et al. (2004a) showed that thermal damage is negligible at moderate temperature below 60 °C, hence changes in structural properties in this temperature range could be mainly explained by electroporation effects. However, besides thermal and electric field cell disintegration effects, heating may also lead to a modification of cell wall properties, alteration of binding of intracellular compounds and tissue softening (Hoff & Castro, 1969; Linehan & Hughes, 1969). Moderate electrothermal treatment application investigated by Wang & Sastry (2002) resulted in an increase in the apple juice yield of around 23 %, and in particular the yield increased with OH pre-treatment instead of microwave heating at 50 °C which could be an indication of additional electropermeabilization effects occurring for the applied treatment.

In addition, as reported by Jaeger at al. (2012), particle size, mash structure and de-juicing system need to be taken into account in order to convert achieved cell disintegration into higher juice yield.

3.2.2 Juice quality parameters

The results of total suspended solids (TSS), pH and total dissolved solids (TDS) are reported in Table 2.

Table 2. Total suspended solids TSS [%], pH and total dissolved solids TDS [°Brix] of pre-treated carrot and apple samples.

Samples	Carrot			Apple		
	TSS (%)	**pH**	**TDS [°Brix]**	**TSS (%)**	**pH**	**TDS [°Brix]**
Control	3.5 ± 0.5^{a}	6.6 ± 0.2^{a}	8.1 ± 0.3^{cd}	3.5 ± 0.4^{a}	3.68 ± 0.06^{a}	11 ± 2^{a}
PEF (20 °C)	3.5 ± 0.4^{a}	6.50 ± 0.05^{a}	8.8 ± 0.3^{abcd}	4.1 ± 0.4^{a}	3.7 ± 0.1^{a}	11.6 ± 0.8^{a}
40 °C-PEF	3.2 ± 0.4^{ab}	6.2 ± 0.3^{abc}	9 ± 1^{abcd}	4.1 ± 0.8^{a}	3.73 ± 0.02^{a}	12 ± 1^{a}
60 °C-PEF	2.6 ± 0.2^{ab}	5.89 ± 0.05^{bc}	9.4 ± 0.5^{abcd}	4.4 ± 0.1^{a}	3.72 ± 0.04^{a}	12.4 ± 0.7^{a}
80 °C-PEF	$2,6 \pm 0.6^{ab}$	5.86 ± 0.06^{bc}	10 ± 1^{ab}	2.9 ± 0.3^{a}	3.68 ± 0.01^{a}	13 ± 1^{a}
(20 °C-40 °C) OH	3.1 ± 0.5^{ab}	6.4 ± 0.2^{ab}	7.9 ± 0.3^{d}	3.0 ± 0.6^{a}	3.7 ± 0.1^{a}	11 ± 1^{a}
(20 °C-60 °C) OH	3.4 ± 0.4^{a}	6.09 ± 0.16^{abc}	8.2 ± 0.7^{bcd}	2.8 ± 0.6^{a}	3.7 ± 0.2^{a}	10.3 ± 0.4^{a}
(20 °C-80 °C) OH	2.8 ± 0.2^{ab}	5.8 ± 0.3^{c}	8.6 ± 0.8^{abcd}	3.2 ± 0.2^{a}	3.64 ± 0.08^{a}	12 ± 1^{a}
40 °C-80 °C OH	2.6 ± 0.4^{ab}	5.8 ± 0.2^{c}	9 ± 1^{abcd}	3.0 ± 0.1^{a}	3.76 ± 0.01^{a}	11.1 ± 0.1^{a}
60 °C- 80 °C OH	3.0 ± 0.4^{ab}	5.8 ± 0.1^{c}	10.1 ± 0.8^{abc}	3.6 ± 0.8^{a}	3.84 ± 0.06^{a}	11.6 ± 0.3^{a}
80 °C	2.2 ± 0.3^{b}	6.0 ± 0.1^{bc}	10.6 ± 0.4^{a}	3.0 ± 0.4^{a}	3.7 ± 0.2^{a}	13 ± 1^{a}

Data are reported as average values and standard deviations are indicated.
Different letters mean significant differences ($p<0.05$) between samples.

The TSS consists of solid particles being tissue and cell wall fragments that are transferred into the juice during grinding and pressing operations.

For carrot, higher temperatures applied for the mash treatment (80 °C with or without PEF treatment as well as OH up to 80 °C) slightly decreased the TSS content compared to the control samples. The TSS content mainly depends on particle generation during grinding and further mechanical processing of the mash, the filtration properties of the press cake, the tissue textural properties, the mash structure and also the mash and juice viscosity (Jaeger et al., 2012). Compaction of the press cake leads to a reduced porous structure that increases the retention of fine particles. Softening of the carrot tissue and mash particles by heat or PEF treatment results in a different mash structure and higher compaction during pressing which may have lowered the transfer of suspended solids from the mash into the juice and probably result in lower TSS values for all pre-treated carrot juice samples at the different temperatures (40, 60 and 80 °C) either with PEF or OH compared to the control. However, for apple samples, no impact of the pre-treatment on TSS content was found.

Concerning the pH, a significant decrease was found only for carrot juice obtained after pre-heating 60 or 80 °C coupled with PEF, (20 °C-80 °C) OH, 40 or 60 °C pre-heating combined with OH and the sample heated to 80 °C only. Higher temperatures and the application of additional cell disintegration treatments seem to affect the transfer of acids into the juice thus lowering its pH. However, for apple juice, no significant change of pH was found.

The total dissolved solids (TDS) content is mainly represent by sugars and organic acids that are present in the vacuole sap. The application of heating, PEF and OH treatments enhances the disruption of intact cells and vacuoles that allow the release of juice and consequently the leach of the vacuole sap. Also, the filtration properties of the press cake could influence the release of dissolved solids into the juice since the drainage properties depend on the tissue and mash structure.

Pre-heating of carrot mash to 80 °C with or without additional PEF application resulted in a significant increased TDS content compared to untreated samples. For apple juice, no significant differences were detected among all the different samples. Similar results were also obtained by Jaeger et al. (2012) where no impact of PEF treatment was found on TDS content for carrot and apple juices. Turk, Vorobiev, & Baron (2012) also reported no significant differences between PEF treated and control apple mash despite the increase of dry matter for treated samples.

3.3 Carotenoid content

The carotenoid content of carrot juice was determined and is expressed in mg of total carotenoids for one liter of juice (Fig. 3).

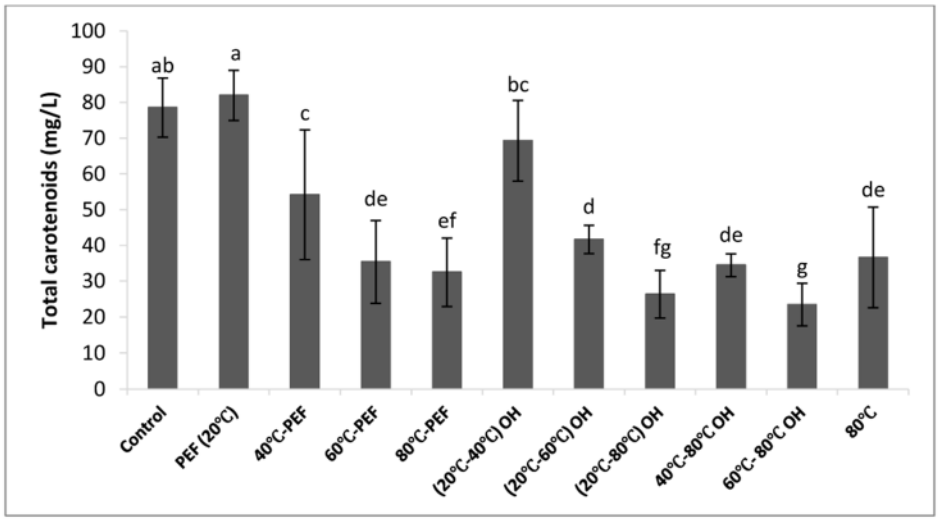

Fig. 3 Carotenoid contents of pre-treated carrot juices.

Different letters means significant different ($p<0.05$) between samples.

PEF treatment at room temperature (20 °C) increased the carotenoid extraction yield compared to the untreated control sample but the increase was of no statistical significance under the tested conditions. All other pre-treatment conditions with temperatures at or above 40 °C led to a decrease in carotenoid content in the final juice. Furthermore, the carotenoid content of the juices decreased with increasing pre-treatment temperatures.

Temperature and PEF treatment lead to a softening of the cell tissue which usually results in an increased extraction of compounds. At the same time, the increase of temperature also promotes oxidative degradation of some ingredients and the cell disintegration promotes the release of enzymes that may contribute to degradation processes.

For the PEF treatment at room temperature, the results are in agreement with Grimi, Praporscic, Lebovka, & Vorobiev (2007) who reported that approximately the same content of carotenoids was obtained in juice, either with or without the application of PEF, using moderate electric field strengths (0.25 – 1 kV/cm).

For treatments involving the pre-heating, small carotenoid losses were also reported during short blanching and heating of intact cell tissue (Britton & Khachik, 2009). However, the combined effect of increased temperature and electroporation of the tissue obviously leads to a loss of carotenoids in an oxygen containing environment.

Oxidative degradation might have taken place in the carrot pomace directly after or during the pretreatment even though, the time to transfer and cooling the mash before the pressing did not exceed 10 minutes.

3.4 Total polyphenols

Total polyphenol (TP) content of differently obtained apple juice samples is shown in Fig. 4.

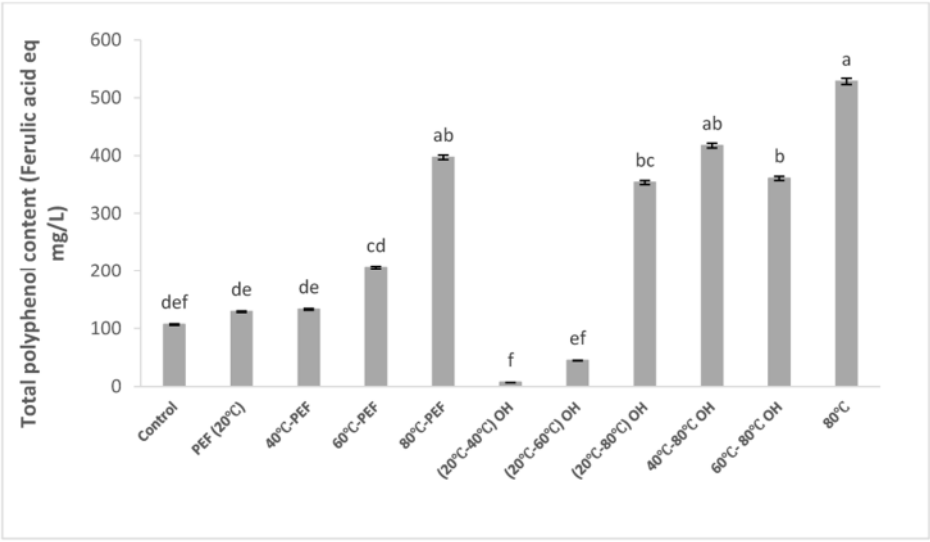

Fig. 4 Total polyphenol content in apple juices obtained from pre-treated apple mash.

Different letters indicate significant differences ($p<0.05$) between samples.

The concentration of phenolic contents in the juice is related not only to the transfer of polyphenols into the juice but also to polyphenoloxidase (PPO) activity that is the degradative enzyme responsible for the oxidation of polyphenols. In the presence of oxygen and PPO, chlorogenic acid is converted into its o-quinone, which further reacts with other phenolic compounds, resulting in the formation of yellow and brown pigments (Oszmianski & Lee, 1990). In addition, the degree of browning of apples was found to be dependent on the relationship between hydroxycinnamic acids and procyanidins (Amiot, Tacchini, Aubert & Nicolas, 1992). Polyphenols are mainly located on the peel of apple fruit and are dissolved in vacuoles of the cell that cover up to 90 % of the total volume of the tissue (Taiz, Zeiger, Møller, & Murphy, 2007). Significantly higher TP values were obtained for apple juices pre-heated to 80 °C with or without additional PEF or OH treatment. However, OH reaching 40 °C and 60 °C seems to reduce the TP content compared to the control juice which might be an indication of insufficient enzyme inactivation still leading to polyphenol degradation. PEF treatment without pre-heating did also not affect the polyphenol content in the juice which is in accordance with Schilling et al. (2007) who reported no significant changes on TP content between control and PEF treated apple mash for different field intensities (1, 3 and 5 kV/cm). Cell disintegration occurring due to PEF or thermal treatment leads to cell permeabilization increasing juice yield and the release of polyphenols into apple juice. In addition, higher temperatures lead to the inactivation of the oxidative enzymes such as polyphenoloxidase and peroxidase, thus reducing their degradation effects and resulting in higher polyphenol concentrations in the juice. Additional effects other than the permeabilization need to be taken into account since the thermal treatment may affect the binding properties of selected compounds leading to an increased release but higher enzymatic or non-enzymatic degradation processes at the same time (Shilling et al., 2007).

4. Conclusion

In general, higher detected CDI for pre-treated juice samples at 40, 60 and 80 °C coupled with PEF or OH also contributed to higher juice yield of around 10 % for carrot and 5 % for apple compared to untreated samples. Even at higher pre-treatment temperature, the application of PEF and OH provided additional benefits leading to a further increase of juice yield compared to the thermal reference sample that was only heated to 80 °C. This finding was even more pronounced for PEF than for OH indicating its stronger electropermeabilization effect. While an increase in temperature can increase the juice yield, the degradation of sensitive bioactive compounds such as carotenoids may be triggered due to high temperatures and the presence of oxygen. Further models need to be developed in order to take into account thermal and oxidative degradation kinetics competing with the improved release due to cell disintegration effects. This study showed that a thermal treatment followed by PEF or conducted by OH can still improve the cell disintegration and yield; moreover the application of high temperature may contribute to the fast inactivation of enzymes, leading to the release of polyphenols. Hence, raw material dependent processes need to be designed taking into account i) tissue textural properties and the degree of cell disintegration for the release of juice and valuable compounds, ii) thermal effects that may influence not only the cell disintegration but also binding properties, solubility and release of ingredients, iii) thermal effects promoting the degradation of heat or oxygen sensitive compounds and iv) thermal effects contributing to the inactivation of enzymes also making use of fast heating rates that can be achieved by OH.

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Influence of pulsed electric field (PEF) and ohmic heating (OH) pre-treatment on enzyme and antioxidant activity of fruit and vegetable juices

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Influence of pulsed electric field and ohmic heating pre-treatments on enzyme and antioxidant activity of fruit and vegetable juices

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Abstract

The objective of this work was to optimize Pulsed Electric Field (PEF) or Ohmic Heating (OH) application for carrot and apple mashes treatment at different pre-heating temperatures (40, 60 or 80 °C). The effect of tissue disintegration on the properties of recovered juices was quantified taking into account the colour change, the antioxidant activity and the enzyme activity of peroxidase (POD) in both, carrot and apple juice, and polyphenol oxidase (PPO) in apple juice. Lower ΔE* and an increase of the antioxidant activity were obtained for juice samples treated with temperature at 80 °C with or without PEF and OH pre-treatment compared to untreated samples. The inactivation by 90 % for POD and PPO was achieved when a temperature of 80 °C was applied for both carrot and apple mash. Obtained results are the basis for the development of targeted processing concepts considering the release, inactivation and retention of ingredients.

Keywords PEF; OH; POD; PPO; extraction; juice.

1. Introduction

Novel technologies such as pulsed electric field or ohmic heating have already been introduced in order to replace or complement existing processing concepts.

Low intensity pulsed electric field (PEF) can enhance the mass transfer during extraction by increasing cell membrane permeability, known as electroporation. Therefore, PEF treatments can enhance the release of specific intracellular compounds from plant tissues (Fincan & Dejmek, 2002; Toepfl, Mathys, Heinz, & Knorr, 2006; Jaeger et al., 2012; Moussa-Ayoub et al., 2016).

At the same time, ohmic heating (OH) can be used as an alternative thermal pre-treatment prior to extraction. The volumetric energy dissipation and the rapid and uniform heating represent advantages especially for high viscous, particulate products such as fruit or vegetable mash (Jaeger et al., 2016). In addition, the short processing times during the OH treatment may cause less degradation of colour and heat sensitive substances.

In plant cells, antioxidant compounds are mainly located in the vacuoles, whereas the enzymes peroxidase (POD) and polyphenoloxidase (PPO) are found in plastids (Terefe, Buckow & Versteeg 2014).

Processing of plant tissues compromises the internal compartmentalization that allows the contact between degradative enzymes and their substrates (phenolic compounds), implying the reaction known as enzymatic browning. In the case of POD, phenolic compounds are oxidized at the expense of H_2O_2 leading to flavour changes (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998). Instead, PPO is an oxidoreductase, which catalyses the oxidation of phenolic compounds in *o*-quinones, which are subsequently polymerized into brown pigments (Jayaraman, Ramanuja, Dhakne, & Vijayaraghavan, 1982). Therefore, the inactivation of POD and PPO enzymes is a crucial prerequisite and indicator of quality in the processing of fruit and vegetables.

Moreover, the activation of enzymes including an increased release and the enhancement of enzymatic reactions by the cell disintegration applied at early stages during fruit and vegetable processing might be a limitation for the shelf life of recovered juices. Thermal treatment has been used in order to reduce the enzyme activity, but it causes negative effects on quality and related nutritional compounds (Barsotti, Dumay, Mu, Diaz, & Cheftel, 2001). Non-thermal food preservation technologies are considered to be more efficient in terms of required energy and in terms of avoiding heat induced changes of colour, flavour and nutritional value (Lasekan, Ng, Azeez, Shittu, Teoh, & Gholivand, 2016). However, enzyme inactivation achieved during the non-thermal preservation of juices is rather limited (Surowsky, Fischer, Schlueter, & Knorr, 2013).

Carrot and apple are good sources of carotenoids and phenols, which are located in the chromoplasts and in the vacuoles of the plant cells, respectively (Schweiggert & Carle, 2017; Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999). They are considered to provide health benefits due to the antioxidant properties that also contribute to the colour and sensory quality of fresh and processed products (Khanizadeh, Tsao, Rekika, Yang, Charles & Rupasinghe, 2008).

To promote the selectivity of the extraction of bioactive compounds from plant tissues, pulsed electric field (Schilling et al., 2007; Caminiti et al., 2011; Yan, He, & Xi, 2017) and ohmic heating (Bhat, Saini, Kumar, & Sharma, 2017; Saberian, Hamidi-Esfahani, Ahmadi Gavlighi, Banakar, & Barzegar, 2017) treatment have been already investigated.

Bhat et al. (2017) applied thermal and OH treatment (60 – 90 °C; 1 - 5 min) to bottle gourd and compared the effects on total phenolic content and colour of obtained juices. The total phenolic content increased with OH and thermal application at 80 °C for 4 min and 90 °C for 5 min, respectively and the best colour retention was observed for OH treated juice at 80 and 90 °C.

Saxena et al. (2016) reported the effect of OH treatment on PPO activity in sugarcane juice. A high PPO inactivation was observed by applying 32 V/cm at 90 °C for 5 min.

However, for the optimization of the PEF and OH process parameters with regard to reduce the energy requirement and process time and to increase yield and quality, more information is required on specific effects that may result either from thermal or from electric field cell disintegration. Subsequently, the impact on enzyme activity and the recovery of bioactive compounds from the raw material need to be investigated. A first part of the study focussed on juice yield and selected ingredients (Mannozzi et al., 2018). This second part of the work aimed at understanding the effects of PEF and OH treatments on antioxidant properties, colour and enzyme activity such as peroxidase (POD) for both juices and polyphenoloxidase (PPO) for apple juice. The optimization of the two processing technologies was performed taking into account the modulation of process parameters as well as treatment temperatures by applying a pre-heating step (40, 60 or 80 °C) in order to evaluate the influence of thermal and electric field effects on antioxidant and enzyme activity of recovered apple and carrot juices.

2. Material and methods

2.1 Plant raw material and mash preparation

Fresh commercial carrots and apples were purchased from the local market. The fruit and vegetable matrices were washed and cut into smaller pieces. A mill (Alexanderwerk, Austria) with replaceable stainless-steel screens with a grinding level of 2 mm for carrots and 5 mm for apples was used in order to produce the mash.

2.2 Mash pre-treatment and juice production

For PEF treatment of apple and carrot mash, a batch PEF system (DIL, Germany) equipped with a parallel plate electrode treatment chamber (distance 5 cm) was used and 50 exponential decay pulses (discharge capacity 0.5 µF, pulse energy 4 J, pulse width 10 µs) were applied to 400 g of mash. The output voltage was set to 4 kV in order to achieve an electric field strength of 0.8 kV/cm in the treatment chamber. A total specific energy input $W_{specific}$ of 0.5 kJ/kg was applied. The applied electric field strength was chosen according the threshold for the permeabilization of carrot and apple tissue (0.4-0.8 kV/cm) (Angersbach,

Heinz, & Knorr, 2000). The total treatment time of 0.5 ms was calculated by multiplying the pulse width with the number of pulses.

For the OH treatment, the same treatment chamber was used and connected to a generator (DIL, Germany) to apply the electric field (1.1 A, 572 V, 12 kHz, 0.6 kW), resulting in an electric field of 114 V/cm. Different temperature-time profiles were obtained depending on the selected temperatures for the different treated mashes.

For pre-heating to the different initial temperatures (40, 60 and 80 °C), microwave (MT 267, Whirlpool) heating with a power of 850 W was applied for different predefined times.

After the different pre-treatment, all mash batches were cooled to room temperature and pressed using a manual laboratory juice press (Hafico, Germany) with textile cloth; eleven juice samples were obtained both for carrot and apple in 3 replicates each (Table 1).

The obtained juices were evaluated regarding different analytical parameters. Colour measurement was performed directly in the fresh juice. For the determination of antioxidant activity (DPPH and ABTS method) and enzymatic activity such as peroxidase (POD) for both juices and polyphenol oxidase (PPO) for apple, juice samples were frozen and stored at -30 °C until analysis.

2.3 Colour measurements

Juice colour was measured using a Digieye colour measurement system (Verivide, UK). For each juice sample, L*, a* and b* parameters from CIELAB scale were measured. Total colour difference ΔE* between untreated and treated juice samples and browning index (BI, for apple juice only) were calculated by equations Eq. (1) and Eq. (2) respectively. It has to be stated that the untreated juice showed a high degree of colour change due to oxidation and enzymatic browning. Hence, larger ΔE values, i.e. larger deviations from the untreated juice represent the preferred colour for high quality juices.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

$$BI = \frac{[(100(x-0.31)]}{0.172} \quad (2)$$

where:

$x = (a + 1.75L)/(5.645L + a - 3.012b)$

The colour analyses were carried out in fifteen repetitions from each carrot and apple juice sample.

2.4 Antioxidant activity (DPPH and ABTS method)

The carrot and apple juices were centrifuged for 15 min at 10,000 x g in a centrifuge (Eppendorf, Germany). The supernatants were collected and used to evaluate the antioxidant activity by DPPH and ABTS assays.

The DPPH scavenging activity was based on the method proposed by Amarowicz, Naczk, & Shahidi (2000). Briefly, 0.1 mL of extract was added to 2 mL of methanol and 0.25 mL of DPPH (Sigma-Aldrich, USA), shaken with a vortex for 1 min and kept to the dark for 30 min. The absorbance was measured with a spectrophotometer (Photometer model U-1100, Hitachi, Ltd. Tokyo, Japan) at 517 nm. Antioxidant activity was quantified by plotting a Trolox calibration curve. Trolox concentration range was 10-500 ppm ($r^2 = 0.9880$). The results were expressed as mmol Trolox/L of juice.

The $ABTS^{+\bullet}$ scavenging activity was carried out following the method proposed by Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans (1999). 30 µL of extract were added to 3 mL of diluted $ABTS^{+\bullet}$ solution (Sigma-Aldrich, USA) after mixing and the absorbance was measured with a spectrophotometer (Photometer model U-1100, Hitachi, Ltd. Tokyo, Japan) at 734 nm every 30 s for a total time of 6 min; the results were expressed as mmol Trolox/L of juice. Antioxidant activity was quantified by plotting a Trolox calibration curve. Trolox concentration range was 10-500 ppm ($r^2 = 0.9946$).

The value provided are the average of three replicates from each juice sample.

2.5 Enzyme activity

2.5.1 POD assay

Carrot and apple juices were centrifuged at 10,000 x g and 4 °C for 15 min. The supernatant was collected and analysed for POD activity at 470 nm and 25 °C as described by Morales-Blancas et al. (2002). The enzymatic extract was obtained by mixing 6.25 mL of juice sample with 12.5 mL of cold potassium phosphate buffer 0.1 M (pH 6.5) for 2 min. The POD substrate solution was prepared by mixing 0.1 mL of 99.5 % of guaiacol, 0.1 mL of 30 % of hydrogen peroxide and 99.8 mL of 0.1 M of potassium phosphate buffer (pH 6.5). POD activity was assessed by adding 150 µL of enzymatic extract to 3.35 mL of substrate solution in 10 mm pathlength glass cuvettes. POD activity for carrot and apple juice was calculated based on the slope (ΔA/min) of the linear portion of the plot of absorbance compared with time. An enzyme unit is defined as the enzyme activity that catalyses the conversion of 1 µmol of substrate into product in one minute.

2.5.2 PPO assay

4-Methylcatechol 80 mM prepared in Mcllvaine's buffer solution at pH 7.5 was used as substrate and 12.5 mL of cold Mcllvaine's buffer solution at pH 7.5 was added to 6.25 mL of enzymatic extract. PPO activity for apple juice was determined reading the absorbance at 420 nm and 25 °C and calculated based on the slope of the linear portion of the curve (ΔA/min). An enzyme unit is defined as the enzyme activity that catalyses the conversion of 1 µmol of substrate into product in one minute.

2.6 Data analyses

The obtained data were analysed using parametric analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test was applied to compare the means at the level of confidence of 95 % ($p<0.05$). Conversely, when the normality of the distribution and the homogeneity of the variances were not satisfied, non-parametric ANOVA (Kruskal-Wallis) along with Holm's post-hoc tests were carried out at the same level of significance ($p<0.05$). R statistical software (R foundation for statistical computing, Vienna, Austria) was employed to perform all the tests.

3. Results and discussion

3.1 Colour

Colour changes represent an indicator for enzymatic browning as well as for process induced browning due to heat induced formation of Maillard products. Total colour variation (ΔE*) between untreated and treated carrot and apple juice samples was analysed and is shown in Fig. 1.

Larger ΔE values represent a positive deviation from the untreated control sample that showed undesired browning due to enzyme activity and oxidation.

Juices from both raw materials pre-treated at 80 °C with or without PEF showed higher ΔE* values compared to control samples. According to the classification of Cserhalmi et al. (2006), ΔE* changes above 6 indicate great visible changes. The increase in ΔE* reflects the increase in the lightness and decrease in the a* value of samples (data not shown). Since the untreated juice, which is considered as control sample in this case, showed unwanted browning and colour change due to enzyme activity and oxidation, higher ΔE* values, i.e. higher deviation from the control juice colour indicates beneficial quality.

Lower ΔE* values were observed for mash pre-treated with PEF at room temperature and at 40 and 60 °C with OH.

The lowest ΔE* values (4.36 - 5.49) were observed for pre-heated juice samples at 40 °C and 60 °C coupled with PEF and the highest total colour differences were observed for samples pre-heated to 80 °C with or without additional PEF treatment (18.49 and 17.15 respectively).

The detected ΔE* values between untreated and treated samples were even more pronounced for carrot compared to apple juice. In general, for both juices, higher L* values promoted also higher total colour differences compared to control one. The browning index (BI) is a common parameter to describe colour and juice quality for apple. A decrease of the BI was found for apple samples pre-heated at 80 °C coupled with or without PEF or OH treatment, in which was observed to reach BI from115 to 119 compared to the untreated juice with much higher values of 142 (Fig. 2). Bhat et al. (2017) reported similar results for bottle gourd treated with OH at 80 °C for 1 and 2 min (BI of 111 and 101 respectively). The progressive decrease in BI with increasing treatment temperature in apple mash indicates the relevance of enzymatic browning

in untreated samples and the role of the temperature during mash treatment for the avoidance of unwanted reactions.

The main groups of pigments that are responsible for the characteristic colours in fruits and vegetables are carotenes and carotenoids, anthocyanins, chlorophylls, and phenolic compounds. The main enzymes involved in biochemical degradations of plant compounds are peroxidase and polyphenoloxidase (Terefe et al., 2014). Moreover, another main cause of brown colour formation is non-enzymatic browning occurred in vegetable and fruit product. However, in the current study, the benefit from short time thermal treatment of the juice of up to 80 °C for the inactivation of oxidative enzymes was more pronounced than the occurrence of detrimental colour changes due to non-enzymatic browning.

3.2 Antioxidant activity (DPPH and ABTS method)

Fig. 3 reports the results of antioxidant activity, obtained with DPPH and ABTS antiradical activity methods, of differently obtained apple juices.

For carrot juice (data not shown), a significantly higher antioxidant activity was obtained for carrot juices pre-heated to 80 °C with or without additional PEF or OH treatment with the ABTS method. Higher retention of bioactive compounds with DPPH method was observed for carrot mash pre-treated at 80 °C coupled with PEF treatment. However, the application of OH treatment reaching 40 and 60 °C reduced the antioxidant activity compared to juice from the untreated control carrot mash detected with DPPH method. With ABTS method, no significant difference was found.

Significantly higher antioxidant activity, detected with both DPPH and ABTS method, were obtained for apple juices pre-heated to 80 °C with or without additional PEF or OH treatment (Fig. 3). Instead, OH reaching 40 °C and 60 °C reduces the antioxidant activity, with both used method, compared to the apple control juices, which might be due to the activation of degradative enzymes, such as peroxidase and polyphenoloxidase that leading to bioactive compounds oxidative degradation.

Fruit and vegetable are good sources of natural antioxidants, containing carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione, and endogenous metabolites. However, the majority of the antioxidant activity of fruits and vegetables is derived from phenolic compounds (hydroxycinnamic acids, flavan-3-ols, anthocyanidins, flavonols, and dihydrochalcones) rather than vitamin C and E, or β-carotene, due to their stronger activity against peroxil radicals (Zhang & Hamauzu, 2004). The activation of peroxidase and polyphenoloxidase lead to the degradation of phenolic compounds that resulted in subsequent loss of nutritional and sensorial values such as browning and off-flavour (Vámos-Vigyázó, 1995). Moreover, thermal treatment may affect the binding properties of selected compounds leading to an increased release but at the same time to higher enzymatic or non-enzymatic degradation processes, that can cause subsequent negative effects on quality of processed products (Shilling et al., 2007).

In fact, higher temperature lead to the inactivation of the oxidative enzymes, thus reducing degradation effects and resulting in higher antioxidant activity in the juice.

The detected difference between the two different methods used could be due to the fact that, DPPH method seems to be more prone to detect flavanones, while ABTS method seems to be more suitable to detect the radical scavengers such as vitamin C (Del Caro, Piga, Vacca, & Agabbio, 2004).

For carrot and apple juice, PEF treatment without pre-heating did also not affect the extractability of bioactive compounds, which is in accordance with Schilling et al. (2007), who reported no significant differences on total antioxidant activity between control and PEF treated apple mash for different electric filed (1, 3 and 5 kV / cm).

3.3 Enzyme activity

3.3.1 POD activity

Process pre-treatment for the juice production is an important operation in order to improve the quality of the vegetable and fruit raw materials as well as to avoid the activation of degradative enzymes such as POD and PPO that consequently provoke pigments and nutrients loss (Gonçalves, Pinheiro, Abreu, Brandão, & Silva, 2010).

Peroxidase (POD) activity for differently obtained carrot juice is shown in Fig. 4.

This study revealed that the effect of only PEF treatment at 20°C and 40 °C pre-heating could not reduce the activity of POD in both carrot (Fig. 4) and apple juice (data not shown), while for the samples pre-heated at 60°C the reduction of POD activity was observed.

The highest POD inactivation could be reached by pre-heating to 80 °C with and without additional PEF treatment. All pre-treatment conditions with temperature at or up to 60 °C and 40 °C-80 °C by OH treatment led to a decrease in POD activity in the carrot juice, while for apple juices were achieved a greater reduction of the activity (from 50 % to 90 %) by 60 and 80 °C pre-heating temperatures with and without additional PEF or OH application. Enzyme inactivation in the juice after PEF treatment for microbial inactivation and preservation purposes at higher PEF treatment intensities is mainly related to secondary effects such as local temperature distributions, electrochemical reactions or formation of free radicals instead of primary effects of electric field. For the treatment of mash, the PEF treatment intensity can be considered 10 fold lower and having no effect direct on fruit and vegetable mash ingredients.

POD activity decreased with increasing temperature, and almost no POD activity was detected in juice extracted when the treatment temperature reached 80°C, especially in apple juice samples. High temperature leads to an increase in the internal energy of the enzymes, thus consequently causes the break of bonds that determine the three-dimensional structure of enzymes (Bhat et al., 2017).

Moreover, with the increasing of temperature, the enzyme activity decreased and required a particular temperature-time combination for complete inactivation. Inadequate temperature led to decrease the enzyme activity time rather than complete inactivation, which may cause browning effect. In fact, Bhat et

al. (2017) reported similar results for OH treated bottle gourd juice, where the temperature of 60 and 70 °C seems to be not enough for complete enzyme inactivation, which instead was observed at 80 °C for 4 min. Icier et al. (2006) showed that OH treatment could be used for POD inactivation on pea puree at the range of 30-50 V/cm combined with the water blanching. Elez-Martinez, Soliva-Fortuny & Martin-Belloso (2006) reported a completely POD deactivation in orange juice after the application of PEF treatment at 35 kV/cm for 1500 µs.

Moreover, the variations of colour for carrot and apple juice pre-treated with 80 °C both with PEF and OH applications could be explained by the decrease of enzyme activity.

3.3.2 PPO activity

Polyphenoloxidase (PPO) activity of differently obtained apple juice samples is shown in Fig. 5. PPO is an oxidoreductase enzyme, which catalyses the oxidation of phenolic compounds in *o*-quinones, which are subsequently polymerized into brown pigments (Murata, 1995).

Heating treatment seems to be the most effective applied treatment for the stabilization of food products against microbial and enzyme activity. Nevertheless, thermal treatment has been shown to cause negative effects on quality and related nutritional compounds (Barsotti et al., 2001). The mechanism of enzyme inactivation is not completely clear, current results show empirical proof of protein modification by electrical fields (Freedman et al., 2013) that may provoke a deformation or structural change of a protein, due to the interaction between the external electric field and the functional groups of the protein that allow its unfolding (Elez-Martinez et al., 2006).

PPO activity significantly decrease by PEF application at room temperature compared to the untreated control sample. In addition, a greater inactivation was achieved when the treatment temperature reached 80 °C as well as with OH treatment and just pre-heating. Moreover, PPO inactivation was even more effective when combination of temperatures and PEF or OH applications were used.

Similar results were reported for PPO Turk, Billaud, Vorobiev, & Baron (2012), PPO activity was reduced in apple cider mash pre-treated with PEF at 1 kV/cm for100 µs. The loss of PPO activity was explained by the inhibition of the enzyme with oxidised phenolic compounds (Le Bourvellec, Le Quere, Sanoner, Drilleau, & Guyot, 2004).

Previous work reported similar results for PPO deactivation; the residual PPO activity was 35 % after 14 min at 70 °C with OH treatment by applying 35 V/cm in grape juice (Icier et al., 2005).

Liang, Cheng & Mittal (2006) found a significant decrease (33 %) in PPO activity in freshly squeezed apple juice when pre-heated at 50 °C and treated with PEF at 27 kV/cm for 58.7 µs.

Saxena, Makroo & Srivastava (2017) found a reduction of PPO activity up to 97.8 % by applying 32 V/cm with OH treatment at 90 °C for 5 min in sugarcane juice. Moreover, a greater increase in residual PPO activity was visible at 90 °C by increasing the holding times of OH treatment (5, 10, 15 and 20 min). The increase of the enzyme activity with the holding time at constant temperature was attributed to the pulsating

OH treatment that may cause biochemical reactions by changing the molecular spacing and may result in a better interaction between substrate and enzyme (Castro, Macebo, Teixeira, & Vicente, 2004).

4. Conclusion

Obtained results emphasize the role of thermal treatment for the inactivation of enzymes reflected by improved colour values for juices exposed to 80 °C independent of the PEF or OH application.

However, a better retention of plant secondary metabolites from carrot and apple mashes could be achieved by additional PEF or OH application. PEF treatment was found to improve the release of such compounds whereas OH contributed to a very fast volumetric heating that reduces the overall thermal load the sample is exposed to. Based on the results, a combination of thermal and electric field pre-treatments is required for the controlled release, inactivation and retention of ingredients. Thermal effects contributing to the colour, bioactive compounds retention and enzyme inactivation were found to still be important when applying non-thermal cell disintegration techniques such as PEF. However, both electrotechnologies, PEF and OH were found to positively contribute to improved juice quality by enhanced ingredient release and retention.

The inactivation of POD and PPO was more pronounced when a temperature of 80 °C was achieved for both carrot and apple mash (around 90 %).

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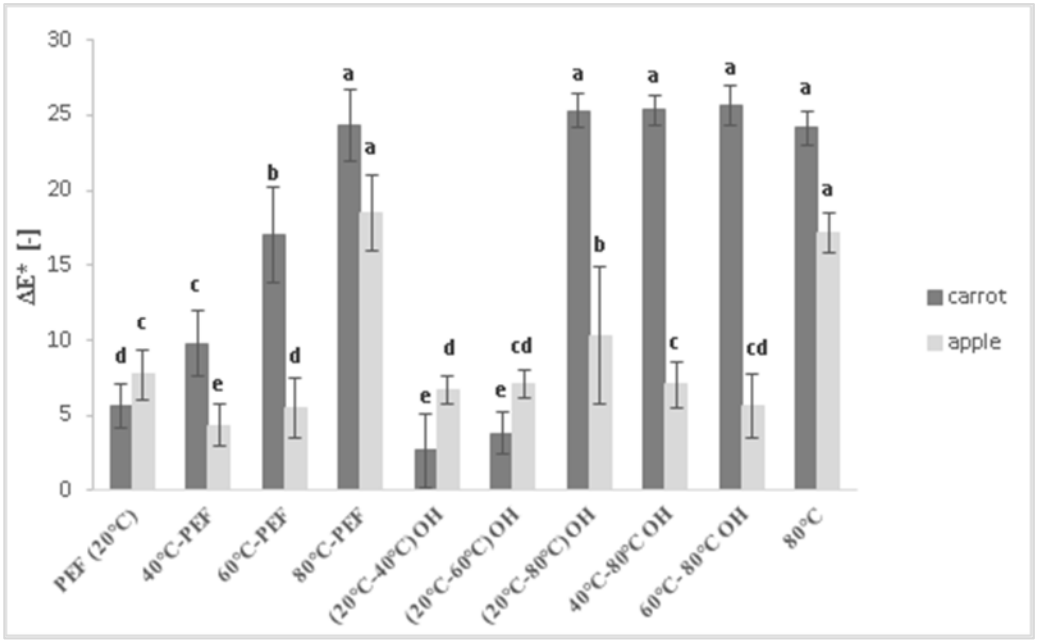

Fig. 1 Total colour variation-ΔE* between of apple and carrot juices obtained from untreated and treated mash.

Different letters indicate significant differences ($p<0.05$) between samples.

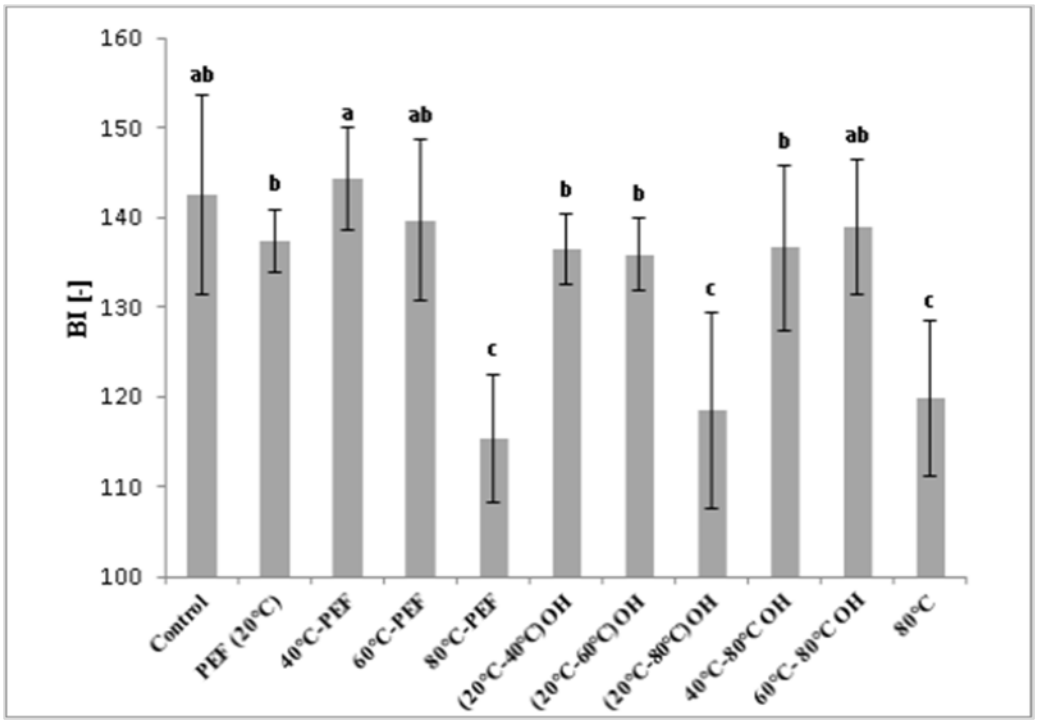

Fig. 2 Browning index-BI in apple juice obtained from pre-treated apple mash.

Different letters indicate significant differences ($p<0.05$) between samples.

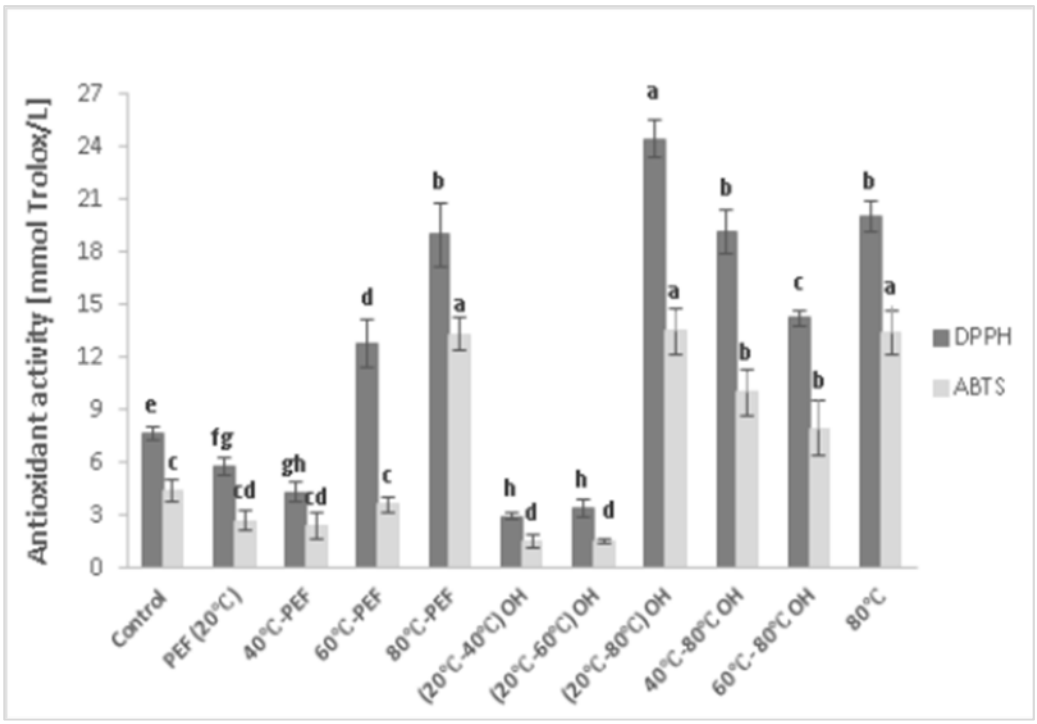

Fig. 3 Antioxidant activity (DPPH and ABTS method) of juices of pre-treated apple mash.

Different letters indicate significant differences ($p<0.05$) between samples.

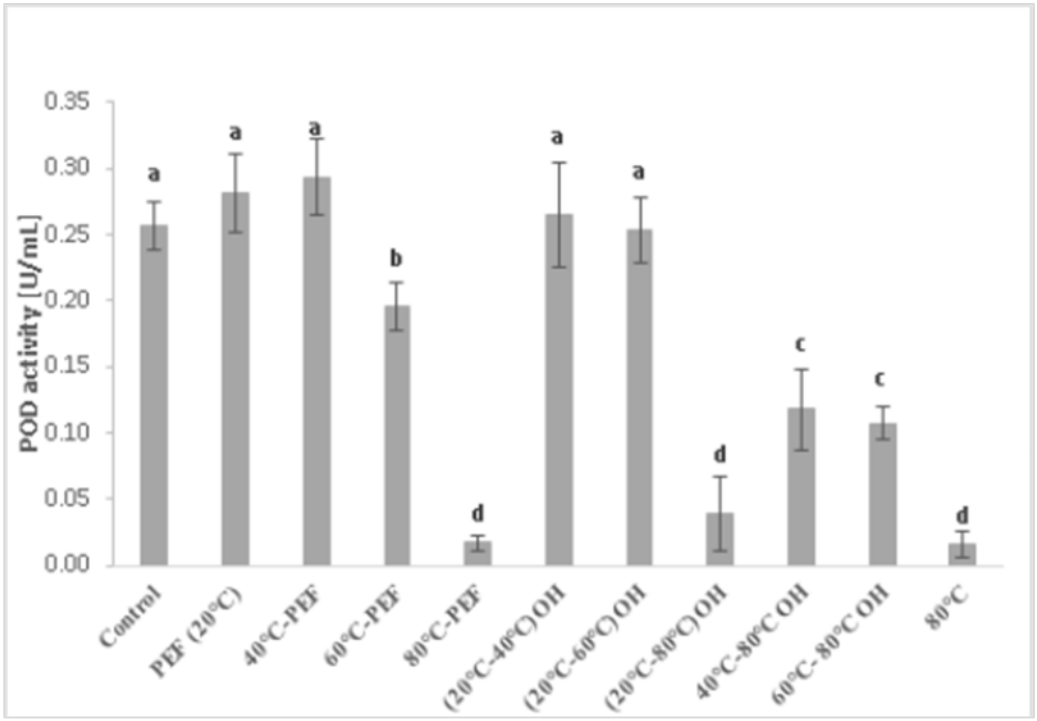

Fig. 4 Peroxidase (POD) activity in carrot juice obtained from pre-treated mash.

Different letters indicate significant differences ($p<0.05$) between samples.

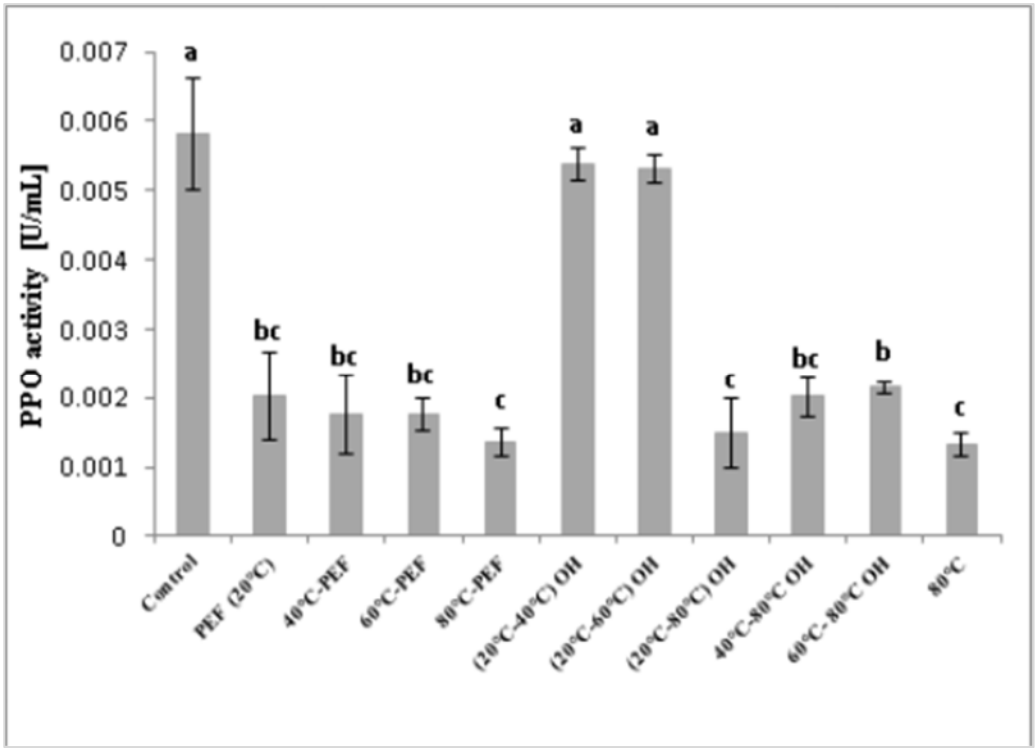

Fig. 5 Polyphenoloxidase (PPO) activity of apple juice obtained from pre-treated mash.

Different letters indicate significant differences ($p<0.05$) between samples.

Table 1. Overview on mash treatment conditions applied for apple and carrot mash

Treatment	Sample	$W_{specific}$ [kJ/kg]
Untreated	Control	0
PEF at 20 °C	PEF (20 °C)	0.5
Pre-heating 40 °C + PEF	40 °C-PEF	192.5
Pre-heating 60 °C + PEF	60 °C-PEF	382.5
Pre-heating 80 °C + PEF	80 °C-PEF	765.5
OH from 20 °C to 40 °C	(20 °C-40 °C) OH	110
OH from 20 °C to 60 °C	(20 °C-60 °C) OH	222
OH from 20 °C to 80 °C	(20 °C-80 °C) OH	355
Pre-heating 40 °C + OH to 80 °C	40 °C-80 °C OH	402.5
Pre-heating 60 °C + OH to 80 °C	60 °C- 80 °C OH	497.5
Pre-heating 80 °C	80 °C	765

Effect of (ultra)-high pressure homogenization on the shelf-life and functionality of organic kiwifruit juice

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Effect of high pressure homogenization on the shelf-life and functionality of organic kiwifruit juice

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Abstract

The present research was aimed to evaluate the effects of high pressure homogenization treatments performed at 200 MPa for 2 and 3 cycles on the safety, quality, and functionality of organic kiwifruit juice stored at three different temperature, i.e. 5, 15 and 25 °C. The results showed that the treatment performed at 200 MPa for 3 cycles was able to significantly increase the shelf-life of organic kiwi fruit juices when stored at refrigeration temperature, avoiding also product separation that occurred in the sample treated at 0.1 MPa (control) after 20 days of refrigerated storage. The data obtained showed also that the highest high pressure homogenization treatment was able to increase some juice quality parameter such as viscosity, luminosity (L*) and also the availability of polyphenols.

Keywords: high pressure homogenization; organic kiwi juice; shelf-life; colour; antioxidant activity, viscosity

1. Introduction

High pressure homogenization (HPH) is a non-thermal technology potentially exploitable at industrial level for pasteurization of liquid foods. The microbial inactivation mechanisms are the result of different events such as cavitation, shear stress, turbulence and impingement which arise during the food treatment (Patrignani & Lanciotti, 2016; Zamora & Guamis, 2015). HPH is also told to inactivate or modulate the activity of enzymes responsible for the separation of fruit or vegetable juices, to maintain the original juice colour, flavour, and aromas and, finally, to preserve the nutritional and functional features of the treated matrices (Błaszczak, Amarowicz, & Górecki, 2018; Patrignani, Tabanelli, Siroli, Gardini, & Lanciotti, 2013a). Several authors verified the efficacy of this treatment on several matrices such as vegetable milks (Gul, Saricaogl, Mortas, Atalarb, & Yazici, 2017), vegetable and fruit juices (Briñez, Roig-Sagues, Herrero, & Lopez, 2006; Betoret, Betoret, Carbonell, & Fito, 2009; Donsì, Esposito, Lenza, Senatore, & Ferrari, 2009; Patrignani, Vannini, Sado Kamdem, Lanciotti, & Guerzoni, 2009b; Bevilacqua, Corbo, & Sinigaglia, 2012; Patrignani et al., 2013a), milk (Lanciotti et al., 2004a; Hayes, Fox, & Kelly, 2005), milk-based products (Lanciotti, Vannini, Pittia, & Guerzoni 2004b; Patrignani et al., 2009a; Massoud, Belgheisi, & Massoud, 2016), and liquid whole egg (Patrignani et al., 2013b; Velazquez-Estrada, Hernandez-Herrero, Lopez-Pedemonte, Guamis-Lopez, & Roig Sagues, 2008), proposing also to combine HPH with additional hurdles such as food low pH and low temperature of food storage to increase food shelf-life (Bernat, Chafer, Rodríguez-García, Chiralt, & Gonzalez-Martínez, 2015; Huang & Kuo, 2015). However, the research was also encouraged toward an improvement of the existing HPH technology, resulting in the production of new types of homogenizers and valves, able to reach levels of pressures between 200-400 MPa, resulting in shelf stable products without negative effects on their quality (Zamorra & Guamis, 2015). Indeed, according to the literature data, HPH is able to diminish the loss of food nutritional compounds (Velázquez-Estrada et al., 2013; Gul, Saricaoglu, Mortas, Atalar, & Yazici, 2017). In fact, some authors evaluated the polyphenol composition, vitamin C, antioxidant capacity, and provitamin A content of apple (Suarez-Jacobo et al., 2011) and orange juices (Velázquez-Estrada et al., 2013) when treated by HPH, reporting that this treatment significantly preserved these compounds differently from samples subjected to pasteurization. However, according to our knowledge, scarce references are available in the literature concerning the use of HPH and its effect on the quality and functionality of kiwifruit juice (Yi et al., 2018).

In this framework, the principal aim of this research was to evaluate the effects of HPH treatments performed at 200 MPa for 2 and 3 cycles on quality and safety of organic kiwifruit juice, immediately after the treatments and during the storage at three different temperatures (5, 15 and 25 °C). Immediately after the treatments and during the storage, the naturally occurring microbial population,

pH, colour, viscosity, antioxidant activity and total phenol content were investigated on the HPH juice samples and their controls (samples treated at 0.1MPa) in order to assess the effects of the proposed treatments on the juice quality and functionality.

2. Material and Methods

2.1 *Kiwifruit juice preparation and high pressure homogenization (HPH) treatment*

Organic kiwifruits (Actinidia deliciosa cultivar ‘‘Hayward’’) were bought on a local market located in Cesena (Italy) and properly stored until the laboratory trials. They were sorted by homogeneous size of 8 mm diameter and a length of 10 mm and refractometric index of 13±1 ºBrix. The raw organic kiwifruit juice was obtained by using a lab extractor (Russel Hobbs, 27700-56) and divided in three 5-liter batches and subjected, after eliminating the seeds, to different HPH treatments performed at 0.1 MPa (used as control), 200 MPa for 2 cycles and 200 MPa for 3 cycles. For all the HPH treatments, a PANDA high pressure homogenizer (Gea, Parma, Italy), provided of a thermal exchanger and a R-type valve was used; the valve assembly comprised a ceramic ball-type impact head, a stainless steel large inner diameter impact ring and a carbide passage head made of tungsten. The homogenizer was previously washed with 1% NaOH water solution, hot water and finally refrigerated sterilized water. Before the treatments, the kiwifruit seeds were removed. The inlet temperature of the juice was about 4°C and the increase rate of temperature was about 2°C/10 MPa. The controls and treated samples were collected in 250 ml-sterilized glass bottles, stored at 5, 15 and 25 °C and analyzed over time. The maximum temperature reached during the most severe HPH treatment was about 44 °C.

2.2 Microbiological analyses ad pH

The cell loads of naturally occurring yeasts, total coliforms and lactic acid bacteria were counted by plate counting on Sabouraud Dextrose Agar (Oxoid Ltd, Basingstoke, United Kingdom), Violet Red Bile Agar (Oxoid Ltd.) and de Man Rogosa and Sharpe Agar (Oxoid Ltd.), respectively. Decimal dilutions of the samples, performed in ringer solution (0.9% (w/v) NaCl), were inoculated in Petri dishes and incubated at 25°C for 48 h for yeasts, 37°C for 48 h for Lactobacilli, and 37°C for 24 h for total coliforms. Moreover, at each time of sampling, the presence of *L. monocytogenes*, *Salmonella enteritidis* and *E. coli* was assessed in all the juice samples during their storage. The presence of the three pathogenic species was investigated according to the ISO methods 11290, 6579, and 16649, respectively.

The pH was measured immediately after juice treatments and during the storage by using a pH-meter Basic 20 (Crison Instruments, Barcelona, Spain).

2.3 Viscosity and colour analyses

Viscosity of juices was measured by a vibrational viscometer (Viscosilite 700 Hydramotion), previously calibrated with distilled water (viscosity = 1cP).

Colour of kiwifruit juice samples were measured using a spectrophotocolorimeter HUNTERLAB ColorFlexTM, mod. A60-1010-615 (Reston, Virginia). For each sample, L*, a* and b* parameters from CIELAB scale were measured.

2.4 Determination of total phenolic content (TPC) and total antioxidant capacity (TAC)

Kiwifruit juice samples were analysed without any extraction using a UV-1601 spectrophotometer from Shimadzu (Duisburg, Germany). Each sample and calibration point were analysed in three replicates (n = 3). The TPC of samples was assessed by means of the Folin-Ciocalteu method (Singleton & Rossi, 1965). The samples absorbances were measured at 750 nm and the phenolic content was calculated on the basis of the gallic acid calibration curve (from 30 to 1000 μg/mL). The results were expressed as mg/100mL of juice.

To determine the TAC, the ABTS and DPPH assay were performed. The ABTS assay was performed as described by Laporta, Perez-Fons, Mallavia, Caturla, &. Micol, (2007), while the DPPH assays was evaluated according to Bonoli, Verardo, Marconi, & Caboni (2004). The decrease in absorbance was assessed at 517 nm in the 0–30 min range (at 25 °C). The values obtained for both TAC assays were compared to the concentration–response curve of the standard Trolox and expressed as μmol of Trolox equivalent (TE)/100 mL.

2.5. Data analysis

The data are the means of two independent experiments and three repetitions. The data were analyzed using Statistica software (8.0; StatSoft., Tulsa, Oklahoma, USA) by two way-ANOVA followed by Tukey honest significant difference (HSD) test at $p < 0.05$ level to monitor changes over time as well as differences among treatments.

3. Results

3.1 Microbial inactivation and pH

In table 1, the inactivation level of naturally occurring yeasts and their re-growth kinetic in organic kiwifruit juices, in relation to the adopted HPH treatments and storage temperature, are reported. The HPH treatments adopted were able to reduce the initial level of naturally occurring yeasts (2.4 log CFU/mL) under the detection limit (1 log CFU/ml), immediately after the treatments. During the storage at 5°C, the control juice spoiled from a microbiology point of view between 27 and 32 days, since the yeasts reached the microbiological spoiling threshold fixed at 6 log CFU/mL. On the contrary, the naturally occurring yeasts present in organic kiwifruit juice were not able to recover after the treatment at 200 MPa for 3 cycles at 5°C, while their potential growth was reduced after the treatment at 200 MPa for 2 cycles and in 40 days the spoiling threshold was not achieved.

As expected, as the sample storage temperature increased, it determined a decrease of the juice shelf-life. The most treated samples (200 x 3 cycles) spoiled after 14 and 9 days when the storage

temperature was 15 and 25 °C, respectively. The control sample (treated at 0.1 MPa) spoiled between 7 and 9 days at 15°C while at 25°C, after 5 days of storage, it resulted already spoiled. In the kiwifruit juice treated at 200 MPa for 2 cycles, the yeast cell loads reached the spoilage threshold between 10 and 12 days at 15°C. The use of the refrigeration temperature determined a significant increase of the shelf-life of the samples. Although the control reached the microbiological threshold for yeast between 24 and 28 days, at 20 days of storage at 5°C it resulted separated. The sample treated at 200 MPa for 3 cycles maintained a yeast cell load always under the detection limit for all the considered storage at 5°C, while the sample treated at 200 MPa resulted in yeast cell load of 4 log CFU/mL after 40 days of storage.

For all the considered samples and storage temperature, total coliforms and lactic acid bacteria never exceeded 1 log CFU/mL and 1.5 log CFU/mL, respectively (data not shown). *L. monocytogenes*, *Salmonella* spp and *E. coli* were never found in the samples (data not shown).

In table 2, the samples pH values, in relation to the HPH treatments applied and the storage temperature, are reported. The application of the HPH treatments in the juice determined a decrease in pH values, which decreased by increasing the severity of the HPH treatment. However, independently on the storage temperature, the sample pH decreased over time.

3.2 Viscosity and colour analyses

In table 3, the viscosity values recorded for organic kiwifruit juice, in relation to the HPH treatments applied, are reported. Treatments at 200 MPa both for 2 and 3 cycles resulted in a higher viscosity compared to the control kiwifruit juice. In general, during storage at 5°C a decrease of viscosity was observed in all samples, which was more pronounced for untreated samples and those treated with 200 MPa x 2 cycles. Moreover, while in the control sample the separation of the phases was observed at 20 days, the reduction of the macromolecules size in the treated samples induced a delay in separation and sedimentation. Juices stored at higher temperatures maintained a similar viscosity during the whole period, which was 14 days for samples stored at 15°C and 7 days for those stored at 25°C.

Table 4 shows the colour parameters measured in control and treated samples during storage at three different temperatures. Lightness (L*) of fresh kiwifruit juice was 33.40. The HPH treatments caused a significant increase of this parameter in comparison to the control samples. Concerning a* and b* parameters, respectively the red/green and the yellow/blue parameter, both samples treated at 200 MPa showed lower values compared to the control sample. During the storage at all considered temperature a slight decrease of L* together with increasing of a* was observed, while b* remained almost unchanged in control and 200MPa *2cycles treated samples. The samples pressured with 3 cycles presented similar colour during the whole storage.

3.3 Total phenolic content (TPC) and total Antioxidant capacity (TAC)

The total phenolic content of most HPH treated kiwifruit juices significantly increased with respect to the controls from 35 to 42 mg/100mL of juice. During the storage at 5° C, TPC decreased slightly, mainly during the first 15 days, although samples treated at 200 MPa for 3 cycles did not show significant differences ($p < 0.05$) (Figure 1a). During the storage at different temperatures, TPC values decreased in all the samples although the highest values were found in HPH samples (Figures 1b, 1c). About the DPPH assay, both treated juices presented a significant higher antioxidant activity compared to the control sample. As previously observed for TPC, also the antioxidant activity decreased over storage in all the samples, independently on the temperature (Figure 2a,b,c). ABTS results (data not shown) followed the same trend with an interesting positive Pearson's correlation with the DPPH method: $r2 = 0.913$ $p < 0.0001$, $r2 = 0.923$ $p < 0.0001$ and $r2 = 0.983$ $p < 0.0001$, for 0.1 MPa, 200 MPa x 2 cycles and 200 MPa x 3 cycles, respectively.

4. Discussion

In the present research, the effects of two HPH treatments performed at 200 MPa for 2 and 3 cycles were investigated on the organic kiwifruit juice microbiological stability, colour, texture and functionality (availability of polyphenols and antioxidant activity) during the juice storage at 5, 15 and 25 °C. The HPH treatments performed caused an instantaneous reduction of yeasts, naturally occurring, under the detection limit. Although the microbial inactivation caused by the application of HPH can be affected by several factors, the level of pressure and the cycles applied, the chemico-physical features of the food matrix and the different sensitiveness of the microorganisms present in the treated food are the principal factors to take into consideration (Diels & Michiels, 2006; Zamora & Guamis, 2015; Patrignani & Lanciotti, 2016). Also, the effects induced by the temperature have to be necessarily taken into account in HPH treatment, since, during homogenization, increase of temperature (about 2.0° C per 10MPa), related to the fluid food employed, can be observed. However, according to Floury, Bellettre, Legrand, & Desrumaux (2004; Pinho, Franchi, Tribst, & Cristianini, 2011) such temperature augment did not result in HPH treated food samples probably due the flash time of treatment of the food matrices (lower than 1 s). However, in the present research, to minimize as much as possible the product temperature increase, generated during the treatment, and its effects, a thermal exchanger was applied avoiding to exceed temperature of 44 °C. As microbiological threshold for the kiwi fruit spoilage, in accordance with the literature data, a level of yeasts of 6 log CFU/mL was fixed since these microorganisms represent the main spoiling agents for this kind of products, characterized by low pH and high sugar content (Patrignani et al., 2009b, 2010, 2013; Donsì et al., 2009). Although the HPH treatments reduced under the detection limit the yeast cell loads, the

applied levels of pressures induced sub-lethal damages on yeast population, suggesting that HPH effectiveness for microbial inactivation is affected by several parameters, including not only process factors and aspects related to the features of the treated matrix, but also the physiological diversity within a population (Ferragut et al., 2015), probably characterized also by different stress resistance and ability to recover. This suggests that in order to validate the effectiveness of a new treatment also the estimation of resistant cells, at the viable but not culturable (VBNC) state, within a microbial population, must be taken into consideration. In the present research, the combination of a HPH treatment at 200 MPa for 3 cycles and the product refrigeration temperature resulted in a stable and safe organic kiwifruit juice for more than 40 days, without detrimental effects on colour, viscosity and antioxidant activity. The decrease in pH observed in HPH treated kiwifruit juices is in accordance with the data obtained by several authors and it can be attributed to the modification of the equilibriums between salts induced by the HPH treatment (Patrignani et al., 2009b; Patrignani et al., 2013a).

From a technological point of view, the increase of viscosity of organic kiwifruit juice in relation to the HPH treatment applied is a very promising result. Treatments at 200 MPa both for 2 and 3 cycles resulted in a higher viscosity compared to the control kiwifruit juice. This increase was probably due to the structural modification of kiwifruit juices induced by HPH treatment, as observed also by Yan et al. (2017) in tomato juice. HPH promotes the disarrangement of the cell clusters into single cells and/or cell fragments (Moelants et al. 2014). The release and solubilization of cell wall constituents, such as pectin and proteins, cause the increase of the volume fraction of particles and lead to the improvement of particle interactions, thus increasing viscosity (Thakur et al., 1995). However, a decrease in viscosity after HPH treatment has been reported for orange juice (Soares et al., 2014) as well as for banana juice (Calligaris et al., 2012).

Karacam et al. (2015) observed a higher viscosity (gel like structure) in strawberry juice treated at 100 MPa for 2 passes, compared to 5 passes. According to the authors the temperature increase during the treatment at 100 MPa x 2 passes reached the optimal temperature for the activation of PME (43°C). Also, other authors observed an initial increase in viscosity of mango and apricot juice after HPH followed by a decreasing trend along with pressure increase, inlet temperature, and passes number (Zhou at al., 2017; Patrignani et al., 2013). In the present study, increasing the number of passes (cycles) promoted few differences, not always statistically significant. This may be due to the fact that the temperature increase was similar for both treatments.

Lightness of fresh kiwifruit juice was similar to the value reported by Islam et al. (2012) for organic kiwifruit juice (L*=32.00). HPH treatment caused a significant increase of this parameter in comparison to the control samples, which could be attributed to the higher light scattering attributed

to smaller size particles. (Calligaris et al., 2012). Also, Yi et al. (2018) observed and increase of lightness of apple juice with 50% of kiwifruit addition upon the application of HPH. Although some authors observed a fair decrease in L* and an increase in a* parameter in kiwifruit puree (Fernández-Sestelo at al., 2013) and mango juice (Zhou et al., 2017), in our research, the hyperbaric treatments demonstrated to be able to enhance the typical green colour of kiwifruit juice. The samples pressured with 3 cycles presented similar colour during the whole storage. Similar results were observed by Calligaris et al. (2012) in banana juice stored for 30 days. Lightness of the homogenised banana juice samples decreased only after 20 days of storage, however, homogenised juice remained always lighter than the untreated one during the whole period of storage. In our study the evolution of colour in samples stored at the higher temperatures (15 and 25°C), could not be verified due to the juice spoilage already after few days. However, Guan et al. (2016) observed that storage of mango juice at room temperature promotes a greater decrease of lightness and increase of redness compared to storage at 4 °C, induced by faster browning reactions. According to the literature data, kiwifruit juice includes a large variety of functional components such as phenolic compounds, antioxidants, potassium, vitamin C, vitamin E, and fibres (Fernández-Sestelo, et al. 2013). Moreover, kiwifruit intake is reported to increase cytokine production and exert antioxidant effects (Iwasawa et al., 2010). Unfortunately, processes involving thermal treatments strongly decrease the product's quality and functionality due to changes induced in thermolabile phytocompounds (Błaszczak et al., 2017). In the present research, the application of HPH determined a significant increase of the availability of total polyphenols. These data are in agreement with the literature data which suggest that the HPH process can increase extractability of antioxidant components by breaking down of compound cell walls (Patras et al. 2009a,b). Moreover, an increase in homogenization pressures results in a decrease of bioactive compound degradation during storage in low pulp mandarin juice at 20 and 100 MPa (Betoret et al., 2017). Also in the present research, the use of 200 MPa for 3 cycles determined, during the storage, a slower reduction of total polyphenols in kiwifruit juice. Bot et al. (2018), treating apple juice at 150 MPa for 10 passes, achieved the inactivation of 50 % of polyphenoloxidase. In the present research the initial antioxidant activity raised in the samples treated by HPH, independently by the level of pressure applied, suggesting that the increase could be due to the partial inactivation of the activity of polyphenol oxidase and peroxidise enzymes involved in the decay of phenolic compounds in vegetable matrix (Guan et al., 2016). On the other hand, the decrease of antioxidant activity of the HPH treated kiwifruit, during storage, could be due to the natural degradation of some bioactive compounds and to their role in food oxidation prevention, not completely inactivated by the treatment (Betoret et al., 2017).

5. Conclusion

Since on the market there is absence of juices stabilized by HPH, this research can be of great importance in order to understand the process conditions that can guarantee safety and shelf-life of kiwifruit juices. The application of a treatment at 200 MPa for 3 cycles allowed to obtain a stable kiwifruit juice for more than 40 days under refrigerated storage and to extend the shelf-life with respect to the control of one week at room temperature, increasing at the same time its antioxidant activity, the availability of polyphenols and its brightness. A further challenge to implement this technology in food process as full alternative to thermal treatment could be represented by the adoption of level pressure up to 400 MPa and the packaging of foods in aseptic conditions.

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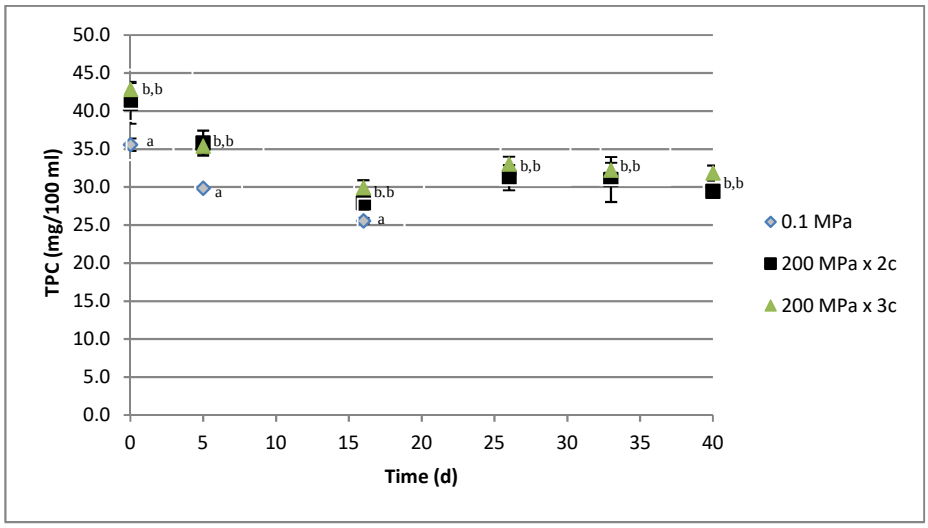
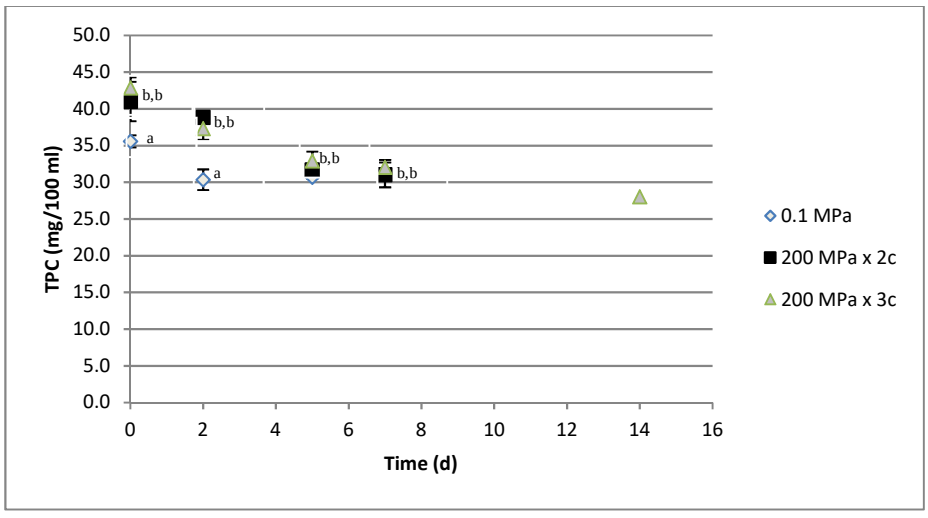
Figure 1. Total phenolic compounds of organic kiwifruit juices in relation to the high pressure applied, during the storage at 5 (1a), 15 (2a), 25 (1c) °C. Means followed by different letters means significant different ($p<0.05$) between samples at each day of storage.

Figure 1.

(a) 5°C



(b) 15°C



(c) 25°C

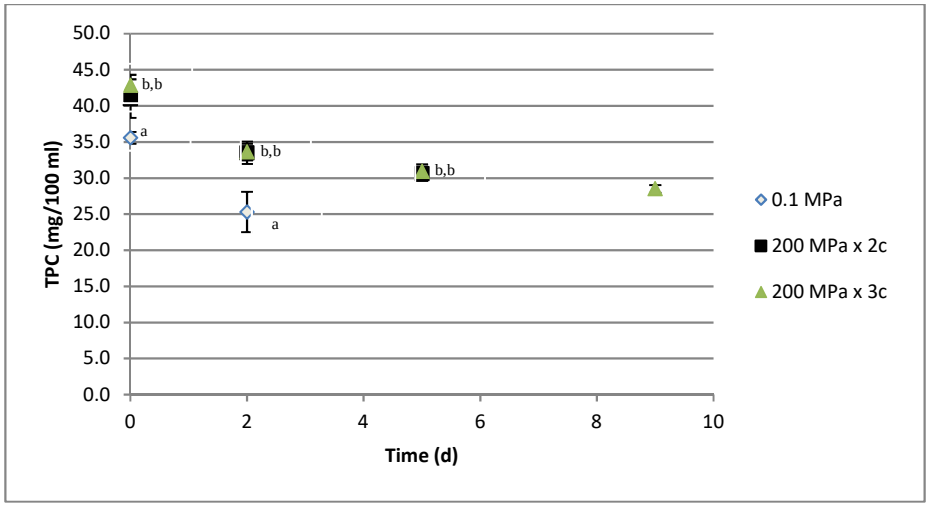
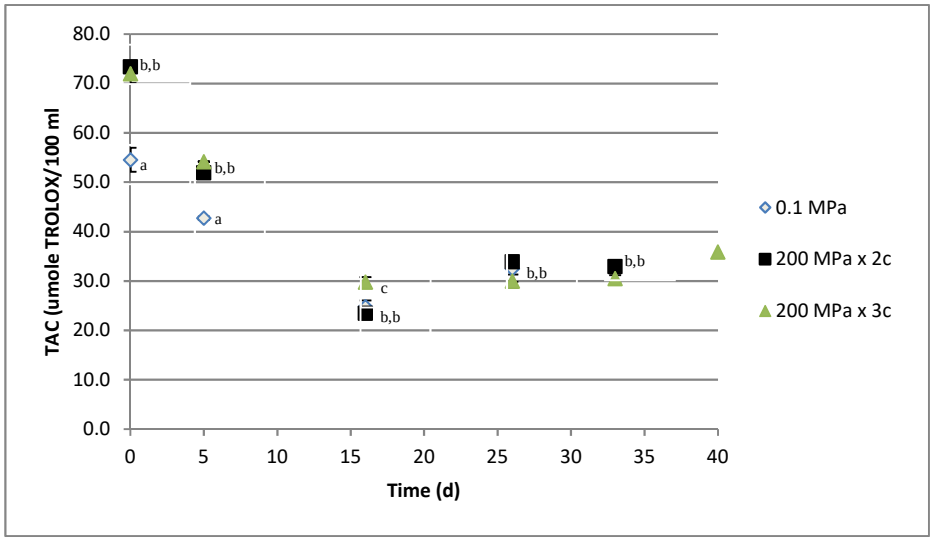
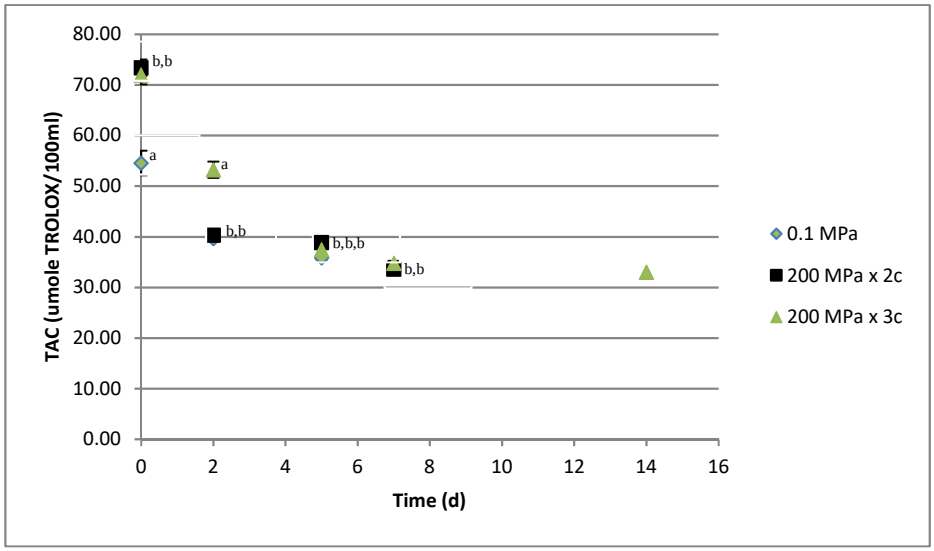

50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
TPC (mg/100 ml)
0
2
4
6
8
10
Time (d)
b,b
a
b,b
a
b,b
0.1 MPa
200 MPa x 2c
200 MPa x 3c

Figure 2. Total antioxidant activity by DPPH assay of organic kiwifruit juices in relation to the high pressure applied, during the storage at 5 (1a), 15 (2a), 25 (1c) °C. Means followed by different letters means significant different ($p<0.05$) between samples at each day of storage.

(a) 5°C



(b) 15°C



(c) 25°C

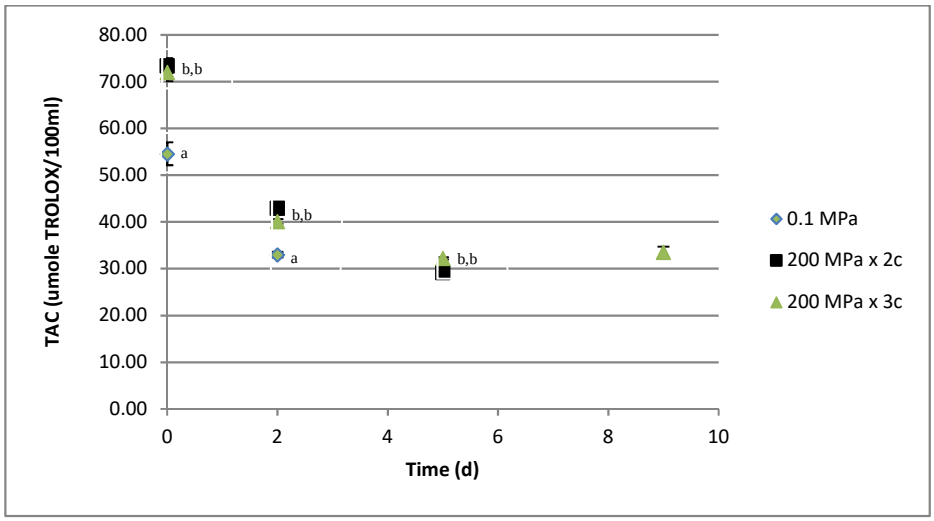


Table 1. Yeast cell loads (log CFU/mL) detected in organic kiwifruit juices immediately after the treatments and during the storage at 5, 15 and 25 °C in relation to the pressures applied.

Cell load (log CFU/mL)							
5 °C							
	T0	T5	T16	T26	T33	T40	
0.1 Mpa	2.4±0.2	2.5±0.1	3.9±0.2	4.6±0.6 [a]	-*	-*	
200MPa *2 cycles	**	**	**	1.5±0.1 [b]	2.3±0.2	4.0±0.5	
200MPa*3 cycles	**	**	**	**	**	**	
15 °C							
	T0	T2	T5	T7	T9	T12	T14
0.1 Mpa	2.4±0.2	3.0±0.3	3.2±0.6	4.7±0.4	-*	-*	-*
200MPa *2 cycles	**	**	**	**	1.5±0.1	-*	-*
200MPa*3 cycles	**	**	**	**	**	4.4±0.4	5.9±0.3
25 °C							
	T0	T2	T5	T7	T9		
0.1 MPa	2.4±0.2	4.7±0.2	-*	-*	-*		
200MPa *2 cycles	**	**	-*	-*	-*		
200MPa*3 cycles	**	**	**	2.0±0.3	5.4±0.4		

*not performed because the juice spoiled

**under the detection limit

Means followed by different letters means significant different ($p<0.05$) between samples at each day of storage

Table 2. pH values detected in organic kiwifruit juices immediately after the treatments and during the storage at 5, 15 and 25 °C in relation to the pressures applied.

			5 °C			
	T0	T5	T16	T26	T33	T40
0.1 MPa	3.34±0.01 [a]	3.28±0.02 [a]	3.14±0.01	3.10±0.03	-*	-*
200MPa *2 cycles	3.27±0.01[b]	3.17±0.02 [b]	3.12±0.01	3.09±0.01	3.09±0.02	3.17±0.02
200MPa*3 cycles	3.25±0.02 [b]	3.15±0.02 [b]	3.03±0.01	3.06±0.01	3.07±0.01	3.15±0.02
			15 °C			
	T0	T2	T5	T12	T14	
0.1 MPa	3.34±0.01 [a]	3.24±0.02 [a]	3.19±0.01 [a]	-*	-*	
200MPa *2 cycles	3.27±0.01 [b]	3.24±0.01 [a]	3.18±0.01 [a]	-*	-*	
200MPa*3 cycles	3.25±0.02 [b]	3.22±0.01 [a]	3.13±0.01 [b]	3.10±0.02	3.05±0.01	
			25 °C			
	T0	T2	T5	T7	T9	
0.1 MPa	3.34±0.01 [a]	3.20±0.01 [a]	-*	-*		
200MPa *2 cycles	3.27±0.01 [b]	3.19±0.02 [a]	-*	-*		
200MPa*3 cycles	3.25±0.02 [b]	3.20±0.02 [a]	3.18±0.01	3.04±0.02	3.02±0.02	

*not performed because the juice spoiled

Means followed by different letters means significant different ($p<0.05$) between samples at each day of storage.

Table 3. Viscosity (cP) of organic kiwifruit juices immediately after the treatment and during the storage at 5, 15 and 25 °C in relation to the pressure applied.

5 °C						
	T0	T5	T16	T26	T33	T40
0.1 Mpa	1.6±0.1^{b}	1.7±0.1^{c}	1.4±0.1^{b}	1.1±0.0^{b}	-*	-*
200MPa *2 cycles	2.0±0.1^{a}	2.5±0.2^{a}	2.3±0.2^{a}	1.2±0.1^{a}	1.2±0.1^{b}	1.3±0.1^{b}
200MPa*3 cycles	1.8±0.1^{ab}	2.2±0.1^{b}	2.1±0.1^{a}	1.3±0.1^{a}	1.5±0.1^{a}	1.7±0.2^{a}
15 °C						
	T0	T2	T5	T12	T14	
0.1 Mpa	1.6±0.1^{b}	1.5±0.2^{b}	1.5±0.1^{a}	-*	-*	
200MPa *2 cycles	2.0±0.1^{a}	2.2±0.1^{a}	1.5±0.1^{a}	-*	-*	
200MPa*3 cycles	1.8±0.1^{ab}	2.1±0.1^{a}	1.6±0.2^{a}	1.8±0.1	1.9±0.1	
25 °C						
	T0	T2	T5	T7		
0.1 Mpa	1.6±0.1^{b}	1.5±0.1^{b}	-*	-*		
200MPa *2 cycles	2.0±0.1^{a}	1.8±0.1^{a}	-*	-*		
200MPa*3 cycles	1.8±0.1^{ab}	1.8±0.1^{a}	1.8±0.2	1.8±0.1		

*not performed because the juice spoiled

Means followed by different letters means significant different ($p<0.05$) between samples at each day of storage.

Table 4. Lightness (L*), a* and b* values of organic kiwifruit juices immediately after the treatment and during the storage at 5, 15 and 25 °C in relation to the pressure applied.

L*						
5 °C						
	T0	T5	T16	T26	T33	T40
0.1 MPa	33.4±0.7[b]	34.6±0.6[b]	30.3±0.4[b]	30.5±0.2[b]	-*	-*
200 MPa *2 cycles	38.68±0.08[a]	38.6±0.6[a]	36.5±0.2[a]	35.5±0.8[a]	35.4±0.3[a]	35.2±0.4[b]
200 MPa *3 cycles	38.9±0.2[a]	38.5±0.5[a]	36.2±0.1[a]	36.5±0.8[a]	36.9±0.4[a]	37.7±0.2[a]
15°C						
	T0	T2	T5	T12	T14	
0.1 MPa	33.4±0.7[b]	32.3±0.8[b]	-*	-*	-*	
200 MPa *2 cycles	38.68±0.08[a]	38.1±0.4[a]	-*	-*	-*	
200 MPa *3 cycles	38.9±0.2[a]	38.9±0.2[a]	37.99±0.01	36.3±0.2	37.1±0.5	
25°C						
	T0	T2	T5	T7		
0.1 MPa	33.4±0.7[b]	33.4±0.7[b]	-*	-*		
200 MPa *2 cycles	38.68±0.08[a]	38.68±0.07[a]	-*	-*		
200 MPa *3 cycles	38.9±0.2[a]	38.9±0.2[a]	37.5±0.3	36.4±0.2		
a*						
5 °C						
	T0	T5	T16	T26	T33	T40
0.1 MPa	-2.4±0.3[a]	-1.8±0.1[a]	-2.7±0.2[a]	-2.1±0.2[a]	-*	-*
200 MPa *2 cycles	-3.7±0.2[b]	-4.6±0.2[b]	-3.4±0.1[b]	-2.9±0.1[b]	-2.8±0.2[a]	-2.7±0.1[a]
200 MPa *3 cycles	-3.4±0.3[b]	-4.4± 0.2[b]	-3.7±0.2[b]	-3.2±0.2[c]	-3.2±0.2[b]	-3.15±0.07[b]
15°C						
	T0	T2	T5	T12	T14	
0.1 MPa	-2.4±0.3[a]	-3.4±0.2[a]	-*	-*	-*	
200 MPa *2 cycles	-3.7±0.2[b]	-4.3±0.2[b]	-*	-*	-*	
200 MPa *3 cycles	-3.4±0.3[b]	-4.2±0.1[b]	-3.7±0.1	-3.6±0.1	-3.7±0.2	
25°C						
	T0	T2	T5	T7		
0.1 MPa	-2.4±0.3[a]	-2.4±0.3[a]	-*	-*		
200 MPa *2 cycles	-3.7±0.2[b]	-3.7±0.1[b]	-*	-*		

200 MPa *3 cycles	-3.4±0.3[b]	-3.4±0.3[b]	-3.0±0.2	-2.8±0.1		
b*						
5 °C						
	T0	T5	T16	T26	T33	T40
0.1 MPa	16.3±0.6[a]	10.9±1.0[b]	13.9±0.5[a]	15.0±0.3[a]	-*	-*
200 MPa *2 cycles	14.0±0.5[ab]	13.8±0.7[a]	13.1±0.6[a]	15.1±0.2[a]	15.3±0.3[a]	15.9±0.3[a]
200 MPa *3 cycles	12.6±0.6[b]	12.6±0.7[a]	11.6±0.2[b]	13.1±0.4[b]	14.7±0.2[b]	15.37±0.04[b]
15°C						
	T0	T2	T5	T12	T14	
0.1 MPa	16.3±0.6[a]	15.1±0.3[a]	-*	-*	-*	
200 MPa *2 cycles	14.0±0.5[ab]	13.2±0.4[b]	-*	-*	-*	
200 MPa *3 cycles	12.6±0.6[b]	13.0±0.4[b]	11.1±0.2	11.0±0.2	12.5±0.5	
25°C						
	T0	T2	T5	T7		
0.1 MPa	16.3±0.6[a]	16.3±0.6[a]	-*	-*		
200 MPa *2 cycles	14.0±0.5[ab]	14.0±0.3[ab]	-*	-*		
200 MPa *3 cycles	12.6±0.6[b]	12.6±0.6[b]	12.3±0.2	11.9±0.4		

*not performed because the juice spoiled

Means followed by different letters means significant different ($p<0.05$) between samples at each day of storage.