UNIVERSITÀ DEGLI STUDI DI PARMA
Dipartimento di Scienze degli Alimenti
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Ciclo XXVII

Effect of thermal and nonthermal processes on selected physicochemical parameters of vegetables

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Ad Annarita e Tina,
per l’intensità di questi anni

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Summary

The increasing request of fruit and vegetables is bringing the food industry, together with the academia, to improve the processes targeted to prolong the crops shelf-life. Preservation of foods usually involves technologies that prevent microbial growth and retard enzymatic quality degradation reactions. Traditionally, thermal processing (≥90 °C) and freezing (≤−40 °C) have formed the core of food preservation. During freezing, the ice crystal formation causes damages of vegetables cells walls. Furthermore, frozen vegetables are previously blanched and consumed cooked, so they are subjected also to the detrimental effect of thermal treatment.

Novel nonthermal technologies are one of the alternatives currently available for the preservation of vegetables because they accomplish inactivation of microorganisms and enzymes but minimize adverse thermal exposure. Among them, the use of high hydrostatic pressures for food processing is finding increased application within the food industry.

Despite several studies have been conducted on the effect of processes on vegetables, a lack of literature is still present, because of the fragmented investigation of the effect of traditional methods and still poor data on the novel technologies.

Based on these considerations, the present PhD thesis deals with the evaluation of the effect of different conventional and innovative preserving technologies on vegetables by means of selected physico-chemical properties. Different approaches were investigated to reach this goal.

In the first section of this PhD thesis, the evaluation of the effect of different steps of a conventional industrial freezing process was investigated on asparagus, zucchini and green beans. Samples were examined in all the stages of production "from farm to fork" as raw/uncooked (control test), blanched, boiled from the raw samples and after industrial freezing. A deep investigation was carried out to evaluate qualitative (texture and colour), histological and nutritional (antioxidant activity and bioactive compounds) aspects. The obtained results will show how manufacturers and researchers have to join together in order to develop industrial freezing process conditions

according to the matrix of vegetable, with the final aim being to offer the end-user consumer high quality frozen products.

In the second section of the thesis, the evaluation of different cooking methods as preservation technique for vegetables was investigated. There is a general lack of study in literature on the effect of cooking on the main quality attributes (i.e. texture, colour) that have a great impact on the final consumer acceptance of vegetables. When taken into consideration, only a single type of heating treatment or one quality attribute was discussed. Thus, this topic needs to be more deeply investigated with the ultimate goal to offer a higher quality final cooked product to the consumers, achieving a greater retention of the original quality of the processed vegetable. Starting from this observation, in a first study the effect of three common cooking procedures (boiling, steaming and microwaving) on structure, texture and colour of carrots processed in an industrial plant was studied and the obtained results were compared with those obtained from raw carrots provided by the same manufacturer and cooked with the same procedures. Then, a relatively new little explored cooking approach, the combined air/steam cooking, was the object of another investigation. Several air/steam cooking time/temperature conditions were selected and applied on two commonly used vegetables, as pumpkin and Brussels sprouts, selected on the basis of their different nutritional values and physical characteristics. The results obtained in term of cooking (cooking values, weight loss), physical (texture and colour) and nutritional (antioxidant activity and phenol contents) parameters were compared with those obtained cooking the same vegetables with a common steaming procedure.

In the third section of the thesis, the impact of a novel non thermal technology, such as high hydrostatic pressure process was studied at the Centre for nonthermal treatment of food of the Washington State University of Pullman (WA) on beetroot, which presents a well-known high nutritional value. In particular, the effect of pressure treatment realized at 650 MPa and a temperature of 21°C was studied taking into account different times of processing evaluating physico-chemical, enzymatic and nutritional aspects. The obtained results were compared to the raw

product and those treated with classical thermal treatments as blanching and canning. The preliminary findings obtained in this part of the thesis, suggested that the high hydrostatic pressure treatment could be used as valid alternative for the preservation of beetroots, being less invasive than canning and quite comparable with blanching.

In the last part of this PhD work, a novel spectroscopical approach as Raman spectroscopy was used for the evaluation of carotenoid pattern changes after different cooking treatments on carrots. Changes on carotenoid profiles measured by means of Raman spectroscopy were compared with their quantitative determinations acquired by the high-performance liquid chromatography and the colour parameters finding good statistical correlations among techniques.

In conclusion, this PhD thesis revealed the strengths and weaknesses of both the traditional thermal and novel nonthermal vegetable treatments. In general, while the freezing and cooking traditional methods resulted in an extended modification of the quality parameters, the high pressure treated samples revealed a better preservation degree. The extent of the modifications observed after the application of either thermal or nonthermal processes was however related to the type of vegetable and the process conditions, therefore this finding should encourage to research and plan specific processing conditions. A big effort was given to the use of novel analytical technology, such as Raman spectroscopy, for the fast and easy way to obtain real time information on the chemical transformation of the nutrients under treatment.

Introduction

Introduction

1. **Vegetables research overview**

Frequent consumption of fruit and vegetables is recommended in practically all dietary guidelines (USDA, 2010; WHO 2014) in order to maintain a balanced diet and to prevent obesity and chronic diseases. A recent WHO/FAO expert consultation report on diet sets population nutrient goals and recommends intake of a minimum of 400 g of fruits and vegetables per day for the prevention of chronic diseases such as heart diseases, cancer, diabetes and obesity. Vegetables bring us vitamins, minerals and fiber, some energy (mainly in the form of sugar), as well as certain minor components, often referred to as phytochemicals or secondary plant products, which are potentially beneficial for human health (Mobasheri et al., 2014). The increasing attention to health and wellness, in order also to reduce the health public expenditure, is bringing the governments to finance the scientific research in this field.

The United States Department of Agriculture (USDA), included Nutrition and Childhood Obesity among the seven identified priority research topics in 2012. In these efforts, USDA supports nutrition education programs and encourages Americans to consume more nutritious foods like fruits and vegetables. Agriculture, food and health are also significant themes in the last European Union's Seventh Framework Research Programme (FP7) (McCarthy et al., 2011). Based on this supports, several scientific research are being developed to show the evidence of the relationship between vegetables intake and reduction of cardiovascular and chronic diseases in these last years (Boeing et al., 2012; Dauchet et al., 2006).

The strategy for research and innovation of a country, across all sectors, aim also at the increasing commercial investments. Fruit and vegetables have witnessed consistent increases in the net exporter position of many countries. These products have become the most important in value terms. Over the 2000–2010 decade, the fruit and vegetables sector has grown by more than 11 percent per year at the global level, by almost 20 percent in Africa and by 17 percent in Asia, but Europe continues to be the largest exporter (FAO 2013). In 2003, 9.8 and 13.8 million metric tons

of vegetables for freezing and canning, respectively, were produced in the United States with a total farm gate value of US$1.4 billion (De Corcuera et al., 2004).

Internationalization of food supplies, more eating out (and less home-cooking), and growing attention to 'convenience' are all influencing eating habits and deserve close attention. While the retail and catering industries are concerned with supply and choice, food processing creates issues of content, taste and safety. In the field of food processing, the matters of design, preparation, manufacturing and also home cooking, linked to health and disease representing the interface between industry and the scientific sector. With the aim to address the balance between commercial opportunity and consumer protection and satisfaction, the research is investigating also in the vegetable processing field. Several scientific studies are so also focused on the development of novel vegetables industrial processes, the improving of the traditional ones and the impact that they have on the crops (De Corcuera et al., 2004; Oey et al., 2008; Mazzeo et al., 2011).

2. Vegetable processing

Plant-based foods are subjected to cooking or processing to increase their edibility and palatability. Furthermore, because of the varied growing and harvesting seasons of different vegetables at different locations, the availability of fresh vegetables differs greatly in different parts of the world. Processing can transform vegetables from perishable products into stable foods with long shelf-life and thereby aid in the global transportation and distribution of many varieties of vegetables. The goal of processing is to deter microbial spoilage and natural physiological deterioration of the plant cells while the original sensory and nutritional properties are maintained as high as possible. To achieve the balance between food quality and safety, there is a need to optimize conventional processing techniques currently applied in food industries and to develop novel processing technique (Oey 2008).

Generally, the traditional techniques are based on the use of temperature as process variable.

Among the vegetable thermal treatments, in this thesis it will be discussed blanching, canning and freezing and cooking techniques.

The novel technologies could be generally called nonthermal technologies because of the use of process variables alternative to the temperature, such as pressure, electricity, electromagnetic wavelengths etc. In this context, the high pressure technology will be described.

2.1 Thermal treatments

- High temperature

Blanching

Blanching is a pre-treatment for freezing, canning, and drying and it consists in the exposure of the vegetables to heat treatment for a brief period principally to inactivate enzymes, but also to modifying texture; preserving color, flavor, and nutritional value; and removing trapped air (De Corcuera et al., 2004). Depending on its severity, blanching will also destroy some microorganisms. The two most used commercial methods of blanching involve passing food through an atmosphere of saturated steam or a bath of hot water. Microwave blanching is not yet used commercially on a large scale (Fellows, 2009). Water blanching is performed in hot water at temperatures ranging typically from 70 °C to 100 °C, resulting in a uniform treatment. In steam blanchers, a product is transported through a chamber where ‘‘food-grade’’ steam at approximately 100°C is directly injected. It requires less time than water blanching because the heat transfer coefficient of condensing steam is greater than that of hot water. However, because of the high-temperature gradients between the surface and the center of the product, larger products or pieces of product can be *overblanched* near the surface and *underblanched* at the center.

Steam blanching is more energy-efficient and produces lower effluents with less biological oxygen demand (BOD) and hydraulic loads than water blanching. In addition, nutrient leaching is reduced compared to water blanching. Microwave vegetable blanching is a promising technique. Early studies showed the improvement of the blanched product quality, the reduction of the waste production and the very short processing time in comparison to conventional water or steam blanching. These first studies were however carried out in batch ovens, so the technique is still under industrial implementation (De Corcuera et al., 2004).

Practically every vegetable needs to be blanched and promptly cooled prior to every other process. Blanching before canning is carry out for the following reasons: to remove the respiratory gases that would reduce the ultimate vacuum in the can, if they were released during processing; to inhibit enzyme activity that can take place during the time taken to reach sterilizing temperatures, particularly in large cans; to soften the texture of vegetables to facilitate filling into containers prior to canning; to hydrate certain dry products; to preheat the product in order to assist the vacuum formation in the can (Ramaswamy & Chen, 2003). Blanching before freezing furthermore, removes trapped air (e.g., in broccoli florets) and metabolic gases within vegetable cells and replaces them with water, forming a semicontinuous water phase that favors a more uniform crystal growth (De Corcuera et al., 2004).

If vegetables are not heated sufficiently, the enzymes will continue to be active during storage and may cause the vegetables to toughen or develop off flavors and colors. Enzymes, which cause a loss of eating and nutritional qualities in vegetables and fruits, include lipoxygenase, polyphenoloxidase, polygalacturonase and chlorophyllase. Two heat-resistant enzymes, which are found in most vegetables, are catalase and peroxidase. Although they do not cause deterioration during storage, they are used as marker enzymes to determine the success of blanching. Peroxidase is the more heat resistant of the two, so the absence of residual peroxidase activity would indicate that other less heat-resistant enzymes are also destroyed (Fellows, 2009). Blanching also causes wilting or softening of vegetables, making them easier to pack. It destroys some bacteria and helps remove any surface dirt. For some vegetables, the blanching step also serves to remove harsh flavors (Barbosa Canovas et al., 2005). The vegetables are then ready for the various food-processing methods described below.

Canning

With the canning treatment, vegetables undergo sterilization, that is the complete destruction of microorganisms and spores, allowing to obtain stable products at room temperature. Canning is the major food processing method of the world. It is particularly useful in developing countries where

refrigeration is limited or nonexistent. Properly processed canned vegetables, moreover, can be stored at room temperature for years. In the canning process, vegetables are often cut into pieces, packed in cans, and put through severe heat treatment to ensure the destruction of the bacteria spores.

In brief, the steps of the canning treatment are:

-Food preparation by washing, sorting, grading, peeling, cutting to size, pre-cooking and pulping is conducted according to the type of product.

- Can filling of glass or metal containers may be carried out manually or by using sophisticated filling machinery. The filling weight and the volume headspaces are important factor to be considered, because can affect the rate of heat penetration affecting the extent of the treatment.

- Vacuum production can be achieved by filling the already heated product into the can, by heating the can and contents after filling, by evacuating the headspace gas in a vacuum chamber, or by injecting superheated steam into the headspace. In each case, the can is sealed on immediately afterwards. This process, called also exhausting of the container, have the aim to create an anaerobic environment in the can (vacuum), which would inhibit microbial spoilage and minimize the strain on the can seams or pouch seals during the process.

- Thermal processing is usually carried out in an autoclave or retort, in an environment of steam under pressure. The filled sealed can must be heated to a high temperature for a sufficient length of time to ensure the destruction of spoilage micro-organisms.

- Cooling of the processed cans in chlorinated water to a temperature of 37°C. At this temperature the heat remaining is sufficient to allow the water droplets on the can to evaporate before labelling and packing (Ramaswamy & Chen, 2003).

The thermal process is the core of the canning treatment. The times and temperatures required for heat processing have been determined experimentally to ensure that spores of the most heat resistant food poisoning organisms known, *Clostridium botulinum*, are destroyed (Dauthy, M., 1995). For this reasons, the sterilization frequently means a treatment of at least 121° C (250° F) of wet heat

for 15 minutes or its equivalent. This treatments conditions, note as ‘*minimum botulinum cook*’, reduce the chance of survival of *Clostridium botulinum* spores. It also means that every particle of the food must receive this heat treatment (Dauthy, M., 1995).

Bacterial spores have a greater resistance to heat when the growth-medium is neutral or near neutral, and neutrality is normally required for bacterial growth to commence. Because of this, canned foods have been broadly divided into two groups:

a) ‘acid’ foods having a pH of 4.5 or lower and

b) ‘non-acid’ foods having a pH of more than 4.5

‘Non-acid’ foods (vegetables) must, therefore be heat processed at high temperatures using steam under pressure, whereas ‘acid’ foods (fruit) may be processed at the (lower) temperature of boiling water, since this will kill moulds and yeasts. In addition, if any bacterial spores survive the combination of acid and heat, they will be inhibited from growth by the acid environment (Dauthy, M., 1995).

For high quality products, aseptic canning is practiced. Also known as high-temperature–short-time (HTST) processing, aseptic canning is a process whereby pre-sterilized containers are filled with a sterilized and cooled product and sealed in a sterile atmosphere with a sterile cover. The process avoids the slow heat penetration inherent in the traditional in-container heating process, thus creating products of superior quality (Ramaswamy & Chen, 2003).

Cooking

Although cooking has traditionally considered a domestic process, it is also often carried out outside in catering services, restaurants, or school and/or hospital canteens. Nowadays, factory food preparation has become common, with many "ready-to-eat" foods being prepared and cooked in factories. "Home-cooking" may be associated with comfort food, and some commercially produced foods are presented through advertising or packaging as having been "home-cooked".

Most of vegetables can be cooked by different cooking methods. The versatility of preparation, cooking and serving is a great attribute of vegetables. The extent of cooking varies according to

individual taste and regional or traditional dictum. In the habits of the U.S. South, for example, the boiling of vegetables is usually protracted, with a soft consistency and a blend of flavors in combining ingredients considered desirable. In the *nouvelle cuisine* of France, on the other hand, Chinese influence dictates minimal boiling or steaming to preserve fresh color, texture, and flavor. In general it's possible to say that dense vegetables require longer cooking times e.g. carrots, potatoes. Vegetables with higher water content cook faster (e.g. capsicums, leafy greens).

All the cooking methods are based on a heat transfer mechanism between two systems due to the presence of a temperature gradient. There are three ways in which heat may be transferred: radiation, conduction and convection. Radiation is the transfer of heat by electromagnetic waves; conduction is the movement of heat by direct transfer of molecular energy within solids; convection is the transfer of heat based to the density changes caused to the temperature variations in the fluid. At heating the density change will cause the fluid to rise and be replaced by cooler fluid that also will then heat and rise (Fellows, 2009).

Boiling, steaming, microwaving and baking are the most used techniques for vegetables cooking.

During boiling, the food is cooked by immersion in water that has been heated to near its boiling point (100° C) at sea level. The direct contact between the food and the heating medium allows to transfer heat by convection.

During steaming the water is continuously keep at the boiling phase, causing vaporization. Steamed food is cooked by the heat carried by the steam produced by the vaporization of water by convection transfer mechanism.

Microwave cooking is an example of heat transfer by irradiation. In particular, microwave ovens has a device called magnetron that converts electrical energy in oscillating electromagnetic energy at frequency around 2450 megahertz (MHz). These quicker waves are remarkably selective, primarily affecting polar molecules. Dipoles in the water and in some ionic components such

as salt, attempt to orient themselves to the field. Since the rapidly oscillating electric field changes from positive to negative and back again several million times per second, the dipoles attempt to follow and these rapid reversals create frictional heat. The increase in temperature of water molecules heats surrounding components of the food by conduction and/or convection (Fellows, 2009).

The mode of heat transfer can coexist within the same cooking technique. For example, during baking heat is transmitted simultaneously by conduction, convection and radiation: conduction is given by the plate of the oven on which the food is placed, convection is obtained with the air (or with the steam) contained therein and the irradiation by means of electrical resistances or direct flame fueled by fuel gas.

While at the domestic level, cooking conditions are chosen according to the taste of consumers, at the industrial level it's necessary to use cooking indicators standardize the processes.

Many factors affect the performance of a cooking technique rather than another.

In conventional cooking such as steaming for example, heating starts at the surface of the food, and heat is slowly transferred to the center by conduction. Conversely, in microwave cooking, microwaves permeate the center of the food by radiation, and the heat generated within the food is transferred toward the surface of the food. In this respect, an equivalent rise in temperature occurs more quickly in microwave processing than steaming (Knutson et al., 1987). Steam cooking, in turn, has a faster temperature increase than baking or boiling, because latent heat, other than sensible heat, is transferred to food when saturated steam condenses (Fellows, 2009).

Studies on the mechanisms of heat transfer led to calculate, for the convective heat treatments, 'convective heat transfer coefficients' for some common fluids in different conditions (Rohsenow et al., 1998). They are dependent on the type of media, gas or liquid, the flow properties such as velocity, viscosity and other flow and temperature dependent properties.

- Free Convection - air, gases and dry vapors : 0.5 - 1000 (W/(m2K))

- Free Convection - water and liquids: 50 - 3000 (W/(m2K))
- Forced Convection - air, gases and dry vapors: 10 - 1000 (W/(m2K))
- Forced Convection - water and liquids: 50 - 10000 (W/(m2K))
- Forced Convection - liquid metals: 5000 - 40000 (W/(m2K))
- Boiling Water : 3.000 - 100.000 (W/(m2K))
- Condensing Water Vapor: 5.000 - 100.000 (W/(m2K))

These coefficients could be discriminating criterions in the choice of a convective technique or system of cooking food rather than another.

The depth of penetration of microwaves energy instead, is determined by the dielectric constant and the loss factor of the food. They vary with the moisture content and temperature of the food and the frequency of the electric field. In general, the lower are both the loss factor (i.e. greater transparency to microwaves) and the frequency, the greater is the penetration depth (Fellows, 2009).

In this way, it is possible to choose a frequency from the permitted bands that will give a suitable electric field strength for a given loss factor.

The amount of heat penetration in the food is not only due to the medium of heat transfer characteristic, but it is also strictly dependent from the shape and the nature of the product.

To compare the total thermal effect of different processes, the degree of cooking value (C^{z}_{Tref}) can be used (Chiavaro et al., 2006) . Measuring the temperature in the geometric centre of the samples at fixed time intervals, the heat penetration curves are obtained. From the integration of the heat penetration curves it’s possible to calculate the degree of cooking value, expressed using the following equation:

$$C^{z}_{Tref} = \int_{0}^{z} 10^{(T-Tref/z)} dt$$

where t=time; Tref=reference temperature, set equal to 100 °C; z=temperature increase that induces a 10-fold increase of the reaction rate of the chemical reaction taken as reference. Common used

z values are reported in Tab.1 (see below). From these results also the rate of cooking or the time of cooking for fixed degrees can be calculated. In this way, the food business operators can streamline the cooking processes.

- Low temperature

Freezing

Freezing is the unit operation in which the temperature of a food is reduced below its freezing point and a proportion of the water undergoes a change in state to form ice crystals. It is one of the oldest and most widely used methods of food preservation. The immobilization of water to ice and the resulting concentration of dissolved solutes in unfrozen water lower the water activity of the food (Fellows, 2009). Preservation is achieved by a combination of low temperatures, reduced water activity and, for vegetables, pre-treatment by blanching that inactivate the deteriorative enzymes (Barbosa Canovas et al., 2005). The low temperatures avoid the microorganism's growth, the chemical and the cellular metabolic reactions. Freezing preservation allows therefore to the retention of the quality of agricultural products over long storage periods extending the usage during off-season other than the transportation to remote markets that could not be accessed with fresh products. As a method of long-term preservation for fruits and vegetables, freezing is generally regarded as superior to canning and dehydration, with respect to retention in sensory attributes and nutritive properties (Barbosa Canovas et al., 2005). The availability of different types of equipment for several different food products results in a flexible process in which degradation of initial food quality is minimal with proper application procedures. From a technical point of view, it is also one of the most convenient and easiest of food preservation methods. In terms of energy consumption, the freezing process and storage constitute approximately 10 percent of the total cost (Barbosa Canovas et al., 2005). The freezing process starts with the subtraction of sensible heat to the matrix since the first seed of crystal appears. The process producing this seed, upon which the crystal can grow, is called nucleation. In food systems there is the co- existence of free and bound water. Bound water does not freeze even at very low temperatures. During the freezing

process, the concentration of soluble solids increases in the unfrozen water, resulting in an increaseof freezing temperature below the 0 °C. When the product temperature reaches the point where most freezable water has been converted to ice, the process could be considered ended and the temperature is reduced to storage temperature (-18°C).

The freezing time and rate are the most important parameters in designing freezing systems. Freezing time depends on several factors, including the initial and final temperatures of the product and the quantity of heat removed, as well as dimensions (especially thickness) and shape of product, heat transfer process, and temperature. The freezing rate (°C/h) for a product is defined as the ratio of difference between the initial and final temperature of the product to freezing time at a particular location within the product. Since the temperature distribution within the product varies during freezing process, the thermal center is generally taken as reference. Generally, if freezing is instantaneous, there will be more locations within the food where crystallization begins. In contrast, if freezing is slow, the crystal growth will be slower with few nucleation sites resulting in larger ice crystals. Large ice crystals are known to cause mechanical damage to cell walls in addition to cell dehydration.

The industrial equipment for freezing can be categorized in many ways, namely as equipment used for batch or in-line operation, heat transfer systems (air, contact, cryogenic), and product stability. The temperature of freezing is generally around -30, -40°C.

In the equipments in which air is used as freezing medium, the food is placed in freezing rooms (Air-blast freezers), or on trays placed in racks or trolleys which can be moved continuously inside a tunnel (tunnel freezers), or on a multi-tier or a spiral conveyor belt (Belt freezers). Still, air freezing is the cheapest way of freezing and has the added advantage of a constant temperature during frozen storage; however, it is the slowest method of freezing due to the low surface heat transfer coefficient of circulating air inside the room.

The individually quick frozen foods (IQF) technology was developed with the aim of quick freezing. Food is placed on a bed with a perforated bottom through which cold air is blown

vertically upwards and completely surrounds the food resulting very effective especially for particles with a tendency to stick together. Direct immersion of a product into a liquid refrigerant is the most rapid way of freezing since liquids have better heat conducting properties than air. Indirect contact freezers are instead type of freezer, in which materials being frozen are separated from the refrigerant by a conducting material, usually a steel plate (Barbosa Canovas et al., 2005).

The frozen food market is one of the largest and most dynamic sectors of the food industry. The global frozen food market is expected to grow from $218.41 billion in 2010 to $261.50 billion in 2015 at an estimated CAGR (compound annual growth rate) of 3.7% (www.marketsandmarkets.com - Market Research Reports Marketing Research Company, Business Research). During 2013, in Italy the frozen vegetables segment resulted the first in absolute value: it it constitutes about the 43% of the retail volume sales (224.600 tons in 2013) (www.istitutosurgelati.it). Today an increasing demand for frozen foods already exits and further expansion of the industry is primarily dependent on the ability of food processors to develop higher qualities in both process techniques and products. Improvements can only be achieved by focusing on new technologies and investigating poorly understood factors that influence the quality of frozen food products like relative cost and nutritive values.

2.3 Non-thermal

-High pressure technology

High hydrostatic pressure (HHP) is as a novel nonthermal technology recently used for food preservation. In the early 1990s, only a few high-pressure units were available for use; at the beginning of 2000, an important increase in the number of high-pressure units was observed and nowadays is still growing (Bermudez Aguirre & Barbosa Canovas, 2011). Today, the food industry uses some of this equipment in their facilities and now processes and sells high-pressurized products comprise fruit and vegetable products.

During the HHP treatments, the food (liquid or solid) is subjected to pressures above 100 MPa up to 900 MPa, with pressures used in commercial systems between 400 and 700 MPa (San Martin et al.,

2002).The effect of HP on food chemistry and microbiology is governed by Le Chatelier's principle. This principle states that when a system at equilibrium is disturbed, the system then responds in a way that tends to minimize the disturbance (Pauling 1964). In other words, HP stimulates some phenomena that are accompanied by a decrease in volume, but opposes reactions that involve an increase in volume. Consequently, HP can disrupt large molecules of or microbial cell structures, such as enzymes, proteins, lipids and cell membranes, and leave small molecules such as vitamins and flavour components unaffected (Norton & Sun, 2008).

The pressurization is carried out for the duration of the treatment in a confined space (pressure vessel) containing a fluid (usually water) that acts as the pressure transmitting medium. An HHP processing system (Fig.1) consists basically of the pressure vessel, a pressurization system, devices for temperature control (heating/cooling systems), and product handling devices.

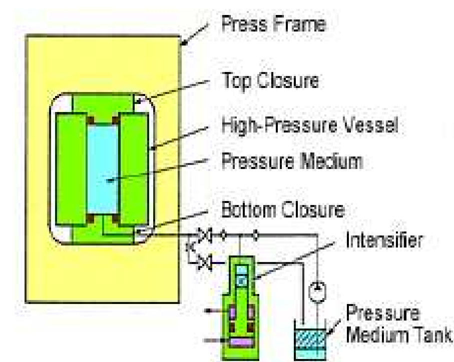

Fig.1 Pressure application system
(modified from: Redie, 2012)

HHP is a batch process, although semi continuous lines may be built by assembling three or more pressure vessels in series (Fig.2).

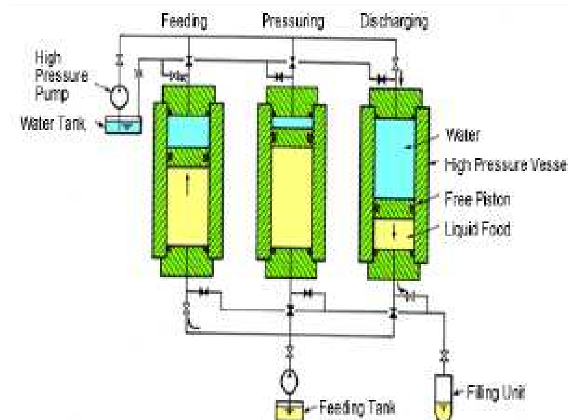

Fig.2 Continuous high pressure processing system
(modified from: Redie, 2012)

According to the isostatic rule on which the HP process are based, the pressure is instantaneously and uniformly transmitted throughout a sample whether the sample is in direct contact with the pressure medium or hermetically sealed in a flexible package (Fig.3) (Norton & Sun, 2008).

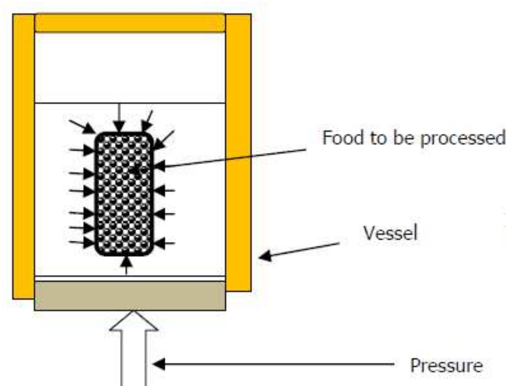

Fig.3 Isostatic pressure application
(modified from: Redie, 2012)

Therefore, the time necessary for HPP should be independent from the sample size and the geometry of the product, which are important limiting factors in thermal processing and frequently

lead to size reduction (Rastogi et al. 2007). Due to the work of compression, HPP causes temperatures to rise inside the HP vessel. This is known as adiabatic heating and should be given due consideration during the preservation process. The value of the temperature increments in the food and pressure transmitting medium will be different, as they depend on food composition as well as processing temperature and pressure and the rate of pressurization (Otero et al. 2007). Improvements are being made in the materials used for construction of the high-pressure vessel and the pumps to reduce come-up times. The pressure is held for the desired treatment time and then released. The applied pressure and the holding time will depend on the type of product treated and the expected final result, that is, generally, enzyme inactivation requires using higher pressures than pressures used for microbial inactivation (San Martin et al., 2002). When conducted at room temperature, the HHP is used for pasteurization; consequently low acid foods need to be stored in refrigerated conditions. Sterilization of products against spore growth was approved by the FDA in 2009 with the use of a combined pressure and heat technique known as PATS (pressure assisted thermal sterilization) (NFSCT, 2009).

In the past, the commercialization of high-pressure products was focused on high value-added foods because of the low availability of HHP equipment in many countries and the scarcity of HHP-processed products. The main cost involved is the equipment and its installation, but because of current availability of high-pressure equipment, the cost of the products processed by this technology has significantly dropped in recent years, making more products accessible to the consumers. However, because of the fast development of HHP technology, the high acceptance of these products and their demand by consumers, more HHP-processed products are now available in the local supermarkets, reducing their cost considerably (Bermudez Aguirre & Barbosa Canovas, 2011). According to Rastogi et al. (2007), the average cost of high-pressure processing is around US$0.05–0.5 per liter or kilogram depending on processing conditions, which is lower than thermal processing costs. However, according to Hernando-Sainz et al., (2008), the cost of high-pressure processing under some common processing conditions, for example, ready-to-eat meats (585 MPa,

3 min, filling vessel 50%), is really between US$0.08 and 0.22/kg, which makes HHP technology suitable for products of premium quality. At the end of 2007, an average of 120 pressurized products was reported around the world, with an estimated production of 150,000 tons/year. Although the incorporation of pressurized products into the global market is generally slow, new products and markets are appearing on a daily and regular basis.

3. Vegetables quality and its measurement

In the past, the first issue of the food industry was the food safety, inactivating or inhibiting pathogenic microorganisms of public health concern. Nowadays, in addition to the first, at the heart of all food control activities there is also the achievement of consumer satisfaction.

Shewfelt (1999) suggests that the combination of characteristics of the product itself be termed quality and that the consumer’s perception and response to those characteristics be referred to as acceptability. Consumers, nowadays, judge food quality based on its sensory and nutritional characteristics (e.g. texture, flavor, aroma, shape and color, vitamins etc.), and alongside shelf life, these now determine an individual’s preference for specific products (Norton et al., 2008).

For vegetables, the concept of quality is often associated with the ones of freshness. This dualism exists because fresh vegetables, other than being more palatable, are also a known source of healthy molecules that may be lost during ripening or industrial treatments.

At factory level, because of the production of standard products, it’s important that the quality perception has the same meaning for everyone using it. Instruments can approximate human judgments by imitating the way people test the product or by measuring fundamental properties and combining those mathematically to categorize the quality. The instrumental measurement of the quality is often preferred over sensory evaluation, because instruments reduce variations among individuals, are more precise, and can provide a common language among researchers, industry and consumers. Only people can judge quality, but instruments that measure quality-related attributes are vital for research and for inspection (Abbot, 1999).

Methods to measure quality and quality-related attributes have been developed over centuries. Colour and texture are probably for vegetables the major physical attributes for the consumer choice. Instrumentally, the color of an object can be described by several color coordinate systems as RGB (red, green and blue), Hunter L a b, CIE (Commission Internationale de l'Eclairage) L* a* b*, etc. and it is measured by colorimeters in which the sensors are filtered to respond similarly to the human eye. Fruit and vegetable color is due to the presence of pigmented molecules in the cellular compartments (Mazzeo et al., 2011).

Mechanical properties are instead related to texture. Mechanical tests of texture include the familiar puncture, compression and shear tests, as well as creep, impact, sonic and ultrasonic methods. Pectic polysaccharides, which are abundantly found in the primary wall and the middle-lamella between cells, are primarily responsible for most of the texture changes of fruits and vegetables (Waldron et al., 1997). The texture of edible vegetables observed at a macroscopic level, can give information at microstructural/cellular level in relation with the activity of certain pectin-related enzymes as pectin methylesterase (Sila et al.,2009). Thus, the study of the microstructural changes and enzymatic activity are important tasks in order to understand and predict the changes occurred in the physical–chemical properties at higher levels of structure.

As mentioned before, the vegetable quality is often associated with their healthy properties. The presence of vitamins, minerals, fibers and bioactive compounds, with high antioxidant properties, allows vegetables to be considered preventive agents for chronic and cardiovascular diseases. The healthy properties of a product unfortunately are not perceived by the consumers senses. At laboratory level, it's however possible to measure the composition or the antioxidant activity of a product, thanks to sophisticated chemical detection equipments or assays. The growing attention of the manufacturers and of the institutions towards consumers leads them to check the nutritional properties of the processed products and for a transparency reason report it in the label.

Fruits and vegetables are notoriously variable, and the quality of individual pieces may differ greatly from the average. It is essential to determine statistically the number of pieces and the

number of measurements per piece required to achieve significant, representative sampling (Abbott, 1999).

A continuing aim of food manufacturers is to find improvements in processing technology to retain freshness creating desirable sensory qualities or reducing the damages to food caused by processing.

4. Vegetable physico-chemical quality modifications under treatment

- *Effect on texture*

Thermal processes of vegetables result in an initial loss of the instrumental firmness due to membrane disruption and the associated to the loss of turgor. Additional softening occurs later as a result of an increase in the cell separation. This is due to the ß-eliminative degradation of the pectic polymers that are involved in cell-cell adhesion. The extent of ß-elimination is related to the degree of methyl esterification of the pectic polymers and to the levels of Ca^{2+} that are available for cross-linking. In some processes such as canning, the extent of softening can be modulated and reduced by the addition of Ca^{2+} (Waldron et al., 1997). These phenomena were observed for canned, cooked and in a less extent, for blanched vegetables. Kidmose and Martin (1999) reported furthermore that the microwave blanching better retains the textural quality than water or stem blanching on carrots.

During freezing, the rigid cell structure of fruits and vegetables may be damaged by ice crystals. The extent of damage depends on the size of the crystals and hence on the rate of heat transfer. While rapid freezing rates retain better texture and high degree of cellular integrity, considerable softening and structural damage were seen at slower rates. Slow freezing can lead to large extracellular ice crystals, increased concentration of solutes, and therefore to cell dehydration and death through osmotic plasmolysis and membrane damage. Upon thawing, extracellular ice does not reenter the cells and may cause extensive drip and texture softening (Fellows et al., 2009).

High pressure treatments have been shown to have a softening influence on texture of fruits and vegetables; tissue firmness may be lost due to cell walls breakdown and loss of cells turgidity (Oey et al., 2008). Basak and Ramaswamy (1998) found that pressure-induced textural changes occurred in two phases: instantaneous drop after pressure application followed by a gradual texture recovery

or further loss during pressure holding. Oey et al. (2008) suggested that the disruption of the tissue, after the high pressure process, liberate the pectinmethylesterase (PME) that contacts its substrate, the highly methylated pectin, leading to demethylation. The de-esterified pectin is capable of forming a gel-network with divalent ions resulting in increased hardness during the holding phase.

- *Effect on color*

The heat treatments chemically affect the vegetable pigments allowing changing in colors. Chlorophyll, the major green pigments, wildly distributed in plant species is highly unstable and rapidly changes in color to olive green and brown. This color change is believed to be due to the conversion of chlorophyll to pheophytin by the exchange of Mg^{2+} by H^+ in the center of the porphyrin ring of the pigment. This reaction is accelerated by heat and is acid catalyzed (Turkmen et al., 2006). Several studies reported however that water blanching and sometimes boiling brighten the color of some vegetables by removing air and dust on the surface and thus altering the wavelength of reflected light (Pellegrini et al., 2010). Sodium carbonate (0.125% w/w) or calcium oxide are often added to blancher water to protect chlorophyll and to retain the color of green vegetables.

Carotenoids, in particular α and ßcarotene, are a group of mainly lipid soluble compounds responsible for many of the yellow and red colors of plant. Their colors don't fade much in response to heat transferred by water like in water blanching or boiling. The major cause of carotenoids degradation is oxidation. Its change during processing was related to their isomerization into various cis-isomers (Chen et al., 19995). Mazzeo et al., 2011 reported reduced redness after cooking, more for steaming than for boiling, probably because of the oxygen presence.

Anthocyanins are a group of more than 150 reddish water-soluble pigments that are very widespread in the plant kingdom. They are degraded to brown pigments under the effect of heat and the rate of destruction is pH dependent, being greater at higher pH values.

Poor data are available on the effect of mere freezing on the color of vegetables. It seems that it doesn’t have any effect on the instrumental color perception of the treated product. Some changes

were registered during frozen storage. Chloroplasts and chromoplasts (the cellular organelles in which pigments are compartimentalized) are broken down during freezing. During storage the free chlorophyll is slowly degraded to brown pheophytin even in blanched vegetables. Changes in pH due to precipitation of salts in concentrated solutions change the color of anthocyanins. Residual lipoxygenases activity in inadequately blanched vegetables causes degradation of carotene. Browning during storage are instead due to still high polyphenoloxidase activity (Barbosa Canovas et al., 2005). Pellegrini et al., (2010) reported well retained color for Brassica vegetables when cooked after freezing than cooked from fresh vegetables. They correlated these phenomena with the positive effect of the previous blanching treatment in the deactivation of the enzymes and on the replacement of the oxygen with water, avoiding pigments oxidation.

High pressure treatment (at low and moderate temperatures) has a limited effect on pigments. The color compounds of HP processed fruits and vegetables can, however, change during storage due to incomplete inactivation of enzymes and microorganisms, which can result in undesired chemical reactions (both enzymatic and non-enzymatic) in the food matrix (Oey et al., 2008).

- *Effect on phytochemicals*

The colored molecules, such as carotenoids, are themselves antioxidant molecules, therefore their modification under treatment could be associated with changes in the healthy value.

Vitamins and polyphenols are other high healthy and antioxidant molecules present in vegetables.

Strong thermal treatments generally cause significant losses in all these compounds. The effect of the high temperature processes on phytochemicals is often the chemical degradation. The acceleration of some oxidative modifications can also occur during thermal treatments. The water heat treatments such as blanching or boiling lead to the loss of some water soluble because of the leaching in the heating medium (Fig.5). Vitamin C (ascorbic acid) is the most frequently lost nutrient. Its high solubility and susceptibility to heat and oxygen make it a conservative indicator of nutrient retention (De Corcuera et al., 2004). For some canned foods however, soluble vitamins are transferred into the brine or syrup, which is also consumed thus allowing to a smaller nutritional

loss (Fellows, 2009). The absence of the leaching effect of the "dry" thermal processes, seems to have a better retention of the nutritional molecules. Kidmose and Martin (1999) reported higher nutritional quality for microwave blanched carrots than for water blanched ones. Pellegrini et al., (2010) reported a good retention of phytochemicals in microwave cooked Brassica vegetables than in boiled. They found also that boiling had a more marked effect on nutritional pattern of frozen vegetables in comparison with steaming.

On the other hand, several studies reported also increased antioxidant activity for blanched or cooked vegetables (Mazzeo et al., 2011; Jimenez Monreal et al., 2009). This increment was justified by the cellular release of the molecules with antioxidant activity due to the thermal modification of some components of cell walls or, as suggested by Yamaguchi et al., 2001, by the production of stronger radical-scavenging antioxidants and/or novel products by chemical reaction and/or inactivate oxidative enzymes.

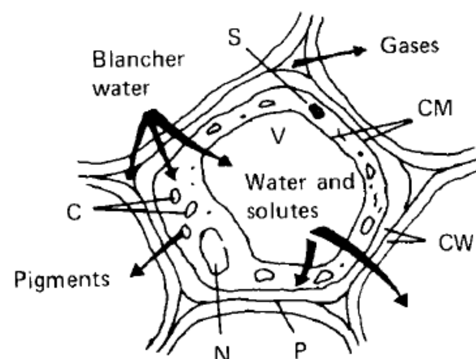

Fig.5 Leaching of the water – soluble molecules during blanching (modified from Dauthy, M. 1995)

The freezing process itself has no effect on nutrients. Losses or increases of the nutritional molecules during the freezing pre-treatments can occur, as said before for blanching.

Most frozen vegetables will maintain a high nutritional quality when stored at -18 °C or lower. At that temperature very slow residual enzymatic activity is registered, allowing, for frozen product, 12 to 18 months of shelf life (Barbosa Canovas et al., 2005). Pellegrini et al., (2010) found that, after cooking, fresh Brassica vegetables retained phytochemicals and total antioxidant activity better than

frozen samples. They justified these results saying that the previous blanching and subsequent freezing softened the vegetable matrix causing more extended losses after cooking.

Many studies on vitamin stability under high pressure treatment (at moderate temperatures) have shown that HP does not significantly affect or affects only slightly the vitamin of phytochemicals content of fruit and vegetable products (McInerney et al., 2007; Oey, 2008b). Increased antioxidant activity have been found on some vegetables treated with high hydrostatic pressures (McInerney et al., 2007). It is possible that changes to the tissue matrix induced by high hydrostatic pressures, for example disruption of plant cell walls, resulted in the release into the extracellular environment of compounds with antioxidant actions. Losses of nutritive molecules of HHP treated vegetables were often reported during storage. The high baro-resistance of some vegetables quality related enzymes (González-Cebrino et al., 2013) lead to an incomplete inactivation and a quality drop during shelf life for the HHP treated vegetables (Suthanthanjai et al., 2005).

4.1 Process quality indicators

The two most important issues connected with food processing are food safety and food quality.

The major safety issue for thermal processes involves inactivating pathogenic microorganisms that are of public health concern. Quality issues revolve around minimizing chemical reactions and loss of nutrients and ensuring that sensory characteristics (appearance, colour, flavor and texture) are acceptable to the consumer. Quality changes, which may result from enzyme activity, must also be considered. Conflicts between safety and quality issues can exist. For example, microbial inactivation and food safety are increased by more severe heating conditions while product quality in general deteriorates.

When heat inactivation studies are carried out at constant temperature, it is often observed that microbial inactivation follows first order reaction kinetics i.e. the rate of inactivation is directly proportional to the population. This can be illustrated by plotting the log of the population against time and finding that there is a straight-line relationship (Fig 6a).

The heat resistance of an organism is characterized by its decimal reduction time (D), which is defined as the time required to reduce the population by 90% or by one order of magnitude or one log cycle, i.e. from 10^4 to 10^3, at a constant temperature, T. Every microorganism has its own characteristic heat resistance and the higher its D value, the greater is its heat resistance. Heat resistance is also affected by a wide range of other environmental factors, such as pH, water activity and the presence of other solutes, such as sugars and salts.

Food scientists use a parameter known as the *z* value, to describe temperature dependence. This is based on the observation that, over a limited temperature range, there is a linear relationship between the log of the decimal reduction time and the temperature. The *z* value represents the temperature change which results in a tenfold change in the decimal reduction time (Fig.5b). The *z* value for most heat-resistant spores is about 10 °C, whereas for vegetative bacteria it is considerably lower, usually between 4 °C and 8 °C. A low *z* value implies that the reaction in question is very temperature-sensitive. In general, microbial inactivation is very temperature-sensitive, with inactivation of vegetative bacteria being more temperature- sensitive than heat-resistant spores. In contrast to the microbial inactivation, chemical or physical reaction rates are much less temperature-sensitive than microbial inactivation, having higher *z* values (20– 40 °C). This is also the case for many heat-resistant enzymes, although heat-labile enzymes. Differences between the D and *z* values of micro-organisms, enzymes and sensory or nutritional components of foods are exploited to optimize processes for the retention of nutritional and sensory qualities (Fellows, 2009). Some known D and *z* values are reported in Tab.1.

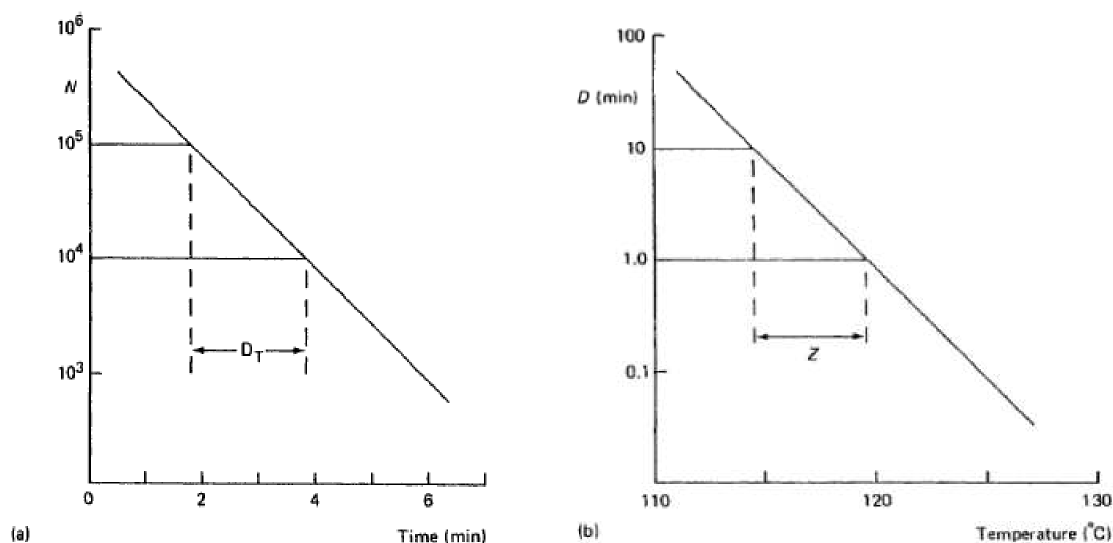

Fig.6 (a) Relationship between the population of microorganisms and time at a constant heating temperature. (b) relationship between the decimal reduction time and temperature, to determine the z value. (modified from Brennan J.G., 2006)

D and *z* values could be used also for processing in which safety is not the principal issue.

For example, the time-temperature combination used for blanching is a compromise which ensures adequate enzyme inactivation, but prevents excessive softening, losses of ascorbic acid or pigments changes, according to the priorities (Fellows, 2009). The use of the *z* value is also at the base of the cooking value calculation, as described before (Par.2).

Microbe	D_{121} (°C)	z (°C)
Bacillus stearothermophilus NCDO 1096, milk	181.0	9.43
B. stearothermophilus FS 1518, conc. milk	117.0	9.35
B. stearothermophilus FS 1518, milk	324.0	6.7
B. stearothermophilus NCDO 1096, milk	372.0	9.3
B. subtilis 786, milk	20.0	6.66
B. coagulans 604, milk	60.0	5.98
B. cereus, milk	3.8	35.9
Clostridium sporogenes PA 3679, conc. milk	43.0	11.3
C. botulinum NCTC 7272	3.2	36.1
C. botulinum (canning data)	13.0	10.0
Proteases inactivation	0.5–27.0 min at 150 °C	32.5–28.5
Lipases inactivation	0.5–1.7 min at 150 °C	42.0–25.0
Browning	–	28.2; 21.3
Total whey protein denaturation, 130–150 °C	–	30.0
Available lysine	–	30. 1
Thiamin (B_1) loss	–	31.4–29.4
Lactulose formation	–	27.7–21.0

Tab.1 Values of D and z for microbial inactivation, enzyme inactivation and some chemical reactions (modified from Brennan J.G., 2006)

Although high pressure in combination with heat was permitted by FDA for sterilization of food products, the inactivation kinetics of microorganisms or the quality changes do not follow the

traditional linear relationship that it has been assumed for many years thermal processing. As in most emerging technologies, other alternatives must be used to model and describe the inactivation patterns of microorganisms and quality factors under pressure (Bermudez-Aguirre and Barbosa-Canovas, 2011).

5. Future perspectives

As described in the preceding paragraphs, all the vegetable stabilization and preservation processes have still big limitations due to their effects on product quality. Researchers, together with industry are still implementing these techniques.

The main challenges for the heat treatments are the microbial and/or the enzymatic inactivation with the retention of the nutritional and sensory properties. The use of higher temperatures and shorter times during heat processing seems to give good results in this sense. The selection of particular time-temperature combinations to optimize a process is at the base of individual quick blanching (IQB), ultrahigh-temperature sterilization (UHT). In addition, results seem to indicate that for several traditional canned products heat transfer rates can be greatly improved by rotary agitation processing aided to minimize product degradation at the container surfaces (Richardson P., 2001).

Other promising technologies, nowadays being developed as alternative to the thermal treatments are radio frequency pasteurization or sterilization (Marra et al., 2009), microwave pasteurization, sterilization or blanching (Rames et al., 2002) and ohmic heating pasteurization, sterilization or blanching (Icier et al., 2006). The potential of these techniques are the rapid and uniform heat distribution, large penetration depth and lower energy consumption other than a consistent reduction of the produced effluents.

High hydrostatic pressure is still considered a novel technology; further basic advancements in research are thus needed. Balasubramaniam et Farkas, (2008) individuated the needs for research in this field. Kinetic models are needed for describing bacterial inactivation under combined pressure-thermal conditions and for microbial process evaluation. The identification of suitable surrogate organisms are necessary to be used as indicator for process validation studies.. Combinations of

different nonthermal technologies, such as pulsed electric field or ultrasound, with high pressure could reduce the severity of the process pressure requirement. Processing equipment requires improvements in reliability and line-speed to compete with heat pasteurization lines. More studies are also needed to document the changes in food tissue and nutrient content during pressure processing.

Conventional cooking methods have been reported to affect bioactive compounds, other than the sensory quality of the products. Vacuum-based cooking treatments such as vacuum boiling, vacuum deep-frying, sous vide and sous vide microwaving may be innovative combined techniques with high potential application on an industrial scale. Furthermore, they can provide benefits in microbiological, nutritional, physicochemical and sensorial quality compared to conventional cooking methods, since the partial vacuum generated allows cooking at lower temperatures. The low oxygen atmosphere during vacuum-based cooking methods may minimise the enzymatic and non-enzymatic aerobic degradative reactions; this usually occurs during conventional cooking procedures and affect the quality of the cooked product (Martínez-Hernández et al, 2013).

One of the principal challenges for the improvement of the freezing technology is the avoiding of the tissue damages with consequent texture softening because of the ice formation. It’s already known that in fast freezing, smaller ice crystals form within both cells and intercellular spaces with consequent less tissues damages. Consequently, greater advancement in freezing and refrigeration is mainly characterized by the advancement in the development of novel techniques aimed to enhance the freezing rate. In recent years, application of novel processing techniques such as ultrasound and high pressure-assisted freezing, to produce better-quality frozen vegetables with extended shelf life, will help the frozen vegetable industry (Fellows, 2009).

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Objective

Objective

High consumption of fruit and vegetables is worldwide recommended because of their composition and content of bioactive compounds, strictly correlated with the prevention of chronic and cardiovascular diseases. The growing demand for fruit and vegetables is entailing a rapid expansion of global markets with consequent intensification of the imports and exports. Moreover, the perishable nature of the crops and their seasonality require to the food industry to process these products in order to increase their *shelf-life* and allow a continuous and constant trade.

The main objective of the vegetable processing is therefore to supply wholesome, safe, nutritious and acceptable food to consumers throughout the year. Those objectives can be achieved through the deactivation or inhibition of pathogens and spoilage microorganisms, the inactivation or inhibition of deteriorative enzymes and avoiding of the oxidative reactions.

Several techniques are today available for the vegetable stabilization or preservation. Depending on whether they use or not the temperature as processing variable, such processes can be classified into thermal or nonthermal.

Thermal processes are the traditional food preservation techniques and are based on the use of high temperature (e.g. blanching, canning, cooking) or low temperature (e.g. freezing). Despite their proven effectiveness, the thermal processes have some detrimental effects on the physico-chemical properties of the vegetables. Nonthermal processes are very promising novel techniques based on the use of process variables different from the temperature, like pressure (high pressure), electricity (pulsed electric field) or electromagnetic wavelength (e.s. ultrasounds) to stabilize the food products. The high potential of these techniques is related to their lower impact on the physico-chemical properties of the vegetables.

Despite several studies have been conducted on the effect of processes on vegetables, a lack of literature is still present, because of the fragmented investigation of the effect of traditional methods and still poor data on the novel technologies.

On the base of these premises, the aim of this PhD thesis is a deep evaluation of the effect of some traditional thermal and innovative nonthermal preservation technologies on the physico-chemical quality parameters of selected vegetables. In particular, a traditional complete industrial freezing process, different cooking methods and high hydrostatic pressure treatments were tested on selected vegetables in terms of organoleptic and nutritional parameters using classical and alternative analytical approaches.

Section I

Impact of the industrial freezing process on selected vegetables — Part I. Structure, texture and antioxidant capacity

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Published in Food Research International
DOI:10.1016/j.foodres.2014.04.019

Abstract

In this work, the impact of the industrial freezing process on structure, texture and total antioxidant capacity was studied using green asparagus stems, zucchini and green beans. Samples were analysed as raw/uncooked, blanched, raw/boiled and industrially frozen/boiled. A consistent damage of the vegetable tissue was revealed by the histological analysis on vegetables boiled after freezing. The cells appeared to be dehydrated, contracted and separated at different levels depending on the anatomical structure of each vegetable. The initial textural quality was partially retained in all blanched vegetables, and enhanced in cut tested asparagus stems, in relation to the action of phenolic acids at cell wall level. Raw/boiled and industrially frozen/boiled asparagus stems exhibited comparable forces of penetration and cut tests. On the other hand, zucchini, both raw and frozen, completely softened after boiling making the texture measurement impossible. Industrially frozen/boiled green beans showed higher values of cut and penetration forces, probably due to a higher presence of swollen cell walls, in comparison to those raw/boiled.

Blanching and boiling significantly increased the ferric reducing antioxidant power values of asparagus stems and green beans compared to uncooked/raw samples, while boiling after the freezing process significantly deprived both vegetables of the initial antioxidant capacity. On the other hand, boiling the frozen zucchini proved to be detrimental to the antioxidant capacity.

In conclusion, manufacturers and researchers should join together to develop specific industrial freezing process conditions according to the matrix of each vegetable.

Keywords: Vegetables; Cooking; Histological structure; Industrial freezing; Texture; Total antioxidant capacity

Introduction

A high consumption of fruits and vegetables is recommended worldwide as some of their constituents (e.g. vitamins, minerals, trace elements, dietary fibre, and phytochemicals) are considered to be responsible for beneficial effects on human health. In particular, the World Health Organization (WHO) recommends consuming at least 400 g a day (WHO, 2005) as their intake was epidemiologically associated with a decreased mortality from cardiovascular diseases, certain cancers and obesity (Martin, 2013). National Authorities regularly collect data on the food consumption at household levels through the budget surveys and data showed that processed, semi-processed and pre-packaged vegetables play an important role in a standard human diet. Data obtained from the DAta Food NEtworking databank (DAFNE, 2008), a joint effort of 24 European countries that compared the food habits of their populations and monitored the trends in food availability, showed that the total vegetable consumption (excluding potatoes and pulses) varied from 284 g a day in Cyprus to 84 g a day in Norway. The data related to Italy demonstrated a high daily consumption per capita (about 56 g) of processed vegetables (excluding potatoes), such as frozen, canned, pickled, dried and ready-to-eat meals. Freezing is recognized worldwide as one of the best method available in the food industry for preserving food products of high quality, such as vegetables. The decrease of temperature inhibits metabolic processes occurring in vegetables after harvesting, as well as slows down the rate of microbiological growth that compromises the material quality. In addition, the short heat treatment that precedes freezing (blanching) enhances colour and texture of vegetablesextending their shelf life (Jaiswal, Gupta, & Abu-Ghannam, 2012). However, during freezing, the transformation of liquid water into ice leads to a variety of potential stress mechanisms for vegetable tissues due to several factors such as the volumetric change of water converting into ice, the spatial distribution of ice within the system, and the size of individual ice crystals (Van Buggenhout et al., 2006). These stress mechanisms can deteriorate frozen products mainly influencing their quality in terms of texture and vegetable structure. The texture of plant foods is attributed mainly to the structural integrity of the cell wall and the middle lamella, as well

as to the turgor pressure generated within the cells (Waldron, Parker, & Smith, 2003). Both factors can be affected during blanching, freezing and frozen storage following freezing, making the preservation of desirable texture of frozen vegetables very complex and difficult to understand (Van Buggenhout et al., 2006). The relationship between morphological and structural changes and the final textural properties, as well as the maintenance of a high nutritional value during industrial freezing, should always be taken into consideration when defining the proper conditions to retain the best quality of such processed vegetables. Freezing vegetables are generally consumed cooked. Thermal treatment is well known to greatly influence vegetable structure and texture causing an initial loss of instrumental firmness due to the disruption of the plasmalemma and subsequently, to an increase of the ease of cell separation often accompanied by the swelling of the cell walls (Waldron et al., 2003). All these structural changes could be worsened on the tissues of a previously frozen vegetable.

To the best knowledge of the authors, the industrially freezing process of vegetables was not previously debated in literature in terms of a step by step evaluation of qualitative and nutritional aspects. Freezing effects were mainly discussed taking into consideration the changes of the organoleptic and nutritional qualities on the final commercial samples (Mazzeo et al., 2011; Murcia, Jiménez, & Martínez-Tomé, 2009; Pellegrini et al., 2010), the simulation of a homemade freezing process (without the blanching step) (Danesi & Bordoni, 2008), and the evaluation of different blanching procedures under laboratory scale (Canet, Alvarez, Luna, Fernández, & Tortosa, 2005). In this framework, the aim of this study is to evaluate and relate the structural, textural and antioxidant capacity changes occurring during the different steps of the freezing process on three commonly consumed vegetables: green asparagus, zucchini and green beans. Samples were analysed as raw/uncooked, blanched, boiled after the industrial freezing process and a storage period of two months at −18 °C, commonly performed in the industrial plants, mimicking an entire “from farm to fork” process. Boiling was also performed on the raw vegetables to be compared with those boiled after freezing and storage.

Materials and methods

Samples

Green asparagus stems (Asparagus officinalis L., var. Grande), zucchini (Cucurbita pepo L., var. Quine) and green beans (Phaseolus vulgaris L., var. Giamaica) were analysed in this study. These vegetables were selected taking into account the same seasonality, their large consumption in the Italian diet as frozen food items, and their different morphological and antioxidant features. The vegetables were provided by a local manufacturer, having been harvested in the production site and processed within 24 h from harvesting. Four types of samples were analysed for each vegetable; raw/uncooked (R), raw/boiled (B), blanched (BL) and industrially frozen/ boiled (FB). Raw (10 kg of each vegetable), blanched (5 kg of each vegetable) and industrially frozen samples (5 kg of each vegetable) were transported to the University of Parma laboratories under adequate refrigerated conditions (2–4 °C for R and BL samples, −18 °C for frozen vegetables) within 72 h from harvesting. The blanched samples were cooled immediately after blanching in an ice-water bath for 3 min (Canet et al., 2005) and then transported to the laboratories. Raw and blanched samples were analysed in the laboratories within 24 h. Raw samples were also boiled (as described below) and analysed within 48 h from the arrival at the laboratories. Frozen samples were stored for two months at −18 °C in a thermostatic chamber to better mimic the storage condition commonly applied to the processed vegetables in the industrial plants prior to the final commercialization. Then, they were boiled and analysed (as described below).

Processing and cooking

Vegetables were prepared as follows: asparagus stems of uniform diameter (10 ± 0.1 mm) at the base and length (120 ± 0.1 mm) were washed with the tap water and drained after being sorted for size and length. Zucchini (diameter of 30 ± 0.1 mm) were washed and drained, the top and the bottom were removed and the samples were cut into slices of 8 mm of thickness before processing. Green beans (diameter 10 ± 0.1 mm and length 80 ± 0.22 mm) were also collected to be processed and analysed.

The industrial process was carried out by the manufacturer starting from blanching to peroxidase inactivation (Gonçąlves, Pinheiro, Abreu, Brandaõ, & Silva, 2007), by immersing samples in a hot water bath at the following conditions: 30 s at 90 °C for asparagus, 2 min at the same temperature for zucchini and 2 min at 96 °C for green beans. Then, green beans and zucchini were frozen by forced convection in an industrial freezing tunnel with air at –40 °C for 6 min, while asparagus samples were frozen in an industrial spiral freezer with air at –40 °C for 15 min. Boiling was realized in hot water (100 °C) adding material to boiling tap water in a covered stainless-steel pot (1:5 food/water). For each cooking trial, approximately 20 pieces of each sample were boiled. The cooking conditions for raw samples were optimized by preliminary experiments carried out according to the judgement of a large group of semi-trained panellists, as previously reported (Mazzeo et al., 2011; Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008). In particular, asparagus samples were cooked for 15 min, green beans for 20 min and zucchini for 12 min.

Frozen vegetables were not defrosted before cooking, as suggested on the package. Asparagus stems were cooked for 10 min, zucchini and green beans for 8 min, respectively, according to the suggestions labelled by manufacturer. All the cooking procedures were performed in triplicate.

Analyses

The histological and textural analyses were carried out approximately on the same vegetable position; asparagus stems were analyzed in a zone around 10 cm from the top, zucchini in a central zone of fruit and green beans in a point of the pod between two seeds. The total antioxidant capacity was carried out as on the whole vegetable.

Histological analysis

The samples were fixed in FAA solution (formalin: acetic acid: 60% ethanol solution, 2:1:17 v/v) (Ruzin, 1999). After two weeks, they were dehydrated with gradual alcohol concentrations. The inclusion was made in a methacrylate resin (Technovit 7100, Heraeus Kulzer & Co., Wehrheim, Germany) and the resulting blocks were sectioned at 4 μm thickness (transversal cuts) with a semithin Leitz 1512 microtome (Leitz, Wetzlar, Germany). The sections were stained with

Toluidine Blue (TBO) solution (Ruzin, 1999), Periodic Acid Schiff (PAS) reagent and Amido Black (Ruzin, 1999). The inorganic dye Ruthenium Red (Ruzin, 1999) was used in fresh sections of each vegetable to detect the pectin degradation in cell wall after cooking. Six pieces of each vegetable were sampled for each treatment and for each stain. Sections were observed with a Leica DM 4000B optical microscope (Leica Imaging Systems Ltd., Wetzlar, Germany) equipped with a Leica DC 100 digital camera (Leica Imaging Systems Ltd., Wetzlar, Germany). The tissues were measured using an image analysis system (QWIN 5001 Leica Imaging Systems Ltd., Wetzlar, Germany). The microscopic observations were carried out by observing at least ten slides carrying ten sections each, for each specimen per each vegetable. The image analyses were carried out using a manual configuration of the image analysis system.

Texture analysis

The texture of raw and treated vegetables was analysed using a TA.XT2 Texture Analyzer equipped with a 245.2 N load cell (Stable Micro Systems, Godalming, UK), a force resolution equal to 0.01 N and an accuracy value of 0.025%. The parameters were quantified using the application software provided (Texture Expert for Windows, version 1.22). Puncture, penetration and cut tests were done on asparagus and green beans, while zucchini were only subjected to a puncture test. The puncture and penetration tests were performed on the samples in a radial direction using a 2 mm diameter stainless steel needle probe and 3 mm diameter cylinder probe, respectively, driven up to the centre of the sample at a speed of 3 mm s^{-1} The following parameters were extracted from the curves; the first peak force (FP1 given in N) and the maximum puncture force (Fmax given in N) from the force vs the time curves for the puncture test; the maximum penetration force (Fmax, given in N) from the force vs the time curves for the penetration test. The non-destructive elastic deformation of the sample was also obtained (Slope, given in N mm^{-1}) from the force vs the distance curves. The cut test was performed using a 3 mm thick stainless steel knife blade driven through the entire diameter of the vegetable, positioned on a slot surface, at a speed of 3 mm s^{-1}. The maximum cut

test force Fmax (N) was obtained from the force vs time curves. For all tests, a total of 10 vegetables was analysed for each processing step.

Total antioxidant capacity (TAC)

All samples were extracted for the measurements of the TAC values as previously described in Miglio et al. (2008). Extracts were kept in the dark at −20 °C prior to their analysis and analysed for their antioxidant capacity by Trolox equivalent antioxidant capacity (TEAC) assay (Pellegrini, Del Rio, Colombi, Bianchi, & Brighenti, 2003) and ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1999). TEAC values were expressed as millimoles of Trolox equivalents per 100 g of dry matter. FRAP values were expressed as millimoles of Fe2+ equivalents per 100 g of dry matter. For moisture determination, 3–4 g of raw and treated homogenized sample (as triplicate) was dried in a convection oven at 105 °C for at least 16 h until reaching constant weight according to the AOAC (2002) method. All analyses were carried out in triplicate.

Statistical analysis

SPSS statistical software (Version 20.0, SPSS Inc., Chicago, IL, USA) was used to perform a one-way analysis of variance (ANOVA) among samples from different treatments. The least significant difference (LSD) at a 95% confidence level ($p \leq 0.05$) was performed to further identify differences among groups. A t test ($p \leq 0.05$) was also performed to analyse differences among raw and blanched samples for the puncture test.

Results and discussion

Histological analysis

Asparagus

The raw asparagus stems had the typical monocotyledon anatomical structure (Fig. 1A), as previously described (Villanueva, Tenorio, Sagardoy, Redondo, & Saco, 2005). The hypoderma was composed of collenchyma and parenchyma. Below the epidermal layer (a monocellular layer with cell wall cutinization), the angular collenchymas became visible (from 8 to 10 cell layers, with a total thickness ranging from 227.6 to 268.5 μm) (Fig. 1A). Beneath the hypodermal tract a

meristem layer was present, while the rest of the fundamental system of tissues (stem) was composed of large and long-celled parenchyma.

The parenchyma was made of large isodiametric cells with an average diameter ranging from 20.5 to 74.6 µm and narrow intercellular spaces (Fig. 1A). Sclerenchymatic fibres were present around each vascular bundle.After the blanching treatment (Fig. 1B), the cells of the outer tissues (epidermis and collenchyma) showed a different degree of plasmolysis (see at the Supplementary material 1), while those of collenchymas showed the initial signs of separation (Fig. 1B and Supplementary material 2). On the other hand, the stem structure presented more important changes after boiling. Plasmolysed cells (see at the Supplementary material 3), especially those from parenchyma, were found, while epidermal cells appeared completely detached from the cortical layers (Fig. 1C). The cells of more internal tissues, particularly parenchyma, became more separated presenting wide intercellular spaces (Fig. 1C). The anatomical observation indicated that the cell separation did not involve any visible change in cell walls. Ruthenium Red occurrence (Fig. 2) showed that pectic substances in the cell wall did not visibly decrease after the thermal process. It can therefore be hypothesized that cell separation was due to a breakage of chemical bonds between the pectic components of middle lamellae of adjacent cells and/or a hydrolysis of some other components of the cell wall (i.e., pectin, hemicelluloses, and cellulose). This would confirm what observed by different authors (Lecain, Ng, Parker, Smith, & Waldron, 1999; Van Marle et al., 1997; Waldron et al., 2003), who showed that in most thermally processed fruit and vegetable products there is a partial degradation of the water-soluble pectin, possibly due to a trans-elimination reaction, also called β-elimination, and a consequent depolymerization. In our study, the separation of cells after cooking might be ascribed to a decrease of the strength of cell–cell interactions in the middle lamella adjacent to the intercellular spaces. After both freezing and boiling, the cells of all tissues presented a marked dehydration. In the internal parts of the parenchyma, deep fissures were present across the tissues (Fig. 1D), as a consequence of the ice formation during freezing and the following thermal treatment during cooking. Some authors

asserted that during the freezing of vegetables, ice crystals begin to form in the liquid between the cells, probably because of the higher freezing point of this extracellular liquid, compared to the intracellular liquid (Silva, Goncąlves, & Brandão, 2008). During freezing, the ice crystals grow between the cells making the extracellular liquid more concentrated. Thus, the cells will lose water by osmosis, and this leads to an extensive dehydration and contraction of the cells resulting in relatively few large ice crystals among shrunken cells (Silva et al., 2008). In FB, the sample epidermis was absent over a great part of the sample surface and the collenchyma was swollen, due to very large intercellular spaces that made it to appear as an aerenchyma (Fig. 1D).

Zucchini

The outer layer of zucchini pericarp was a single layer epidermal tissue (average thickness 21.1 μm), whose outer and radial walls were covered by a thick cuticle (Fig. 3A). The more internal hypoderm consisted in isodiametric, thickened cells: few and small intercellular spaces were present. The mesocarp may be divided into three parts: outer, middle and inner mesocarp (Fig. 3A). The outer mesocarp was a region composed of cells gradually increasing in size from the small cells (12.5 μm) of the hypoderm to the large ones (average 38.0 μm) of the middle mesocarp. The sub-hypodermal cells (outer mesocarp) were strongly adherent to each other with few intercellular spaces. The cells of middle mesocarp were thin-walled and more loosely arranged if compared to the outer mesocarp. Finally, the inner mesocarp consisted of a rather large-celled parenchyma (65.0 μm and more). The endocarp was made of irregular large cells with thin walls. After blanching, the structure of zucchini remained unchanged with respect to raw material, while boiling caused a slight dehydration on the epidermal cells and on the outer hypoderm layer. In this latter condition, more intercellular spaces became visible in all tissues, owing to the phenomenon of cell separation previously observed in thermally processed fruits and vegetables (Lecain et al., 1999; Van Marle et al., 1997). Ruthenium Red staining showed that pectic substances in the cell wall did not visibly decrease after the thermal process (not shown), as found for asparagus stems. The anatomical structure of frozen boiled zucchini presented significant changes after the thermal processes when

compared to raw samples. Indeed, in addition to the enhanced cell separation in all tissues, it was possible to note the presence of extremely large fissures in the mesocarp. The fissures characteristically developed tangentially separating cell layers, parallel to the circumference of the structure (Fig. 3B). Hence, the tangential cell walls appeared to be the most damaged by the thermal processes (freezing followed by boiling), while the radial walls did not detach and keep the cells firmly linked (Fig. 3B).

Green beans

The pericarp of green beans was made of a number of tissues, as previously observed (Stolle-Smits, Donkers, van Dijk, Derksen, & Sassen, 1998), which from the outside inwards were: an outer epidermis made of a single layer of cells, a hypodermis consisting in a three layered collenchyma (total thickness raging from 121.0 to 154.2 μm), an 8–10 cell outer parenchyma, a 3 small cell layer, and finally an inner parenchyma or seed-cushion. The outer parenchyma showed large cells (average diameter ranging from 62.1 to 171.0 μm) with an inclusion of starch grains, clearly visible in this vegetable, adjacent to the 3 small cell layer located between the two parenchyma tissues (Fig. 4A). The inner parenchyma was made of large cells (average diameter ranging from 44.3 to 172.4 μm) with thinner cell wall, if compared to the outer parenchyma cells. It appeared more compact than the outer parenchyma, due to smaller intercellular spaces (Fig. 4A). After blanching, the anatomy of the green beans (Fig. 4B) seemed unaltered compared to untreated samples, as previously observed for asparagus (Fig. 1B) and zucchini. The outer and inner parenchyma cells were turgid, but the starch grain content was lower; some starch gelatinization was detected. The cytoplasm of outer parenchyma cells appeared granular and this modification was also described (Canet et al., 2005; Grote & Fromme, 1978; Stolle-Smits et al., 1998). In B samples, the cell walls became less compact and less-densely stained (due to the swollen cell wall, Fig. 4C); some cells showed the evidence of the onset of separation in the middle lamella regions (Fig. 4C). This condition was quite evident in the outer parenchyma cells. In this tissue, the number of starch inclusions decreased. On the contrary, the inner parenchyma cells seemed to retain an intact cell

wall. After frozen boiled treatments, the cells of all tissues showed a marked dehydration. The epidermis detached from the collenchyma layer, the outer parenchyma appeared completely plasmolysed, with pronounced swollen cell walls (Fig. 4D). These modifications were similarly described by Stolle-Smits et al. (1998) and by Grote and Fromme (1978) for green beans processed by heat treatments. The cells of the outer and inner parenchyma became separated without a visible decrease of cell wall pectin substances (not shown) and this phenomenon could be ascribed to a modification of the middle lamella composition, as demonstrated by Stolle-Smits et al. (1998) on green bean pods. Therefore, the industrial freezing and subsequent cooking treatments appeared, also in this case, to be severe to the structural integrity, as well; both parenchyma tissues (outer and inner) displayed cell lysis and fissures (Fig. 4D).

Texture analysis

Asparagus

The puncture test curves obtained for all asparagus stem samples were shown in Fig. 5A. R and BL samples showed similar profiles with a first peak force (FP1) ascribable to the resistance offered by the epidermal and collenchyma tissues to the probe penetration (Fig. 1A) This first peak approximately occurred at a distance value comparable to the collenchymas thickness (when force was reported vs distance, graph not shown). Values of this force (Table 1) significantly differed between R and BL samples, showing the softening effect of the blanching on the vegetable structure. After this first peak, there was a progressive increment of the recorded force for R and BL, probably due to the friction exerted by parenchyma cells on the needle, as previously shown for other species with the same probe (Maury et al., 2009). From a histological point of view, the softening effect of blanching could be justified with the plasmolysis and collenchyma cell separation (Fig. 1B) that suggested a modification of chemical bonds or other pectic constituents of middle lamella (Waldron et al., 2003). Pectic substances of the cell wall did not visibly decrease, instead (Fig. 2). B and FB samples did not exhibit any peak force as a consequence of freezing step and/or thermal treatment. The first peak disappeared due to the detachment of the epidermal layer

and the separation of the collenchyma cells. In addition, the curve flattened, due to the dehydration and separation of the cells, as well as to the formation of deep fractures in the parenchyma tissue that allowed an easy penetration of the probe (Fig. 1C and D). Fig. 5C showed the force–time penetration curves obtained for all samples. The maximum peak was clearly distinguishable only for R and BL, due to the presence of the whole external mechanical tissue (Fig. 1A and B). It also significantly decreased for boiling and freezing treatments, as also shown by the extrapolated force values (Fmax) reported in Table 1. This decrement could be attributed to the loss of the adhesion of cell walls, as shown by the histological observation above described and reported in literature (Rodriguez-Arcos, Smith, & Waldron, 2002). In addition, freezing produced slight fractures in the tissues (Fig. 1D), due to the ice formation, that significantly affected texture.

The slope of the force deformation curve (N/mm), related to the apparent elastic properties and considered as an index of sample stiffness (Nguyen et al., 2010), was also calculated in this study (Table 1) and resulted significantly different among samples. It could be hypothesized that all treated samples have been subjected to a greater deformation with respect to raw vegetables. This deformation rose increasing the thermal treatment, being also the highest in FB, where a freezing step and related storage were added, and samples broke without a signifi- cant elastic deformation. Blanched and frozen vegetables were previously found to undergo to deformation under compression test during the following frozen storage, with a gradual tissue softening (Martins & Silva, 2003). Finally, the force–time curves obtained by cutting samples were reported in Fig. 5E and extrapolated maximum shear force values (Fmax) were shown in Table 1. BL showed significantly higher Fmax values than the other samples. Several authors (Parker & Waldron, 1995; Rodríguez et al., 2004; Waldron et al., 2003) indicated that phenolic acids (especially ferulic acid) present in the cell wall polysaccharide matrix of vegetables play a key role in maintaining the thermal stability of cell–cell adhesion and consequently the vegetable texture. The collenchyma tissue, present in the asparagus stems (Fig. 1A), was characterized by a thick cell wall richer in polysaccharides (pectin and cellulose) with respect to the cell walls of the other tissues. This

mechanical tissue could be responsible of the asparagus stem firmness after the blanching treatment, due to its chemical (presence of phenolic acids) and mechanical properties. On the other hand, B and FB exhibited significantly lower Fmax. This softening occurred as a result of the cell separation (Fig. 1D), as previously described by Lau, Tang, and Swanson (2000) for thermally treated asparagus, due to the β-eliminative degradation of the pectic polymers (Waldron et al., 2003) and it was more severe for FB where the freezing process and the following storage were added.

Zucchini

The textural analysis of zucchini was only possible with the puncture test, because of the thermal damage suffered by the structure under the applied processing conditions. A first peak force was clearly distinguishable only from curves of R and BL (figure not shown) and it was due to the resistance offered by the skin and the outer mesocarp (Fig. 3A). After boiling and freezing–boiling treatments, the zucchini tissues appeared dehydrated and with an extensive cell separation (Fig. 3B). This condition justified the absence of the first peak force (Fp1) in these two processed samples.

BL samples were apparently not damaged by the thermal treatment (figure not shown), but they showed significantly lower Fmax than R (Table 1). The decrease of the instrumental firmness could be dependent on the loss of cell turgor due to the membrane disruption, as stated by Greve et al. (1994) for carrots, associated with the pectin dissolution. Moreover, a mechanical tissue (Fig. 3A) or other cells with thickened walls were not present in zucchini. The structural and textural properties of vegetable tissues are dependent, largely, on the cell wall; hence, the collenchyma tissue in asparagus stems (Fig. 1A) played a fundamental role in the maintenance of vegetable firmness after cooking. For this same reason, B and FB zucchini did not exhibit any peak force, differently from the above discussed asparagus stems.

Green beans

Fig. 5 (insets B, D, F) showed the curves obtained for green bean samples. The puncture test showed similar profiles to the asparagus stems (Fig. 5A), being the first peak force only evident for R and BL (Fig. 5B). This was most likely related to the presence of three layers of collenchymatic

cells (mechanical tissue) (Fig. 4A). A marked flattening was clearly visible also in this case for B and FB sample curves, being related to the cell separation due to the depolymerisation and/or solubilisation of the component of middle lamella, more evident for the outer parenchyma (Fig. 4C, D). The histological analysis did not show any clear difference (Fig. 4A, B) in R and BL, although they significantly differed for Fmax (Table 1). The curves obtained under penetration test were quite different among samples (Fig. 5D) as well as the Fmax values (Table 1). Significant differences were found in R and BL samples both for Fmax and slope values. This suggested a major deformation of the BL samples, as a consequence of the thermal treatment, that significantly affected the adhesion of the cell walls, as previously shown by Canet et al. (2005) on the same vegetable. It is to be noted that FB showed a higher Fmax than B. Both freezing and cooking treatments softened the green bean tissues, although with a different degree. Freezing–boiling caused a cell wall rupture and an extensive cell separation, as shown by the histological analysis (Fig. 4C and D), producing a more extensive damage. The histological study also showed the presence of swollen cell wall in B and more evidently in FB samples (Fig. 4D). It could be hypothesized that the swelling phenomenon was crucial for the obtainment of higher Fmax values of FB compared to B and for the maintenance of similar slope values in these two samples (Table 1). The presence of swollen cells were previously related to a harder texture in green beans thermally treated (Leadley, Tucker, & Fryer, 2008). The curves obtained from the cut test significantly flattened in B and FB in comparison to the other two samples, due to the lower blade resistance of these two tissues that showed a higher degree of broken cells (Fig. 4, insets A, B, C, D). Cut test forces were also significantly lower for these two samples (Table 1) in comparison to R and BL that exhibited comparable values.

Total antioxidant capacity

Asparagus

TEAC and FRAP values of uncooked asparagus stems (Fig. 6, insets A and B) were slightly lower than those found in a previous paper (Pellegrini et al., 2009, referred to fresh weight), due to

agronomical factors, such as different genotype, growing conditions, season and maturity. Boiling increased TAC compared to R samples, but this increase was significant only when TAC values were determined by the FRAP test (Fig. 6). The same effect was already observed for boiled asparagus stems by Fanasca et al. (2009) using the FRAP assay, in relation to an increase of bioactive compounds, such as total phenols, rutin, quercetin and carotenes (i.e. β-carotene and lutein). Thus, this increment could be justified by the release of molecules with antioxidant activity (i.e. carotenoids) due to two possibilities: i) the thermal modification of some components of cell walls (Fig. 1D), such as the middle lamella, and ii) the related softening of the matrix for this kind of vegetable (Table 1), as also hypothesized by Yamaguchi et al. (2001). The increase of antioxidant capacity was observed also in blanched samples; although the tissue suffered lower changes than in B (Fig. 1B) due to the less severe thermal treatment. In addition, Yamaguchi et al. (2001) theorised that other phenomena could be involved when an increase of radical-scavenging activity was observed in some vegetables after boiling, as was found for asparagus stems. The thermal treatment can produce stronger radical-scavenging antioxidants and/or novel products by chemical reaction and/or inactivated oxidative enzymes that oxidise the antioxidant molecules. However, the extent of each contribution to the TAC increase cannot be quantified (Yamaguchi et al., 2001). Differently, FB asparagus stems showed significantly lower TAC than BL and B. The largest fissures formed into the tissue after the freezing and boiling steps (Fig. 1D) may have facilitated the penetration of water by capillarity, enlarging the tissue–water contact surface and also allowing the leaching of antioxidant soluble compounds in the cooking water. With respect to B, the FB samples suffered a greater thermal stress, because these samples underwent the blanching step before freezing and cooking.

Zucchini

The uncooked zucchini presented TEAC and FRAP values slightly higher than those previously found by Miglio et al. (2008). A significant increase was obtained after boiling for both TEAC and FRAP assays as for asparagus stem and in accordance with a previous study (Miglio et al., 2008). In

that study, the cooking conditions were approximately the same as the present one. On the other hand, the total antioxidant capacity showed a slight, but significant decrease for BL and FB samples. The processed zucchini presented a high degree of softening for all steps of treatment also after blanching due to the absence of a mechanical tissue, as discussed above. Thus, it is possible to hypothesize that the structural damage induced by heating and/or freezing, such as the cell separation and the dehydration discussed above, could have caused a partial leaching of antioxidants in FB. The lixiviation could also have occurred under the industrial condition for blanching since zucchini are not preserved by a mechanical tissue, although the thermal damage suffered by tissue was lower for BL than for FB. Contrarily, it appears that some of the conditions previously described by Yamaguchi et al. (2001) in relation to a TAC increase could have occurred under the homemade boiling of unprocessed samples.

Green beans

The total antioxidant capacity measured for unprocessed green beans by TEAC and FRAP tests was higher than those previously found for the same vegetable (Pellegrini et al., 2009, referred to fresh weight) probably due to the above cited agronomical factors. B samples showed an increase of the antioxidant capacity as for the other two vegetables and the increment was particularly high for FRAP values. This was in accordance with previous studies in which TAC was found to increase (Pellegrini et al., 2009) or to remain unaltered after boiling (JiménezMonreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009). BL displayed a significant increase of TAC, as it did for asparagus. Also in this case, it is possible to hypothesize a release of the antioxidant components by cells as well as the occurrence of one or more of the other factors theorised in literature (Jiménez-Monreal et al., 2009; Yamaguchi et al., 2001). Similar to the other analysed vegetables, boiling of frozen green beans caused a significant reduction of TAC values with respect to all the other samples. The loss of the antioxidant molecules, probably due to the leaching, could be related to the great damage suffered by tissues. These displayed cell lysis and fissures, also showing a consistent softening both by penetration and cut tests, as seen for the other vegetables.

Conclusions

The industrial conditions applied during the freezing process and the consequent storage period, with the end-user consumer cooking, determined a consistent damage to the anatomical structure of vegetables. The cells were dehydrated, contracted and separated, probably in relation with a partial degradation of cell-wall components as pectin. The extent of this damage appeared to be different depending on the characteristic of the vegetable anatomical structure, as asparagus stems and green beans present a mechanical tissue that did not exist in zucchini. This statement was critical for the partial preservation of the textural quality during the entire process, too. After blanching, the initial textural quality was partially retained in all vegetables, but enhanced in cut tested asparagus stems probably in relation to the action of phenolic acids at the cell wall level. After cooking, samples revealed a general softening, as expected, also displaying a different behaviour: zucchini completely softened, while B and FB asparagus stems showed comparable cut and penetration force values. On the other hand, FB green beans exhibited higher cutting and penetration forces in comparison to B samples, with an overall better retention of textural quality than the other vegetables. The industrial freezing and storage processes partially deprived all analysed vegetables of the initial antioxidant capacity. On the other hand, B samples exhibited similar or higher antioxidant capacity values than the raw vegetables. These outcomes need to be strengthened by the further investigation of bioactive compounds. Finally, the findings of this work should encourage manufacturers and researchers to join together in order to develop industrial freezing process conditions according to the matrix of vegetable. The selection of adequate and appropriate time/temperature combinations, the application of new blanching processes (e.g. microwave or ohmic treatments), along with a different length of storage period are all viable options for manufacturers to seriously take into consideration, with the final aim being to offer the end-user consumer a texturally and nutritionally higher quality frozen product.

Supplementary data to this article can be found online at

http://dx.doi.org/10.1016/j.foodres.2014.04.019.

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Captions for Figures

Fig. 1. Transverse sections of asparagus stem samples stained with PAS – Amido Black: A. raw/uncooked; B. blanched; C. boiled from raw; D. frozen boiled.

Legend: c = collenchyma; vp = vascular bundle; p = parenchyma; f = fissure.

Fig. 2. Detail of the Ruthenium Red staining on frozen boiled asparagus stem. Arrows indicate integral pectin in cell walls.

Fig. 3. Transverse sections of zucchini samples stained with PAS – Amido Black: A. raw/uncooked, B. frozen boiled.

Legend: om = outer mesocarp; mm = middle mesocarp; im = inner mesocarp; f = fissure.

Fig. 4. Transverse sections of green bean samples stained with PAS – Amido Black: A. raw/uncooked; B. blanched; C. boiled from raw; D. frozen boiled.

Legend: op = outer parenchyma; ip = inner parenchyma; c = collenchyma; s = starch grains; f = fissure; sw=swollen cell wall.

Fig. 5. Force (N)-time (s) curves obtained for puncture test A. asparagus, B. green beans; for penetration test C. asparagus, D. green beans; for cut test E. asparagus, F. green beans. Abbreviations: R, raw/uncooked; BL, blanched; B, boiled; FB, frozen boiled. F_{P1}, maximum force at the first rupture peak and F_{max}, maximum break force of puncture are shown on inserts A and B. F_{max}, maximum forces of penetration and cut test are also shown on inserts C and D, and E and F, respectively.

Fig. 6. Total antioxidant capacity of the analysed vegetables: A TEAC values. Values are expressed as mmol Trolox/100 g dry weight. B FRAP values. Values are expressed as mmol Fe2+/100 g dry weight. Error bars represent ± 1 SD, (n = 3). Bars of histograms with different letters are significantly different ($p \leq 0.05$). Abbreviations: R, raw/uncooked; BL, blanched; B, boiled; FB, frozen boiled.

Table 1. Textural parameters of analysed vegetables [a].

Test		R	BL	B	FB
Asparagus					
Puncture	F_{P1} (N)	0.4 (0.1)*	0.2 (0.1)	-	-
	F_{max} (N)	1.9 (0.3) *	0.8 (0.2)	-	-
Penetration	F_{max} (N)	11.6 (1.3) a	10.0 (0.8) b	2.2 (0.5) c	0.9 (0.3) d
	Slope (Nmm^{-1})	9.9 (1.6) a	5.6 (0.7) b	2.1 (0.4) c	0.5 (0.1) d
Cut	F_{max} (N)	36.1 (4.7) b	57.4 (5.5) a	34.2 (7.4) b	17.8 (3.9) c
Zucchini					
Puncture	F_{P1} (N)	0.3 (0.01)	0.4 (0.1)	-	-
	F_{max} (N)	3.4 (0.3)*	1.3 (0.1)	-	-
Green beans					
Puncture	F_{P1} (N)	0.9 (0.1)	1.0 (0.1)	-	-
	F_{max} (N)	2.2 (0.2) *	1.2 (0.1)	-	-
Penetration	F_{max} (N)	16.8 (1.4) a	12.8 (1.6) b	1.8 (0.3) d	3.2 (0.7) c
	Slope (Nmm^{-1})	9.6 (0.7) a	7.4 (1.6) b	1.7 (0.3) c	1.6 (0.4) c
Cut	F_{max} (N)	37.2 (2.4) a	32.5 (3.1) a	3.1 (0.5) b	4.5 (1.0) b

[a] Data are expressed as means of 10 samples. Standard deviations are given in parenthesis.
Means in rows followed by different letters or an asterisk (on the highest mean value) are significantly different ($p \leq 0.05$).
Abbreviations: R, raw/uncooked; BL, blanched; B, boiled; FB, frozen boiled.

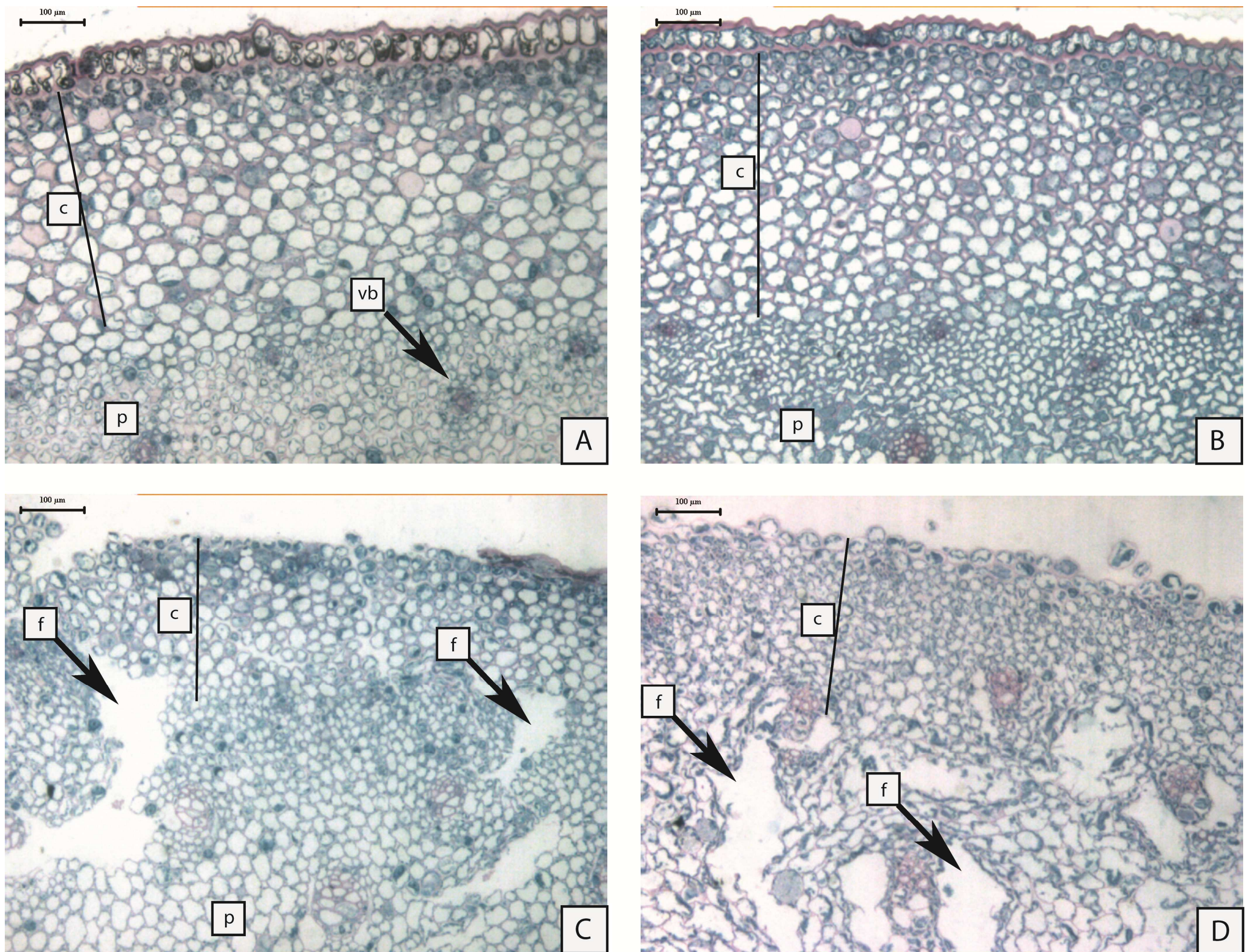

Fig.1

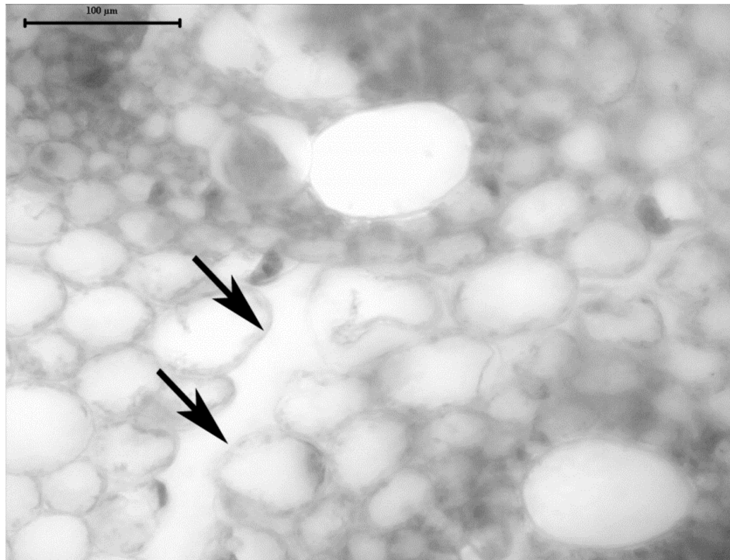

Fig.2

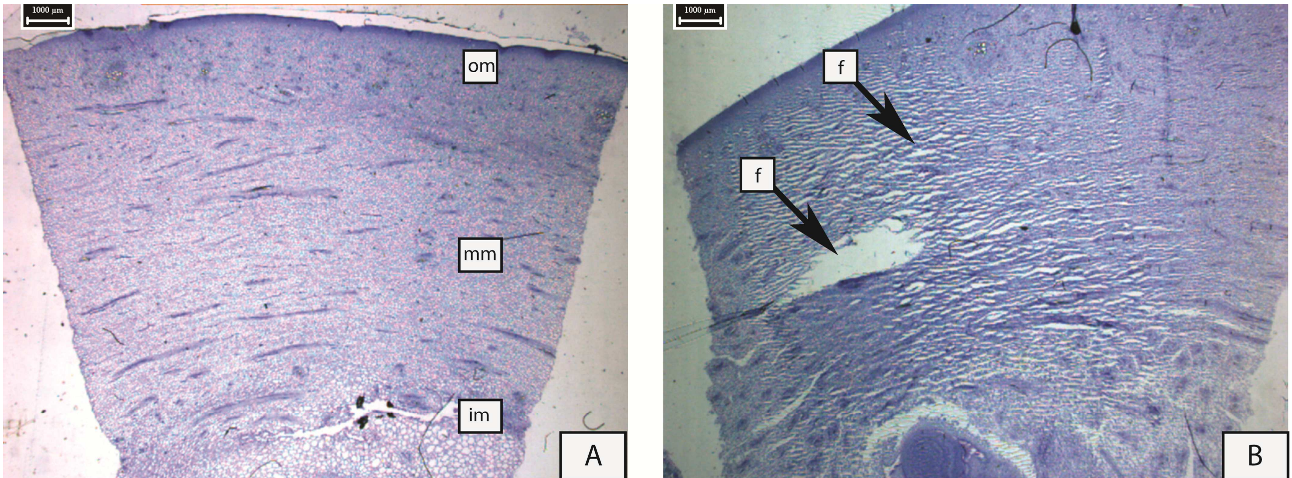

Fig.3

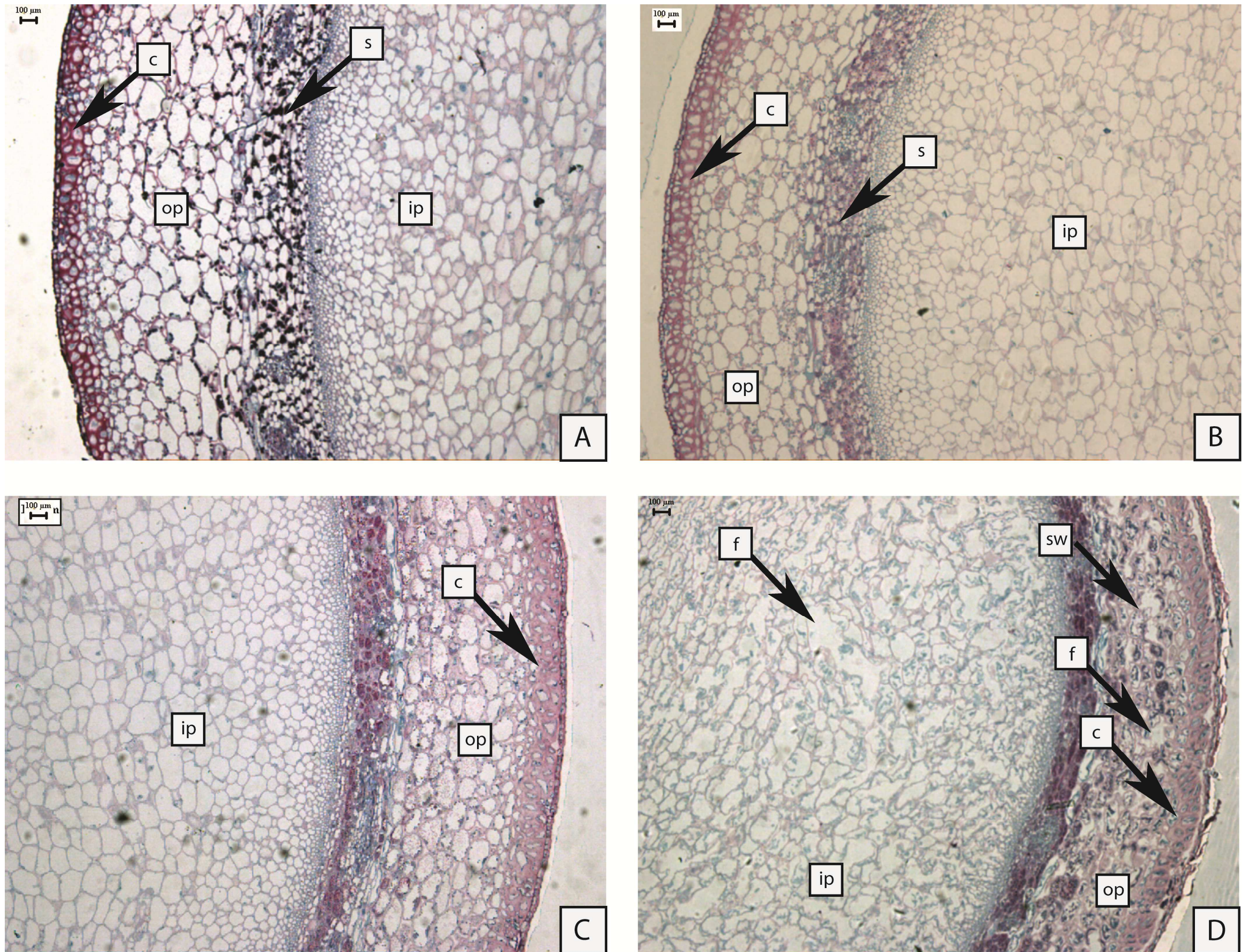

Fig.4

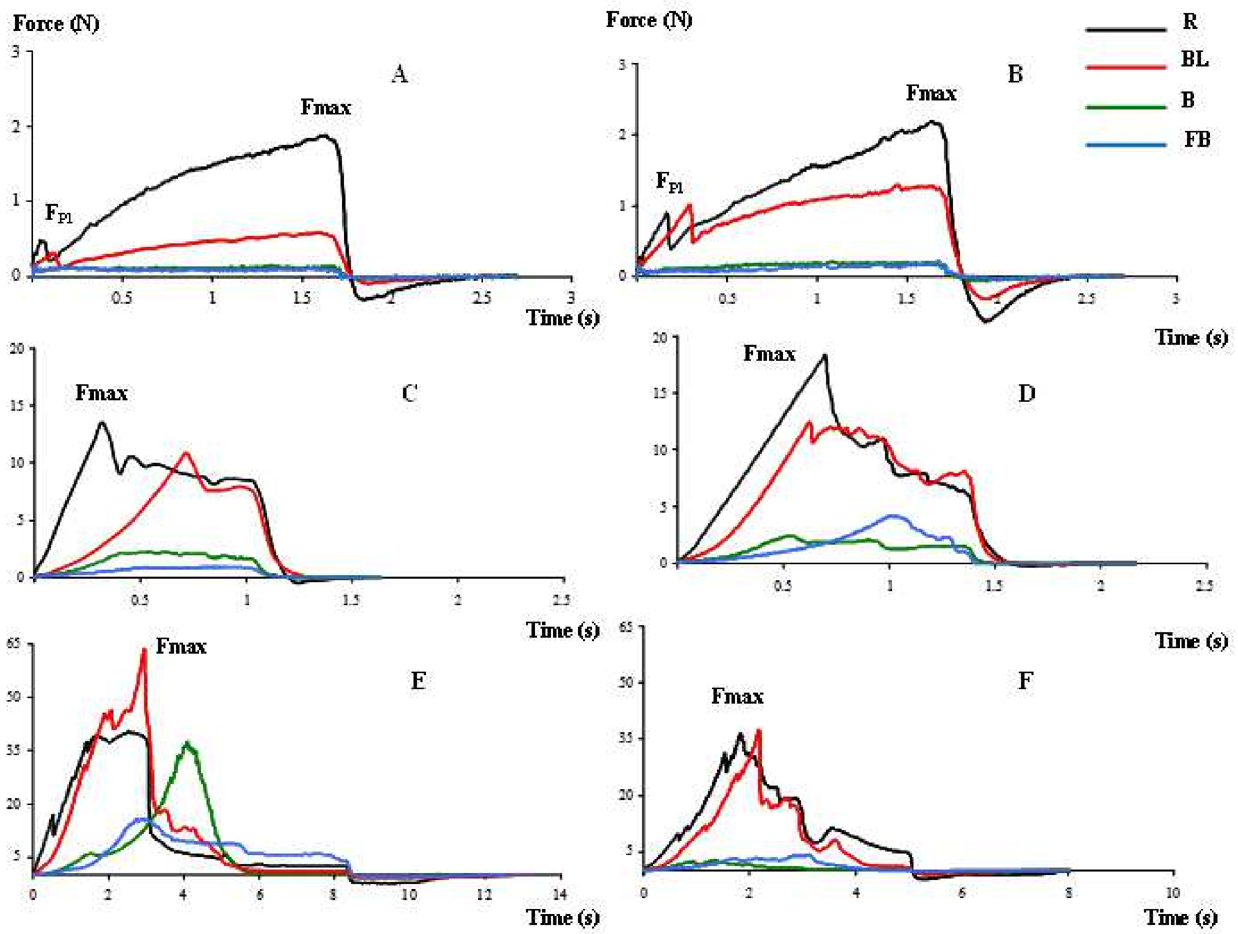

Fig. 5

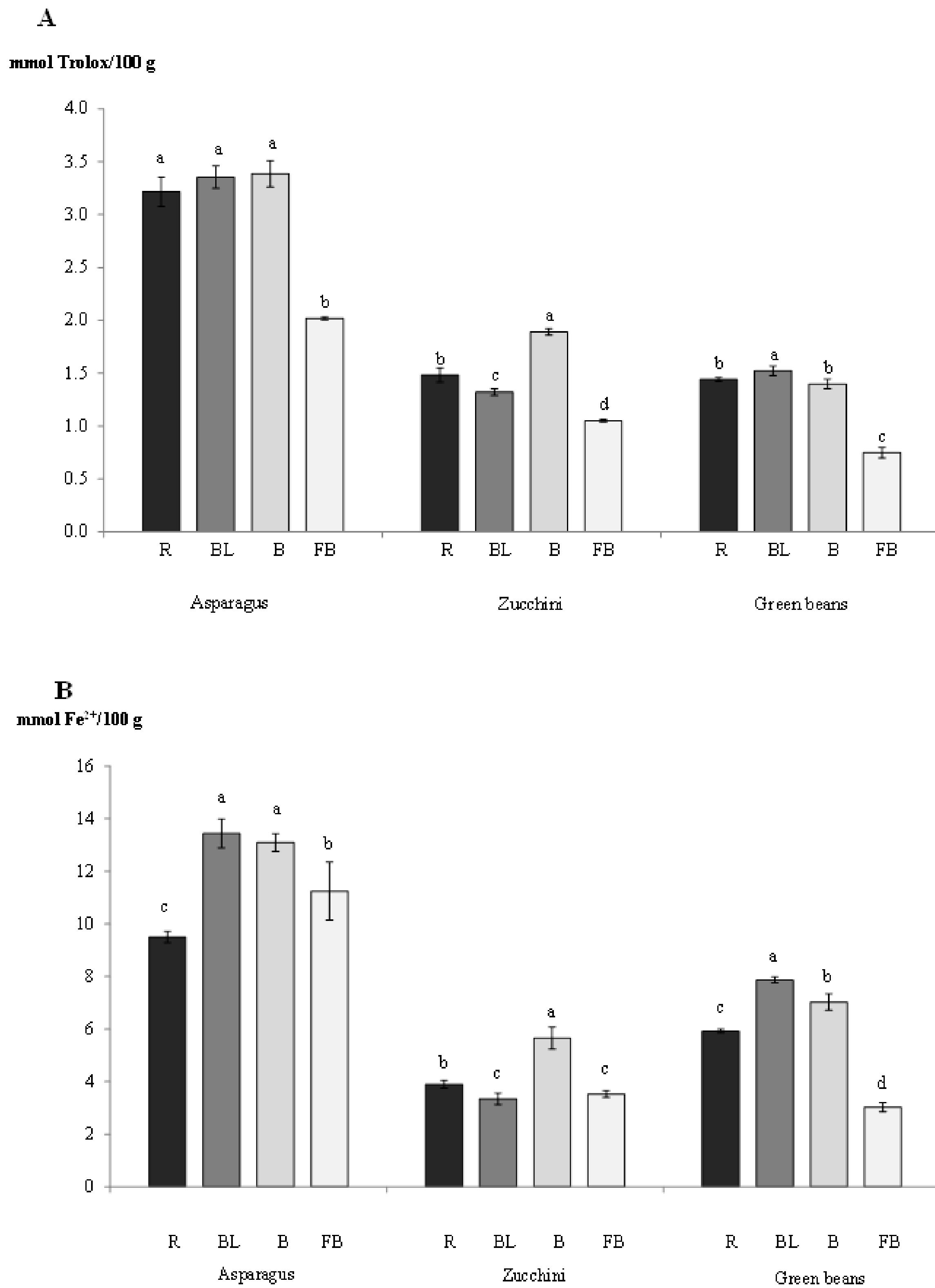

Fig. 6

The impact of the industrial freezing process on selected vegetables

Part II. Colour and bioactive compounds

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Abstract

In the present study the impact of the different steps (i.e. blanching, freezing, storage following the industrial freezing process and the final cooking prior to consumption) of the industrial freezing process was evaluated on colour, chlorophylls, lutein, polyphenols and ascorbic acid content of asparagus, green beans and zucchini. In addition, the domestic boiling of raw samples was compared with the boiling of frozen storage vegetables.

Results showed that the blanching treatment retained phytochemicals in all studied green vegetables and the frozen storage up to 2 months did not negatively affected phytochemicals, in particular lutein and flavonoids in almost all samples. On the contrary, colour significantly changed during blanching and frozen storage. The changes of b* (yellowness) and the shift of H° (hue angle) were not coherent with the increase of pheophytin. In addition, the greenness (- a*) was found to increase with the exception of boiled samples in all vegetables. Generally, in boiled frozen vegetables there was a better or comparable retention of bioactive compounds with respect to raw ones, and this was especially true for green beans and zucchini. Colour changes after cooking did not exhibit the same trends among vegetables, being more remarkable for frozen asparagus in comparison with those boiled from raw, but overall comparable for green beans and zucchini.

In conclusion, the overall results of the present study suggest that, when the industrial freezing process is well performed, the boiled frozen vegetables do not have a lower nutritional value than the fresh ones.

Keywords: asparagus; green beans; zucchini; vitamin C; chlorophylls; polyphenols, lutein.

1. Introduction

Freezing is one of the most used preservation techniques for raw vegetables, which are perishable and seasonal by nature, in relation to the slow rate of deterioration occurring at the low temperature of frozen storage (- 18 °C). Freezing is largely applied in vegetable processing because of its convenience (i.e. fast preparation) and the supposed maintenance of fresh-like characteristics with minimal loss of nutrients and antioxidants over extended time. However, freezing step is not enough to fully stop enzymatic reactions, senescence, microbial growth, and a blanching treatment must be applied (Canet et al., 2004). Blanching consists of a short time exposure of vegetables to a heating treatment and it is generally carried out in water at 85-100 °C. This treatment is reported to enhance vegetable safety (i.e. destruction of surface microflora) and quality attributes (flavour, odour and colour) (Canet et al., 2004). Contrasting results have been published about the blanching influence on loss of thermal instable nutrients such as ascorbic acid and polyphenols. Their contents were found to be unchanged or to have decreased after treatment (Olivera et al., 2008, Patras et al., 2011) Apart from blanching, storage following the industrial freezing process (Lisiewska and Kmiecik, 1997, Martins and Silva, 2003) and especially the final cooking step prior to consumption can affect the quality of the vegetables thus causing a further loss of bioactive compounds (Delchier et al., 2012, Mazzeo et al., 2011, Pellegrini et al., 2010). All these steps should be separately considered from a nutritional standpoint, also keeping in mind that there are significant interactions among them. However, there is a general lack of literature data dealing with the step by step evaluation of the industrial freezing impact on the nutritional quality of vegetables. The steps of process, blanching, freezing and storage, were commonly achieved under a laboratory scale (Martins and Silva, 2003, Canet et al., 2005, Viña et al., 2007), or commercial products were evaluated after cooking without substantial information about the process conditions (Mazzeo et al., 2011, Pellegrini et al., 2010).

Recently, the structural, textural and antioxidant capacity changes of three commonly consumed vegetables (asparagus, green beans and zucchini) in Italy were analysed during the different steps of

the industrial freezing process by Paciulli et al. (2014). The present study continues this former work, analysing the phytochemicals (vitamin C, polyphenols, chlorophylls and carotenoids) and the colour parameters of the same vegetables. Samples were analysed as raw/uncooked, blanched, and frozen after the industrial freezing process and a storage period of two months at – 18 °C. Raw and frozen/stored vegetables were boiled, mimicking the entire "farm-to-fork" process.

2.Materials and Methods

2.1. Sampling and processing

Vegetables used in this study are the same of a previous work (Paciulli et al., 2014). Green asparagus stems (*Asparagus officinalis* L., var. Grande) of uniform diameter (10 ± 0.1 mm) at the base and length (120 ± 0.1 mm), zucchini fruits (*Cucurbita pepo* L., var. Quine, diameter 30 ± 0.1 mm) cut into slices of 8 mm of thickness and green beans (*Phaseolus vulgaris* L., var. Giamaica, diameter 10 ± 0.1 mm and length 80 ± 0.22 mm) provided by a local manufacturer and harvested in the production site, were processed within 24 hours from harvesting. Five types of samples were analysed for each vegetable; raw/uncooked (R), blanched (BL), industrially frozen/stored (FS), raw/boiled (B) and industrially frozen/boiled (FB).

The industrial process was carried out by the manufacturer starting from blanching until to the peroxidase inactivation (Goncąlves et al., 2007), by immersing samples in a hot water bath at the following conditions: 30 s at 90°C for asparagus, 2 min at the same temperature for zucchini and 2 min at 96 °C for green beans. Then, green beans and zucchini were frozen by forced convection in an industrial freezing tunnel with air at – 40°C for 6 min, while asparagus samples were frozen in an industrial spiral freezer with air at – 40°C for 15 min.

Raw, blanched (immediately cooled in an ice-water bath for 3 min) and industrially frozen samples were transported to the University of Parma laboratories under adequate refrigerated conditions within 72 hours from harvesting. Frozen samples were maintained for two months at – 18 °C in a

thermostatic chamber to better mimic the storage condition commonly applied to the processed vegetables in the industrial plants prior to the final commercialization.

Raw and blanched samples were analysed within 48 hours from the arrival at the laboratories while frozen vegetables were studied at the end of storage. Raw and frozen samples were also cooked to be analysed as boiled, as described below, within 48 hours from the arrival at the laboratories.

2.2 Cooking conditions

Raw and industrially frozen vegetables were boiled in hot water (100 °C) adding approximately 20 pieces of each sample to boiling tap water in a covered stainless-steel pot (1:5 food/water). Raw samples were cooked for 15 min, 20 min and 12 min for asparagus stems, green beans and zucchini, respectively. These cooking times were selected from preliminary experiments carried out according to the judgement of a large group of semi-trained panellists, as previously reported (Miglio et al., 2008).

Frozen vegetables were not defrosted before cooking and boiled according to the time labelled by manufacturer (10 min for asparagus and 8 min for green beans and zucchini). All the cooking procedures were performed in triplicate.

2.3. Colour analysis

Colour determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65. The measurements were carried out in five random points on asparagus stems and green bean pods. Colour of zucchini slices was measured both on the skin and the parenchyma of the fruit due to the colour dissimilarity of the two tissues. The instrument was calibrated before each analysis with white and black standard tiles. L* (lightness; black = 0, white = 100), a* (redness > 0, greenness < 0), b* (yellowness > 0, blue < 0), C (chroma, 0 at the centre of the colour sphere), and H° (hue angle, red = 0°, yellow = 90°, green = 180°, blue = 270°) were quantified on each sample using a 10° position of the standard observer (CIE, Paris, France, 1978). ΔE for all the treated samples in comparison to the raw vegetables was also calculated. A total of 10 determinations was performed for each cooking treatment.

2.4. Dry matter determination

For moisture determination, 3–4 g of raw and treated homogenized sample was dried in a convection oven at 105 °C for at least 16 h until reaching constant weight according to AOAC method (2002). Analyses were conducted in triplicate.

2.5. Chlorophyll and carotenoid determination

The determination of chlorophylls and carotenoids was carried out by a high-performance liquid chromatography (HPLC) analysis according to the modified methods previously described (Pellegrini et al., 2010). Lyophilized samples (100 mg) were extracted at least four times (until colourless) with 5 mL of tetrahydrofuran in an ultrasonic bath for 10 min, vortexed for 1 min and centrifuged for 10 min at 1500 g. The supernatants were combined, dried in a Rotavapor (Büchi, model R-200) and stored at -20°C until HPLC analysis. The residue was dissolved in 3 mL of a solution of methanol/tetrahydrofuran (95:5, v/v) before HPLC analysis. Carotenoids were evaluated using a HPLC system (Alliance and DAD model 2996, Waters, Milford, MA) equipped with a Simmetry C18 (4.5 x 150 mm) column. The elution was carried out by linear gradient using acetonitrile (A) and ethyl acetate (B) as eluents. The gradient was as follows: 0 % B for 2 min, from 0 to 40 % B in 23 min, from 40 to 60 % in 0.10 min, 60 % B for 4 min, and then 7 min at start conditions to reequilibrate the column. The flow rate was 1.2 mL/min. Lutein was acquired at 445 nm, chlorophyll *a* was acquired at 663 nm, and chlorophyll *b* was acquired at 645 nm.

2.6. Ascorbic acid determination

Ascorbic acid was extracted using the method proposed by Durust et al. (1997). Briefly, a homogenized portion of raw and cooked vegetables was added to an equivalent weight of oxalic acid solution (0.4%, w/v). The mixture was homogenized in a high-speed blender. A portion of the homogenized sample (~1 g) was subsequently diluted with an appropriate volume (according to ascorbic acid content expected) of oxalic acid solution, shaken, and centrifuged at 1000 *g* for 5 min. All samples were immediately analyzed by HPLC as described by Gokmen et al. (2000).

2.7. Polyphenol determination

One gram of lyophilized sample was extracted with 10 mL of 60% aqueous methanol solution containing 0.25 mg of morin as an internal standard. Then, it was hydrolyzed by addition of 20 mM sodium diethyldithiocarbamate and 5 mL of 6 M HCl and it was refluxed at 90°C for 2 h. A total of 20 µL of the extract was analyzed by HPLC as previously described (Ferracane et al., 2008).

2.8 Statistical analysis

SPSS statistical software (Version 20.0, SPSS Inc., Chicago, IL, USA) was used to perform an one-way analysis of variance (ANOVA) among samples from different treatments. A LSD post hoc test at a 95% confidence level ($p \leq 0.05$) was performed to further identify differences among treatments.

3. Results and Discussion

3.1. Colour determinations

Colour data are summarized in Table 1 for all vegetables.

Blanched asparagus stems became significantly darker (lower L*), greener (higher H°), less yellow (lower b*) and less intense coloured (lower C), in comparison with R samples. An increase of greenness and a decrease of lightness (L*) were previously reported for asparagus stems blanched in boiling water (Begum and Brewer, 1997). The same behaviour was observed for blanched green beans that were found to be darker and greener (lower L* and – a* values, higher H°) in comparison to R samples. The increase of H° angle, the decrease of L* and no significant C variation were previously reported after blanching green beans in boiling water (Brewer et al., 1995; Tijskens et al., 2001; Canet et al., 2005). The colour of zucchini parenchyma did not change after blanching, while skin became significantly greener (higher – a* and H°) and more yellow (higher b*) than R. An initial increase of the green colour (H°) followed by a decrease was previously reported for heating treated green vegetables (Tijskens, et al., 2001). According to these authors (Tijskens, et al., 2001), the colour change was attributed to the air removal around the fine hairs on the surface, the

air expulsion between the cells and its replacement with water and cell juice released from the deteriorated membranes. The surface reflecting properties could be potentially altered by these two phenomena (Canet et al., 2005).

Freezing process and subsequent storage induced further colour changes especially in asparagus and zucchini. Higher ΔE values of FS were found for asparagus compared with R and BL. In particular, FS samples showed significantly lower L*, b* and C* values than in R. Otherwise, FS was greener and less yellow than BL. A decrease of yellowness was also previously reported after the frozen storage of blanched asparagus (Begum and Brewer, 1997). FS zucchini skin appeared significantly greener (higher – a* and H°) and more yellow (higher b*) compared with R. The colour of parenchyma significantly varied during freezing and storage, too, becoming darker, greener and less yellow (lower L* and b*, higher –a* and H°) in comparison with R. To the best knowledge of the authors, no literature data were found on the effect of freezing on the zucchini colour. The tissues of this vegetable were found to be more damaged by freezing treatment (Paciulli et al., 2014) than the other vegetables. Thus, the colour parameters could be altered by the changes of surface reflectance and light penetration above described for BL samples. Conversely, green bean colour appeared to be less influenced by freezing and storage than the other two vegetables; FS samples were found to be less green than BL, without any further significant changes. Patras et al (2011) reported no L* and C changes during short term storage of blanched green beans.

Boiling step after freezing and storage further influenced asparagus colour, but not so consistently, as only a significantly decrease of green colour was observed (Table 1). Comparing both cooked samples, B appeared to be paler (higher L*), less green (lower – a*) and more yellow (higher b*, lower H°) than FB, showing also less colour changes compared to R (lower ΔE value). The same behaviour was shown by zucchini parenchyma while skin showed similar colour parameters between FB and B. In addition, boiling made the raw zucchini parenchyma darker (lower L*), less yellow (lower b*) and greener (higher H°), in accordance with previous results (Miglio et al., 2008). Green beans showed a different behaviour than asparagus with more evident colour changes after

boiling of frozen stored samples. In particular, FB showed higher L*, - a*, b* and lower H° compared to FS. However, comparing both cooked samples, boiling of frozen stored samples induced less colour changes than cooking of raw green beans (ΔE, Table 1). This was related to the loss of lightness, greenness and yellowness shown by B compared to R, in accordance with Turkmen et al. (2006).

3.2. Chlorophylls and carotenoids

Chlorophyll *a*, *b*, pheophytin *a* and lutein values in the three vegetables analysed are presented in Table 2.

Asparagus spears exhibited the high amount of total chlorophylls (*a*+*b*) followed by green beans and zucchini. With the exception of green beans, the amount of chlorophyll *a* in fresh uncooked asparagus and zucchini was found to be higher than that of chlorophyll *b.* A quite similar content of chlorophylls in green asparagus has been reported (Shou et al., 2007), whereas the amount of chlorophylls *a* and *b* found in R green beans was lower than that reported in literature (Turkmen, 2006; Lisiewska, 2011) likely due to a difference in the varieties analysed. To the best knowledge of the authors, no literature data exist on the chlorophyll content of zucchini.

The blanching treatment significantly reduced ($p<0.05$) the content of chlorophylls *a* and *b* in asparagus and green beans, with a greater loss of chlorophyll *a* than that of chlorophyll *b*, while in zucchini the loss was not significant. The loss of chlorophylls after blanching process was already observed by Lisiewska (2011) on green asparagus and on French bean and by Oruna-Concha et al. (1997) on green beans. Oruna-Concha et al. (1997) found that blanching decreased the content of chlorophyll *a* and *b* in green beans by 13 and 28%, respectively. Conversely, Canet et al. (2005) found that short-time blanching (e.g., 75 °C for 2.5 min) increased the coloration and the total chlorophyll content of green beans with respect to fresh vegetable. However, in agreement with our results, when the blanching duration and temperature were increased, the total chlorophyll content decreased, mainly due to the formation of pheophytin.

The frozen storage of the investigated samples did not significantly change the level of chlorophyll *a* compared to the blanched ones and determined a slight increase of chlorophyll *b* with the exception of green beans, in which a significant reduction (14 %) of this chlorophyll was observed. The chlorophyll retention during the frozen storage could be related to the thermal inactivation of enzymes as well as to the deaeration of plant tissue and the consequent reduction of oxygen content induced by blanching. This preliminary process might have limited the degradation of chlorophylls during frozen storage, making the pigments more stable, as previously found in other green vegetables (Vina et al., 2007; Brewer et al., 1995).

Boiling determined a significant decrease of chlorophylls *a* and *b* of all FS vegetables investigated, in agreement with previous observations on frozen vegetables (Ferreira et al. 2006; Turkmen et al, 2006). It is known that both freezing and cooking processes soften vegetable tissue. Paciulli and coworkers (2014) have observed that cooking treatment causes different cellular damage: boiling treatment causes cell separation, freezing causes cell rupture and the freezing and boiling treatment appears to be unfavourable with regard to the anatomical structure. The rupture of cells as a consequence of freezing and boiling treatments can have been responsible to degradation and/or loss of chlorophylls. Moreover, it is known that the most common mechanism of chlorophylls degradation is their conversion into their major derivatives pheophytin *a* and *b* (Turkmen et al, 2006). Accordingly, in all the analysed FS vegetables, the boiling determined a significant increase of pheophytin *a*, which decomposes more rapidly than chlorophyll *b* owing to its greater susceptibility to pheophytinization during heating (Turkmen et al, 2006), whereas pheophytin *b* was not quantifiable in all investigated samples.

The boiling treatment of fresh vegetables determined a general greater retention of chlorophylls with respect to the boiling process on frozen vegetables. This different effect of boiling could be due to the different vegetable structure, being the FB vegetables more softening than B ones due to the effect of both freezing and cooking processes on vegetable tissues, as previously observed (Ferreira et al., 2006; Paciulli et al., 2014).

In contrast to chlorophylls, the lutein content of all fresh vegetables was not altered by blanching as, generally, blanching beneficially inactivates enzymes, such as peroxidase and lipoxygenase, which are involved in carotenoid destruction (Thane and Reddy, 1997). The freezing storage had a significant and positive effect on the lutein content of all vegetables analysed and in particular on green beans. The blanching step, softening slightly the vegetable matrix, could have favoured the lutein release.

The boiling treatment resulted in a significant increase of lutein in almost all the fresh and frozen vegetables. Heat treatment is known to facilitate the extraction of carotenoids by cell walls disruption (Palermo et al 2014). Thus, the increase of lutein could be due to its heat stability, which is higher with respect to others (e.g. β-carotene), and its release from the cell structure promoted by the heating.

3.3. Polyphenols

The phenolic compounds of all the analysed vegetables are shown in Tables 3, 4 and 5.

Among the studied samples, the asparagus was the vegetables richer in polyphenols followed by green beans and zucchini. As already observed in previous works, the main flavonoid in asparagus (Fuleki, 1999; Wang et al. 2003) and in zucchini (Jang et al., 2004; Rodriguez et al., 2005) was rutin. Among phenolic acids, the coumaric acid and its hexoside were the most abundant in asparagus, whereas protocatechuic and sinapinic were the prevalent acids in zucchini and green beans, respectively.

Blanching treatment prior to frozen storage had a general positive effect on the overall phenolic compounds (i.e. phenolic acids and flavonoids) of the three vegetables considered in the present study. In particular, in the case of asparagus, it was interesting to observe that the ferulic acid contents of R asparagus, measured in the aqueous methanol extract, increased several times after blanching treatment. This seems to disagree with previous observations the ferulic acid is involved in links with cell wall polysaccharides (e.g. xylans) and such links increase in the thermal stability

of cell-cell adhesion (Rodriguez-Arcos et al. 2004). A significant increase of total phenolic acids was also observed in green beans and zucchini in comparison to the raw sample.

Similarly to phenolic acids, the blanching treatment determined a general increase of flavonoids in all the studied vegetables. This increase was in agreement to the findings of Kaisier at al. (2013), who reported that the blanching treatment increased the amount of phenolic compounds of coriander. It is stated (Puupponen-Pimiä et al., 2003) that the effect of processing (i.e. blanching) on flavonols is highly plant species-dependent. In cauliflower and cabbage, the flavonol content increased during blanching, while in spinach it decreased (Puupponen-Pimiä et al., 2003). The blanching processes prior to freezing can inactivate enzymes that cause the oxidation of phenolics and, as we hypothesise in the present study, it can also increase the extractability of phenolic compounds.

In general, the frozen storage showed a positive influence on the flavonoid content of the different studied vegetables, in agreement with Olivera et al. (2008), who reported a slightly, even though not significant, increase of total flavonoids after 8 months of frozen storage. For the best author's knowledge, literature data regarding the effect of frozen storage on the phenolic compounds profile of vegetables are very scant. Puupponen-Pimiä et al. (2003) reported in frozen cauliflower, cabbage and spinach a decrease of flavonol during a long-term frozen storage. On the other hand, Prabhu and Barrett (2009) observed a significant increase of total phenolic content in African leafy vegetables during 90 days of storage at −18 °C.

After frozen storage, when vegetables are submitted to boiling process a detrimental effect on the phenolic compounds profile of FS asparagus and FS zucchini was observed. In fact, the boiling decreased the total flavonoid content of FB asparagus and zucchini by about 60 and 15%, respectively. Similarly, total phenolic acids decreased in the two vegetables with losses of about 42 and 16% in asparagus and zucchini, respectively, when compared to FS samples. On the contrary, FB green beans exhibited a slight and significant increase of all phenolic compounds. As expected, among the single phenolic compounds of vegetables a different trend was observed. For example, in

the case of FS zucchini, the boiling process determined a significant increase of caffeic, vanillic, salicylic, gallic, protocatechuic and siringic acids, but a strong decrease of chlorogenic, vanillic glucoside and coumaric acids. The different trend observed for the different phenolic compounds could depend on several factors such as their water solubility, their different localization in the vegetable structure and the structure stability to heat (Palermo et al, 2014). Phenolic compounds are usually stored in pectin or cellulose networks of vegetables and they can be released during thermal processing; as a consequence, individual phenolic compounds may sometimes increase because the heat can break supra molecular structures, releasing the phenolic sugar glycosidic bounds, or also decrease due to the leaking in the water cooking.

Concerning boiling of fresh vegetables, a different influence was shown compared to frozen samples. In fact, boiling had a significant negative effect on both flavonoids and phenolic acids only in the case of R asparagus, whereas a significant increase of total polyphenols for green beans and zucchini was observed. Among single polyphenols, a significant increase of chlorogenic acid content of asparagus and green beans was observed probably due to the ability of cooking processes to disrupt the (covalently or not) interaction of this acid with the polysaccharide moiety of fibre, as already shown in cooked Brussels sprouts (Pellegrini et al., 2010).

The different trend observed in B and FB vegetables should be related to the previous processes (i.e., blanching and freezing storage) on vegetables, which led to a softening of the vegetable matrix; the further process (i.e., cooking) caused major losses of these compounds from the vegetable matrix than those of fresh. This statement is confirmed by our previous histological analyses (Part I) (Paciulli et al., 2014), in which we observed a consistent damage of the tissue of boiled vegetables after freezing, and also partly by the total antioxidant capacity results. In fact, a general decrease of total antioxidant capacity of asparagus, green bean and zucchini was observed in FB samples, whereas the boiling of fresh vegetables determined a general increase of this parameter.

3.4.Ascorbic acid

Figure 1 shows the ascorbic acid content measured in all samples.

Blanching determined an ascorbic acid loss of 28 %, 4.8 %, 15.7 % in asparagus, green beans and zucchini, respectively. A significant loss of ascorbic acid in blanched green beans was also observed by Bahceci et al. (2005) (30 %) and Howard (1999) (<10 %). Ascorbic acid is reported to be the most difficult vitamin to be preserved during blanching (Gupta, 2008). A combination of leaching, enzymatic degradation and destruction of the ascorbic acid by heat during the hot water blanching would explain such loss, as previously reported (Murcia et al., 2000).

Frozen storage resulted in no further losses of ascorbic acid content of blanched asparagus and zucchini, while a slight but significant decrease (about 8%) of this vitamin was measured in FS green beans. Blanching vegetables before freezing purportedly results in an ascorbic acid retention during frozen storage owing to the inactivation of ascorbate oxidase, which causes the oxidation of ascorbic acid to dehydroascorbic acid (Yamaguchi et al., 2003; Favell, 1998). Several previous studies (Howard et al., 1999; Murcia et al., 2000; Lisiewska et al., 2002) have considered the effects of frozen storage for 6–12 months on vegetables, such as broccoli, carrots, green beans, green peas and spinach, reporting between 37 and 80% retention of initial ascorbic acid. The short period (two months) of frozen storage applied in the present study may likely justify the high retention of ascorbic acid observed in our samples.

Boiling process, as involves heat and water, determined significant losses of ascorbic acid both in R and FS samples. In fact, as already showed in other frozen vegetables (Pellegrini et al., 2010, Mazzeo et al, 2011, Prabhu et al, 2009), FB asparagus, green beans and zucchini lost 44%, 34% and 9% of ascorbic acid, respectively with respect to FS vegetables. Accordingly, B vegetables lost between 16 and 60% of ascorbic acid, in agreement with previous results (Fanasca et al. 2006, Miglio et al. 2008, Pellegrini et al 2010). Interestingly, frozen vegetables retained more ascorbic acid than fresh ones and, as a consequence, FB asparagus and zucchini had a similar content of ascorbic acid as their B counterpart. The lower loss observed in frozen vegetables than in fresh ones

could be probably linked to a shorter time of cooking, as the milder the treatment the better retention of this vitamin (Davey et al 2000). Moreover, the inactivation of ascorbate oxidase during blanching may have preserved the ascorbic acid content of frozen vegetables, as a crucial factor of ascorbic retention during heating is the time needed to inactivate such enzyme (Davey et al, 2000). Finally, less exposure of vegetables to atmospheric oxygen and not having chopped the frozen vegetables prior to cooking might all have resulted in a greater retention of the vitamin in frozen vegetables than in the fresh ones, as previously hypothesised (Prabhu et al, 2009).

3. Conclusions

A step-by-step nutritional evaluation of an industrial freezing process on vegetables has not been previously reported in the literature. Thus, the present results, which completed those of a previous paper (Paciulli et al., 2014), could be of great interest for better approaching the whole process with the objective to obtain a high nutritional quality of final products.

The main findings of the present study are summarized in Figure 2 where the percentage variation of target bioactive compounds and of colour index for asparagus (panel A), zucchini (panel B) and green beans (panel C) was reported. The blanching treatment retained phytochemicals in all green vegetables studied. This was particularly true for green beans where a good retention of ascorbic acid and an increase of rutin were found. The frozen storage did not negatively affect phytochemicals. In the case of lutein and rutin there was an increase in all vegetables, particularly evident in the case of green beans. On the contrary, colour significantly changed during blanching and frozen storage, but these changes were only partially in agreement with the increase of pheophytin and the decrease of chlorophyll *a*. In fact, the changes of b* (yellowness) and the shift of H° were not coherent with the increase of pheophytin. In addition, the greenness (- a*) was found to increase with the exception of B samples in all vegetables. These changes were also probably in relation with cellular alteration that influenced light reflectance as well as with the formation of chlorophyll derivatives, which may lead to an increase of green colour intensity.

Generally, in boiled frozen vegetables there was a better or comparable retention of bioactive compounds with respect to raw ones, and this was especially true for green beans and zucchini.

This finding was particularly remarkable for lutein, which was more stable to thermal treatment than the other measured compounds. The observed retention and/or the increase of bioactive compounds were in disagreement with the total antioxidant capacity data obtained in the previous work (Paciulli et al., 2014), as FB samples exhibited significantly lower total antioxidant capacity than B in all studied vegetables. However, the total antioxidant capacity values generally agreed with the trend of phenolic compounds measured in the present study. Colour changes after cooking did not exhibit the same trends among vegetables, being more remarkable for frozen asparagus in comparison with those boiled from raw, but comparable for green beans and zucchini.

In conclusion, the overall results suggest that, when the industrial freezing process is well performed, the boiled frozen vegetables do not have a lower nutritional value than the fresh ones.

Acknowledgement

The authors gratefully acknowledged Dr Maria Zaupa for performing a part of the experiments.

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Captions for figures

Figure 1. Ascorbic acid content of analysed vegetable (mg/100 g d.w.) Error bars represent ± 1 SD, (n = 3). Bars of histograms with the same capital or lowercase letters are not significantly different for the same vegetable ($p \leq 0.05$). Abbreviations: R, raw/uncooked; BL, blanched; FS frozen/stored; B, raw/boiled; FB, frozen boiled.

Figure 2. Variation percentage (%) of the main bioactive compounds for asparagus stems (A), green beans (B) and zucchini (C) samples. Values for raw samples were considered as equal to 100%. Abbreviations: BL, blanched; FS frozen/stored; B, raw/boiled; FB, frozen boiled.

Table 1. Colour parameters of vegetables [a].

	L*	a*	b*	ΔE	C	H°
Asparagus						
R	56.5 (2.6) a	-9.7 (0.6) b	34.3 (0.5) a	-	35.6 (0.4) a	105.7 (1.1) c
BL	48.0 (2.3) b	-10.7 (1.0) b	26.2 (2.8) bc	12.0 (3.0) b	28.3 (2.8) b	112.3 (2.1) b
FS	41.2 (1.3) c	-13.1 (1.0) c	23.8 (1.3) c	18.9 (1.5) a	27.1 (1.5) bc	118.8 (1.5) a
FB	43.3 (2.2) c	-9.1 (.6) b	22.9 (4.1) c	17.7 (3.8) a	24.7 (4.1) c	112.0 (2.2) b
B	50.3 (2.5) b	-4.7 (3.1) a	29.4 (3.3) b	10.2 (3.1) b	29.9 (3.4) b	99.0 (5.6) d
Green beans						
R	52.6 (1.4) a	-10.5(0.6) b	29.9 (1.3) b	-	31.7 (1.3) ab	109.4 (0.6) c
BL	45.3 (1.5) bc	-16.0 (1.7) d	26.8 (3.4) bc	10.3 (1.6) a	31.3 (3.7) b	120.9 (0.8) a
FS	43.0 (1.6) c	-14.2 (0.9) c	27.2 (1.7) bc	10.8 (1.6) a	30.7 (1.8) b	117.5 (0.3) b
FB	47.5 (3.4) b	-10.1 (1.3) b	33.5 (4.2) a	8.1 (2.0) b	35.0 (4.1) a	106.9 (1.4) d
B	43.3 (1.7) c	-6.8 (0.7) a	25.3 (1.6) c	11.2 (1.7) a	26.2 (1.6) c	105.2 (1.6) d
Zucchini						
			parenchyma			
R	81.7 (1.5) a	-1.9 (0.5) a	30.6 (1.1) a	-	30.7 (1.1) a	93.6 (0.9) d
BL	78.8 (2.5) a	-2.4 (0.9) ab	30.9 (1.6) a	3.7 (1.9) d	31.0 (1.6) a	94.5(1.7) cd
FS	61.5 (1.0) c	-3.7 (0.8) cd	26.0 (1.4) b	21.6 (4.0) b	26.3 (6.3) a	98.6 (2.9) b
FB	56.1 (2.0) d	-4.3 (0.9) d	18.5 (1.5) c	28.6 (5.5) a	19.0 (5.4) b	103.7 (4.2) a
B	73.3 (1.6) b	-3.2 (0.8) bc	26.0 (3.2) b	10.4 (2.8) c	26.2 (3.2) a	96.9 (1.2) bc
			skin			
R	39.3 (1.6) a	-7.5 (0.6) a	16.5 (0.6) b	-	18.2 (1.7) b	114.5 (1.4) b
BL	38.9 (1.3) a	-13.3 (1.7) b	19.8 (2.0) a	7.7 (1.4) a	23.9 (2.2) ab	124.2 (2.3) a
FS	37.5 (1.2) a	-14.7 (2.1) b	23.1 (1.9) a	11.3 (3.4) a	27.2 (3.0) a	122.1 (3.5) a
FB	38.4 (1.9) a	-11.9 (1.9) b	20.8 (1.4) a	8.3 (1.4) a	24.0 (2.6) ab	120.0 (3.4) a
B	38.7 (1.8) a	-12.1 (1.5) b	20.9 (1.6) a	8.3 (1.8) a	24.2 (2.6) ab	120.6 (3.6) a

[a] Means in columns followed by different letters, for the same sample, differed significantly ($p \leq 0.05$), standard deviation given in parenthesis.

Abbreviations: R, raw/uncooked; BL, blanched; FS frozen/stored; B, raw/boiled; FB, frozen boiled.

Table 2. Chlorophyll and lutein content of analysed vegetables.[a]

		R	**BL**	**FS**	**FB**	**B**
Chlorophyll *a*						
	Asparagus	24.32 (0.36) a	22.39 (0.41) b	21.03 (2.73) b	17.19 (0.54) d	19.54 (0.45) c
	Green beans	18.75 (0.56) a	14.39 (0.01) b	14.26 (0.64) b	4.45 (0.14) d	8.12 (0.25) c
	Zucchini	14.14 (0.71) a	13.59 (0.19) a	13.85 (0.60) a	8.87 (0.08) c	9.78 (0.75) b
Chlorophyll *b*						
	Asparagus	14.87 (0.20) ab	14.21 (0.40) b	15.09 (0.28) a	9.21 (0.06) c	8.27 (0.26) d
	Green beans	19.18 (1.12) a	15.02 (0.60) b	11.94 (0.08) c	5.16 (0.04) e	7.76 (0.05) d
	Zucchini	8.82 (0.72) ab	8.58 (0.16) b	9.65 (0.32) a	8.25 (0.71) b	6.53 (0.46) c
Pheophytin *a*[b]						
	Asparagus	2.45 (0.02) d	3.78 (0.48) c	3.76 (0.10) c	37.05 (0.39) a	36.02 (1.31) b
	Green beans	2.37 (0.09) e	8.09 (0.03) d	11.75 (0.12) c	18.84 (1.12) b	21.96 (1.41) a
	Zucchini	6.55 (0.03) d	8.35 (0.14) c	8.99 (0.69) c	16.14 (2.67) b	21.97 (0.40) a
Lutein						
	Asparagus	12.27 (0.14) d	12.98 (0.34) d	23.26 (0.42) c	33.63 (2.78) a	28.17 (0.10) b
	Green beans	1.10 (0.04) d	1.14 (0.01) d	8.11 (0.05) a	5.99 (0.20) b	4.10 (1.24) c
	Zucchini	8.96 (0.24) d	9.49 (0.98) d	16.41 (0.52) c	19.47 (0.16) a	17.50 (0.16) b

[a] Values are expressed as mg/100 g of dry weight. Means in rows followed by different letters, for the same sample differed significantly ($p \leq 0.05$), standard deviation given in parenthesis. All compounds were identified by pure standards, unless differently reported. [b] Tentatively identified as pheophytin *a* and quantified as chlorophyll *a*.

Abbreviations: R, raw/uncooked; BL, blanched; FS frozen/stored; B, raw/boiled; FB, frozen boiled.

Table 3. Phenol compound content of asparagus[a].

Asparagus	**R**	**BL**	**FS**	**FB**	**B**
Phenolic acids					
Chlorogenic acid	21.95 (0.16) d	38.02 (0.45) b	80.02 (2.00) a	16.64 (0.94) e	29.57 (0.54) c
Caffeic acid	22.58 (0.54) a	17.19 (0.17) d	19.02 (0.19) b	8.29 (0.21) c	3.88 (0.21) e
Vanillic acid	6.74 (0.89) ab	3.30 (0.04) c	7.27 (0.88) a	5.82 (0.69) b	3.78 (0.07) c
Coumaric acid	57.45 (0.37) b	75.47 (0.20) a	37.96 (2.27) d	21.18 (0.54) e	44.20 (1.91) c
Coumaric acid hexoside	215.50 (0.42) a	116.12 (0.09) c	114.74 (3.83) d	93.92 (2.08) e	141.40 (5.18) b
Salicilic acid	2.13 (0.01) bc	2.41 (0.25) b	7.02 (0.20) a	2.08 (0.02) c	2.36 (0.19) b
Sinapinic acid	1.80 (0.32) b	3.61 (0.24) a	ND[b]	ND	ND
Ferulic acid	10.59 (0.34) b	59.18 (1.78) a	9.84 (0.30) c	8.91 (0.08) c	10.57 (0.62) b
Ferulic acid hexoside	18.54 (0.44) d	42.59 (0.52) b	70.59 (3.24) a	36.88 (0.66) c	33.06 (3.32) c
Gallic acid	5.99 (0.02) e	8.45 (0.15) d	12.28 (0.58) a	11.17 (0.43)b	9.89 (0.46) c
Siringic acid	2.56 (0.03) c	3.36 (0.04) b	2.62 (0.01) c	3.87 (0.07) a	1.58 (0.08) d
Total phenolic acids	*365.03 (1.49) b*	*369.70 (1.10) a*	*361.36 (3.54) b*	*208.76 (3.8) d*	*280.29 (11.32) c*
Flavonoids					
Rutin	747.28 (0.98) c	772.86 (4.74) b	956.48 (7.28) a	357.65 (5.49) e	507.16 (22.23) d
Quercetin -triglycoside (Quercetin-1-2 rhamnose +3glucose)	25.02 (0.26) a	5.35 (0.27) d	26.51 (0.38) a	15.18 (0.32) c	20.26 (0.78) b
Kaempferol	53.84 (0.03) a	36.50 (7.67) b	15.16 (0.35) c	16.77 (0.00) c	12.68 (0.43) c
Isokaempferol	48.30 (1.99) c	61.63 (0.32) b	82.22 (2.19) a	23.14 (0.20) d	13.01 (0.67) e
Kaempferol -3-O-rhamnoglucoside	24.05 (0.06) b	25.24 (0.17) a	3.43 (0.25) e	10.21 (0.07) d	14.39 (0.44) c
Isorhamnetin –triglycoside (Isoramnetin-1 rhamnose +2glucose)	9.17 (0.09) b	8.13 (0.08) c	10.89 (0.12) a	3.51 (0.04) e	4.92 (0.21) d
Isorhamnetin -3-O-rhamnoglucoside	16.55 (0.08) a	14.22 (0.12) b	2.77 (0.05) e	7.45 (0.13) d	11.44 (0.94) c
Isorhamnetin -3-O-glucoside	4.08 (0.15) c	5.36 (0.14) b	7.02 (0.20) a	2.08 (0.02) d	2.36 (0.19) d
Total flavonoids	*928.29 (2.87) b*	*929.29 (12.58) b*	*1104.48 (4.54) a*	*435.99 (6.29) d*	*586.22 (26.03) c*

[a]Values are expressed as mg/100 g of dry weight. Means in rows followed by different letters, for the same sample differed significantly ($p \leq 0.05$), standard deviation given in parenthesis. All compounds were identified by pure standards. [b]ND, not detected. Abbreviations: R, raw/uncooked; BL, blanched; FS frozen/stored; B, raw/boiled; FB, frozen boiled.

Table 4. Phenol compound content of green beans[a].

Green Beans	**R**	**BL**	**FS**	**FB**	**B**
Phenolic acids					
Chlorogenic acid	4.14 (0.47) c	9.70 (0.05) a	1.31 (0.02) d	1.46 (0.09) d	8.64 (0.05) b
Caffeic acid	2.26 (0.03) b	4.93 (0.22) a	1.30 (0.00) c	1.01 (0.05) d	4.80 (0.17) a
Caffeic acid hexoside	4.87 (0.20) b	2.06 (0.11) c	1.32 (0.01) e	1.58 (0.08) d	8.82 (0.06) a
Vanillic acid	4.81 (0.04) b	2.37 (0.38) c	6.25 (0.02) a	4.77 (0.02) b	4.75 (0.02) b
Coumaric acid	3.20 (0.01) a	1.58 (0.35) c	0.90 (0.04) d	1.60 (0.01) c	2.82 (0.11) b
Coumaric acid hexoside	0.67 (0.02) c	2.06 (0.11) a	1.22 (0.02) b	1.10 (0.05) b	2.03 (0.10) a
Salicilic acid	3.15 (0.17) e	4.82 (0.05) d	7.02 (0.53) b	7.70 (0.19) a	6.08 (0.00) c
Sinapinic acid	8.39 (0.24) b	8.90 (0.08) a	1.08 (0.02) c	0.73 (0.08) d	1.02 (0.01) c
Ferulic acid	4.43 (0.26) a	2.73 (0.03) b	1.14 (0.01) d	0.92 (0.03) d	1.80 (0.08) c
Ferulic acid hexoside	1.14 (0.01) d	2.24 (0.05) b	1.27 (0.02) d	1.83 (0.08) c	2.63 (0.28) a
Gallic acid	5.25 (0.07) e	11.38 (0.10) a	7.58 (0.10) c	7.00 (0.14) d	8.42 (0.02) b
Protocatechuic acid	ND[b]	3.28 (0.06) a	0.27 (0.02) d	2.75 (0.12) b	1.76 (0.07) c
Total phenolic acids	*42.31 (0.91) c*	*56.05 (0.85) a*	*30.66 (0.52) e*	*32.45 (0.42) d*	*53.57 (0.47) b*
Flavonoids					
Rutin	13.24 (0.44) e	27.41 (0.32) d	34.35 (0.73) c	39.31 (0.21) a	38.18 (0.52) b
Quercetin	0.77 (0.04) d	1.58 (0.06) a	0.98 (0.02) b	0.89 (0.01) c	0.73 (0.02) d
Quercetin 3-O-xylosyl rutinoside	19.33 (0.94) b	28.11 (0.20) a	0.60 (0.01) d	0.63 (0.02) d	1.36 (0.06) c
Quercetin rhamnoside	2.02 (0.01) a	1.94 (0.00) b	0.77 (0.05) c	0.30 (0.01) d	0.80 (0.00) c
Quercetin 3-galactoside	0.42 (0.06) d	1.16 (0.00) a	0.37 (0.01) d	0.53 (0.03) c	0.60 (0.01) b
Quercetin glucorhamnoside	20.14 (0.61) d	38.05 (0.27) c	45.65 (0.67) a	46.31 (0.24) a	44.33 (0.47) b
Kaempferol	5.95 (0.00) e	7.50 (0.14) c	6.64 (0.12) d	8.74 (0.36) a	7.90 (0.01) b
Kaempferol 3-(2G- xylosyl rutinoside)	2.57 (0.12) b	4.21 (0.05) a	1.85 (0.11) d	1.42 (0.03) e	2.06 (0.12) c
Kaempferol 3 rutinoside	5.53 (0.17) e	6.87 (0.26) d	11.54 (0.35) a	11.39 (0.45) b	8.15 (0.06) c
Kaempferol 3-O-B-glucuronide	3.26 (0.06) e	4.83 (0.04) d	7.02 (0.53) b	7.77 (0.23) a	6.08 (0.00) c
Total flavonoids	*73.23 (2.60) d*	*121.66 (1.34) a*	*109.77 (1.45) c*	*117.29 (0.09) b*	*110.19 (2.10) c*

[a]Values are expressed as mg/100 g of dry weight. Means in rows followed by different letters, for the same sample differed significantly ($p \leq 0.05$), standard deviation given in parenthesis. All compounds were identified by pure standards. [b]ND, not detected.
Abbreviations: R, raw/uncooked; BL, blanched; FS frozen/stored; B, raw/boiled; FB, frozen boiled.

Table 5. Phenol compound content of zucchini.[a]

Zucchini	**R**	**BL**	**FS**	**FB**	**B**
Phenolic acids					
Chlorogenic acid	11.58 (0.43) b	10.42 (0.22) c	12.50 (0.03) a	3.78 (0.15) e	6.96 (0.01) d
Caffeic acid	0.48 (0.02) c	1.45 (0.01) b	1.44 (0.02) b	2.47 (0.06) a	1.44 (0.02) b
Vanillic acid	7.40 (0.30) b	8.53 (0.50) a	4.40 (0.03) d	6.04 (0.51) c	4.74 (0.13) d
Vanillic acid glucoside	8.23 (0.05) c	8.40 (0.03) b	12.44 (0.09) a	4.01 (0.04) e	6.92 (0.05) d
Coumaric acid	10.11 (0.45) d	19.82 (0.34) b	21.02 (0.52) a	15.99 (0.50) c	16.48 (0.18) c
Coumaric acid hexoside	1.68 (0.02) c	2.80 (0.15) a	2.90 (0.00) a	2.25 (0.17) b	1.31 (0.01) d
Salicilic acid	1.48 (0.03) e	3.02 (0.04) c	2.26 (0.09) d	4.15 (0.13) a	3.83 (0.01) b
Sinapinic acid	0.79 (0.02) d	2.13 (0.03) c	2.41 (0.15) b	2.13 (0.04) c	3.35 (0.14) a
Sinapinic acid hexoside	0.60 (0.00) d	3.77 (0.07) ab	3.87 (0.12) a	3.72 (0.08) b	2.53 (0.10) c
Ferulic acid	8.52 (0.06) d	17.86 (0.11) b	22.98 (0.25) b	22.64 (0.54) b	28.33 (1.09) a
Ferulic acid hexoside	1.48 (0.01) d	3.37 (0.24) b	2.06 (0.06) c	1.56 (0.02) d	3.63 (0.15) a
Gallic acid	2.52 (0.11) e	2.75 (0.12) d	3.29 (0.00) c	3.81 (0.01) b	7.22 (0.08) a
Protocatechuic acid	18.45 (0.14) c	24.02 (0.31) a	17.09 (0.09) d	19.02 (0.59) c	21.77 (0.69) b
Siringic acid	6.35 (0.07) a	2.08 (0.00) b	0.47 (0.02) d	0.83 (0.02) c	0.83 (0.01) c
Total phenolic acids	*79.67 (0.04) c*	*110.42 (0.79) a*	*109.13 (1.23) a*	*92.40 (1.93) b*	*109.34 (2.13) a*
Flavonoids					
Rutin	30.51 (0.94) e	39.79 (0.71) b	42.33 (0.38) a	32.45 (0.09) d	33.55 (0.12) c
Quercetin	2.35 (0.13) a	1.31 (0.06) b	0.85 (0.05) c	0.65 (0.04) d	0.96 (0.01) c
Quercetin 3-O glucoside	1.30 (0.06) c	1.22 (0.16) c	2.11 (0.04) b	5.49 (0.02) a	2.10 (0.01) b
Quercetin glucuronide	3.02 (0.01) e	5.77 (0.11) d	10.29 (0.04) b	10.84 (0.13) a	9.70 (0.20) c
Kaempferol	1.54 (0.02) a	0.73 (0.05) d	0.92 (0.01) c	0.60 (0.03) e	1.01 (0.00) b
Kaempferol rutinoside	0.85 (0.00) e	2.58 (0.17) d	3.75 (0.11) b	4.08 (0.47) a	3.09 (0.09) c
Kaempferol O-sambubioside	3.84 (0.02) c	4.29 (0.13) b	5.38 (0.03) a	3.23 (0.02) e	3.66 (0.10) d
Kaempferol O glucuronide	1.40 (0.04) a	0.42 (0.06) c	0.41 (0.00) c	0.59 (0.01) b	0.45 (0.01) c
Isokaempferol rutinoside	14.43 (0.14) b	12.80 (0.15) c	20.63 (0.00) a	12.85 (0.23) c	9.59 (0.36) d
Isokaemferol O-glucuronide	1.47 (0.02) c	1.49 (0.04) c	2.48 (0.00) b	4.94 (0.08) a	2.45 (0.13) b
Isoramnetin 3-rutinoside-7-rhamnoside	3.22 (0.02) e	4.61 (0.04) b	4.79 (0.17) a	3.52 (0.15) d	3.80 (0.07) c
Total flavonoids	*63.93 (1.26) e*	*75.01 (0.76) c*	*93.94 (0.34) a*	*79.24 (0.82) b*	*70.36 (0.01) d*

[a]Values are expressed as mg/100 g of dry weight. Means in rows followed by different letters, for the same sample differed significantly ($p \leq 0.05$), standard deviation given in parenthesis. All compounds were identified by pure standards. Abbreviations: R, raw/uncooked; BL, blanched; FS frozen/stored; B, raw/boiled; FB, frozen boiled.

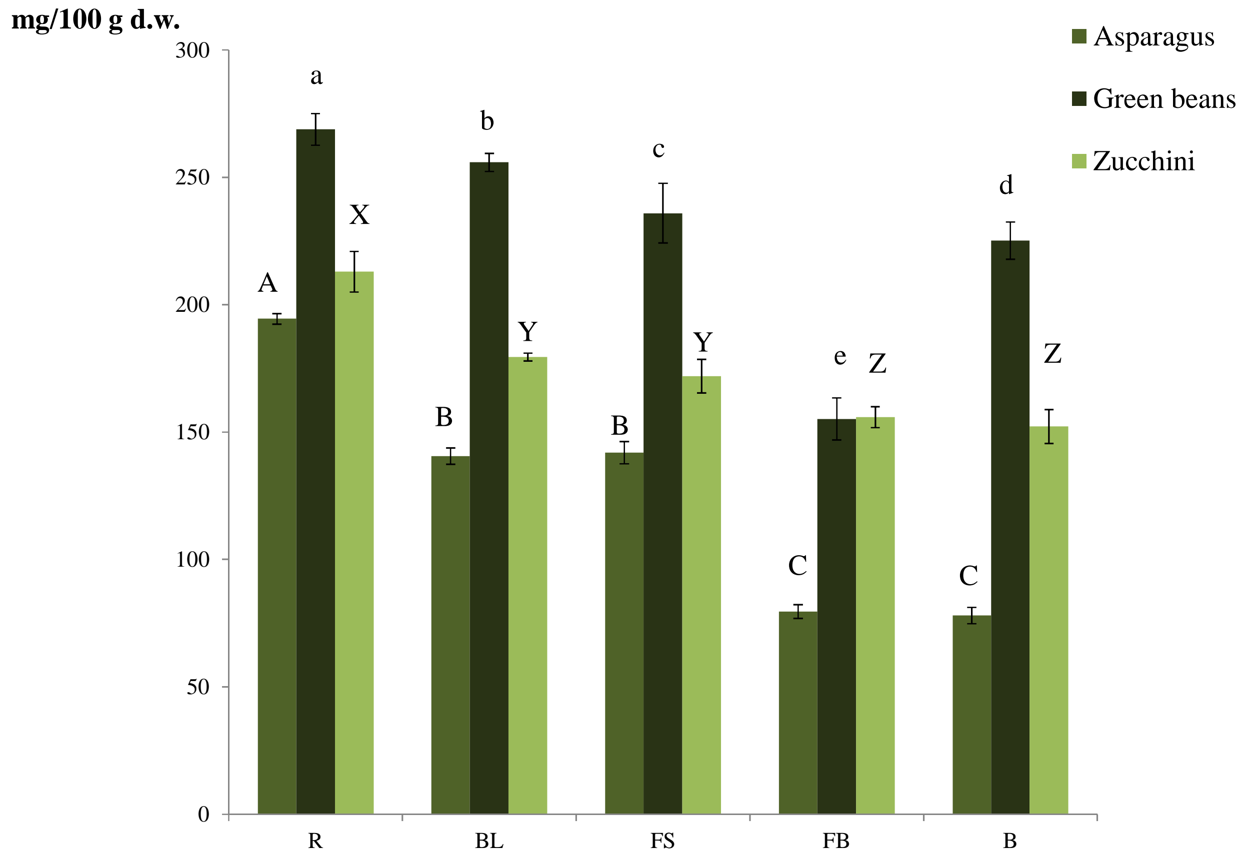

Figure 1

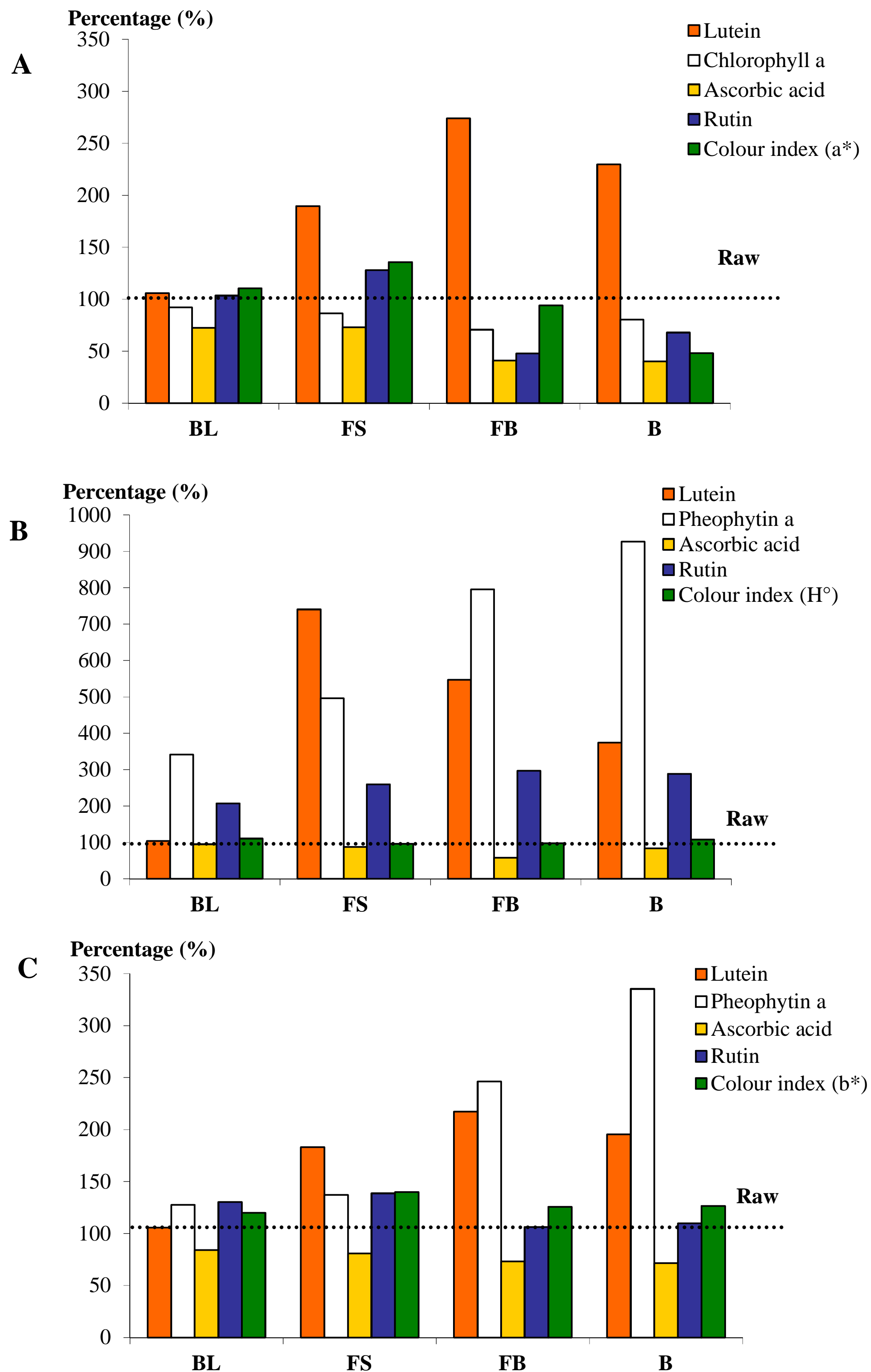

Figure 2

Section II

Effect of different cooking methods on structure and quality of industrially frozen carrots

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Abstract

In this work, the effect of three common cooking procedures (boiling, steaming and microwaving) on structure, texture and colour of carrots industrially processed in an industrial plant was investigated and compared to the results obtained from raw carrots provided by the same manufacturer and cooked with the same procedures.

The anatomical structure of the carrots appeared damaged in the samples subjected to mere freezing and storage, as the freezing process apparently caused the first and most important damage in carrot parenchyma tissues. After cooking, the anatomical structure of microwaved and steamed carrots appeared less damaged than boiled carrots.

Raw carrots resulted significantly firmer than the corresponded cooked samples, as expected, and no significant differences were observed among differently cooked carrots for cut force. On the other hand, microwaved resulted the firmest among the frozen cooked vegetables, being steamed samples the softest and boiled those with an intermediate values

A partial retention of colour was found if the cooking was carried out on frozen carrots, and in particular, when boiling and microwaving methods were used to process samples.

Keywords: carrots; colour; industrial freezing; structure; texture.

1. Introduction

Vegetables are well documented and recognized to beneficially affect the human health as their intake was epidemiologically associated with a decreased mortality from cardiovascular diseases, certain cancers and obesity (Martin, 2013). They are generally consumed thermally processed (either industrially or at household level) other than consumed as raw, to extend shelf-life, preserve quality and to maintain cultural and preference habits. Freezing is one of the most applied methods of processing vegetables and it consists of several different steps (blanching, freezing, frozen storage following freezing, and above all, final cooking prior to final consumption) that greatly impact the final quality of the products (Canet, Alvarez, Luna, & Fernández, 2004).

By a structural point of view, blanching causes an alteration of organelles in the cytoplasm and substantial vesicle formation (swollen walls) in the cell wall (Prèstamo, Fuster, & Risueño, 1998), a gradual breakdown in the protoplasmic structure organization, with subsequent loss of turgor pressure and a softening effect also related to changes in the pectic polymers of the cell wall and middle lamella (Canet, Alvarez, Luna, Fernández, & Tortosa, 2005). Freezing led to major modifications as compared to blanching because it induces the formation of ice crystals that mechanically stress and damage the tissue wall (Van Buggenhout et al., 2006). Frozen vegetables exhibited a weakening of the cell wall, a partial destruction of the cytoplasmatic structure, the depolymerisation of the pectic materials, and an inducing loss of turgor with softening effects, as for blanching (Reid, 1993; Paciulli et al., 2014). Frozen storage induced further tissue softening (Neri et al., 2014).

Finally, high-temperature exposure during industrial thermal processing and/or end-user consumer cooking causes cell separation in vegetables, which is related to the solubilisation of pectin components, often accompanied by the swelling of cell walls (Waldron, Parker, & Smith, 2003).

Carrots are one of the most widespread common consumed vegetables due to the high content of nutrients (*i.e.* carotenoids, B vitamins, minerals). The impact of blanching and/or freezing and/or frozen storage on carrot microstructure was extensively debated in literature in the past (Gómez &

Sjöholm, 2004; Greve et al., 1994a; Greve, McArdle, Gohlke, & Labavitch, 1994b; Prèstamo et al., 1998). On the other hand, the relation of tissue changes, after the freezing process, with the mechanical properties of texture was investigated in few studies (Kidmose & Martens, 1999; Neri et al., 2014; Roy, Taylor, & Kramer, 2001; Van Buggenhout et al., 2006). In these studies, carrot samples were generally treated at laboratory scale and not industrially processed. In addition, the effect of cooking, the final process step, on the main quality attributes (*i.e.* texture, colour) that have a great impact on the final consumer acceptance was scarcely considered (Kidmose & Martens, 1999; Neri et al., 2014; Roy, et al., 2001; Van Buggenhout et al., 2006). When taken into consideration, only a single type of heating treatment or one quality attribute was discussed (Mazzeo et al., 2011; Tanseya, Gormley, & Buttera, 2010). Thus, this topic needs to be more deeply investigated with the ultimate goal to offer a higher quality final cooked product to the consumers, achieving a greater retention of the original quality of the processed vegetable.

In this framework, the aim of this work was therefore to investigate the effect of three common cooking procedures (boiling, steaming and microwaving) on structure, texture and colour of carrots industrially processed in an industrial plant and to compare the results with those obtained from raw carrots provided by the same manufacturer and cooked with the same procedures.

2. Materials and Methods

2.1 Samples and processing

Ten kilograms of carrots (*Daucus carota* L., Napoli variety) were obtained by a local manufacturer, having been harvested in the production site under the same season and agronomical conditions.

Five kilograms of this sample were immediately transported to the University of Parma laboratories under adequate refrigerated conditions (2 - 4 °C) and processed within 48 hours from harvesting as follows: washed with the tap water and drained, sorted for size and length, peeled and cut into slices of 8 mm of thickness. A portion of them was immediately analysed (R) while the other part underwent cooking treatments as described below.

The other five kilograms were processed into the industrial plant within 24 hours from harvesting. Carrots were washed, drained, sorted, peeled and cut as described above. Then, they were blanched by immersion in a hot water bath (100°C) for 2 min 30 sec until to the peroxidase inactivation (Goncąlves, Pinheiro, Abreu, Brandaõ, & Silva, 2007) and frozen in an ammonia forced air cooling tunnel at - 40°C for 6 min. Frozen samples were then maintained for two months at – 18 °C in a thermostat to best mimic the common storage conditions prior to the commercialization. At the end of storage, frozen carrots were transported to the University of Parma laboratories under adequate temperature conditions (- 18 °C). A portion of them was immediately analysed (F), the remaining part was cooked within 24 hours from the arrival.

2.2 Cooking treatments

Boiling, steaming and microwaving were chosen as cooking procedures commonly applied in Italy on raw and frozen carrots. Cooking lengths were optimized for each treatment according to the common Italian habit of palatability and tenderness for this vegetable, obtained by the judgment of a large group of semi-trained panellists. Frozen carrots were not defrosted before cooking.

Boiling was performed by adding carrot slices to boiling tap water in a covered stainless steel pot (1:5, food/water) cooking on a moderate flame. Cooking times, measured when the vegetable was put into boiled water, were 20 min for raw (R_B) and 15 min for frozen carrots (F_B).

Steaming treatments were carried out at 100 °C under atmospheric pressure in a Combi-Steam SL oven (V-Zug, Zurich, Switzerland) that presented an internal volume of 0.032 m^3, an air speed of 0.5 m/s and a steam injection rate of 0.03 kg/min. Oven was pre-heated at the set temperature before inserting samples for each cooking trial. Cooking times were 45 and 30 min for raw (R_S) and frozen carrots (F_S), respectively.

Microwave treatments were carried out in a domestic microwave oven (De Longhi MW651, Treviso, Italy), where frozen carrot slices, placed in a plastic (PP) microwave steamer (1:2, food/water) not in direct contact with water on the rotating turntable plate of the oven, were

exposed at a frequency of 2450 Hz at low power (450 W). Cooking time was 10 min for both raw (R_{MW}) and frozen (F_{MW}) vegetables. All cooking procedures were performed in triplicate.

2.3 Histological analysis

Raw, frozen and cooked samples were analysed. The samples were fixed in FAA solution (formalin: acetic acid: 60% ethanol 2:1:17 v/v) and after at least 2 week they were dehydrated with gradual alcohol concentrations according to Ruzin (1999). Inclusion was made in a methacrylate resin (Technovit 7100 Heraeus Kulzer & Co., Wehrheim, Germany), and the resulting blocks were sectioned at 4 μm thickness (transversal cuts) with a semithin Leitz 1512 microtome (Leitz, Wetzlar, Germany. The sections were stained with Toluidine Blue (TBO) solution (Ruzin 1999), Periodic Acid Schiff (PAS) reagent and Amido Black (Ruzin, 1999).

Six pieces of each vegetable were sampled for each treatment and stain. Sections were observed with a Leica DM 4000B optical microscope (Leica Imaging Systems Ltd., Wetzlar, Germany) equipped with a Leica DC 100 digital camera (Leica Imaging Systems Ltd., Wetzlar, Germany). The tissues were measured using an image analysis system (QWIN 5001 Leica Imaging Systems Ltd., Wetzlar, Germany). The image analyses were carried out using a manual configuration of the image analysis system.

2.4 Texture analysis

Raw, frozen and cooked carrot slices were analysed using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) by means of a cut test. The test was performed using a 3 mm thick stainless steel knife blade driven through the entire diameter of the vegetable, positioned on a slot surface, at a speed of 3 mm/s. The following parameters were obtained from the force *vs* distance curves using the application software provided (Texture Expert for Windows, version 1.22): the maximum force values (F_{max}, given in N), the area under the curve (Area, given in Nmm), the slope of the linear ascendant portion of the curve (Slope, given in Nmm^{-1}) (Canet et al. 2004). Ten slices were analysed for each sample.

2.5 Colour analysis

Colour determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D_{65} on raw, frozen and cooked carrots. L* (lightness; black = 0, white = 100), a* (redness > 0, greenness < 0), b* (yellowness > 0, blue < 0), C (chroma, 0 at the centre of the colour sphere), and H° (hue angle, red = 0°, yellow = 90°, green = 180°, blue = 270°) were quantified using a 10° position of the standard observer. The individual differences in L*, a* and b* values of each cooking treatments in respect of the colour of the raw and frozen samples were evaluated using the ΔE calculation (CIE, 1978).

The assessments were carried out on two pre-selected positions of each slice picking approximately the same points for the outer and the inner parenchyma of the specimens. Fifteen slices for frozen and cooked samples were analysed for a total of 30 determinations for each trial.

2.6 Statistical analysis

SPSS statistical software (Version 20.0, SPSS Inc., Chicago, IL) was used to perform one-way analysis of variance (ANOVA) among samples. The least significant difference (LSD) at a 95% confidence level ($p \leq 0.05$) was performed to further identify mean differences among groups. A t test was performed to compare raw and frozen samples similarly processed.

3. Results and Discussion

3.1 Histological analysis

The general structure of carrot root was in line with that described by Metcalfe and Chalk (1957) for the species. In Fig. 1, the transverse sections of carrot disks samples was shown

The cells presented large vacuoles and in any cases showed a slight plasmolysis due to the anatomical sample preparations (Fig.1A). The wall appeared thin and few intercellular spaces were present. The parenchyma tissues consisted in an outer parenchyma with tangentially oriented, elongated cells and an inner parenchyma with radially oriented, elongated cells. Inner parenchyma presented more large cells in comparison with the outer parenchyma (Fig.1B).

After boiling, the cells of R_B appeared plasmolysed and the cell separation was present (Fig.1C); this change was mainly evident in the outer parenchyma. In this tissue, some cells showed the evidence of the onset of separation in the middle lamella regions; cell separation was due to a breakage of chemical bonds between the pectic components of middle lamellae of adjacent cells and/or a hydrolysis of some other components of the cell wall (i.e., pectin, hemicelluloses, cellulose). This would confirm what observed by several authors in different vegetable structures after thermal treatment (Lecain, Ng, Parker, Smith, & Waldron, 1999; Sila, Smout, Vu, Loey, & Hendrickk., 2005; Paciulli et al., 2014). In our study, the separation of cells after cooking might be ascribed to a decrease of the strength of cell-cell interactions in the middle lamella adjacent to the intercellular spaces. In R_B some cells showed the evidence of swollen cell walls (Fig.1C); this phenomenon was even more evident in the central cylinder. Préstamo et al. (1998) explained the swollen phenomenon as a gelation process of the network of cellulose microfibriles, hemicelluloses and polysaccharides bases on pectins; in particular, the pectins, present in middle lamella, was found to be mainly implicate in this phenomenon. In R_{MW} samples, the phenomenon of plasmolysis was evident; some cells showed swelling cell wall with few cells exhibiting cell wall broken. In any cases, the cell walls became less compact and less-densely stained (due to the swollen cell wall). The cell wall swelling was more evident in the inner parenchyma (Fig. 1D). Moreover, the cell lyses was marked in the outer parenchyma near the epidermis tissue (Fig. 1E). In R_S samples, a strong swelling was observed in cell walls of inner and outer parenchyma, but less evident if compared with R_{MW} samples (Fig. 1F). Moreover, in the inner parenchyma plasmolysis was detected and the structure was not damaged.

The anatomical structure of the carrots appeared damaged in the samples subjected to mere freezing and storage (F) (Fig.2A, 2B), as the freezing process apparently caused the first and most important damage in carrot parenchyma tissues. The damage observed in our study confirmed the results obtained by Fuchigami, Miyazaki, and Hyakumoto (1995) who affirm that the freezing temperature was the most critical factor affecting carrot cell structures. In accord with Préstamo et al. (1998),

frozen caused a physical change in the carrot tissues; in some cases, the cells were clearly disrupted and in the parenchyma appeared fissures (Fig.2A, 2B). The direction of fissures after freezing was variable, according to the different cell orientations in outer and inner parenchyma; in the outer parenchyma, fissures showed a tangential orientation (Fig.2A), while in the inner parenchyma fissures were oriented in a radial way (Fig. 2B).

F_B samples tended to accentuate the structure damages, particularly in the inner parenchyma, near the vascular tissue (Fig.2C). The cells tended to separate (Fig.2C), and this could be due to a partial pectin solubilisation accompanied by depolymerisation, and/or to a partial damage of the cell wall in the middle lamella (Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009). In F_B samples, the starch was partially gelatinized. The anatomical structure of F_{MW} carrots appeared less damaged than F_B carrots (Fig.2D); the structure, in spite of fissures due to the freezing treatment, appeared compact and with no extra intercellular spaces. The most important structural change lied in cell wall thickening after cooking (Fig. 2E). Thus, it appears that microwave heating (before and after frozen) caused some tissue plasmolysis, and that the rapid removal of water may increase the tissue mechanical strength, possibly by increasing the crystallinity of cellulose and hemicellulose in the cell wall (Kidmose and Martens, 1999). An, Yang, Liu, and Zhang (2008) found that in carrot starch gelatinization after microwave cooking was partial; in our study, the starch does not appear to have undergone any gelatinization process. In F_S carrots (Fig.2F), the general structure was preserved and comparable to F_{MW} samples. It can be assumed that in the F_S and F_{MW} cooking treatments, the water present in the tissues was not expelled to the outside, but rather that, due to dehydration, it moved in the matrix phase of the cell wall. This phenomenon did not happen in F_B, as in the boiling conditions cooking takes place in a liquid medium and therefore a balance was threrefore created between external environment (cooking water) and cell sap.

3.2. Texture

Raw and cooked samples showed different cut force vs deformation curves (Fig.3A). In particular, the former evidenced a cutting profile characterised by a fast increase of force before surface

penetration followed by a sudden fall, which indicated a turgid and crunchy structure, as suggested by Neri et al. (2014) and confirmed by the histological observations (Fig.1A, B).

On the contrary, after cooking, only slow force increases are evident. The forces obtained for cooked samples could be due to the sum of resistance opposed to the probe penetration and the deformation caused by the tissue compression. In addition, the decrease of the slope of the rising portion of the curve and the broaden curve profile of the cooked samples suggested the softening of the structure and a rubbery behaviour. The parameters extrapolated from the curves are shown in Table 1. R carrots resulted significantly firmer (71.8±9.4 N) than the corresponded cooked samples, as expected (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008). Tissue softening could be microscopically related to the observed plasmolysis, the presence of swollen cell walls and cell separations (Fig1C-1F), resulting in cell turgidity loss generally related to vegetable softening (Paciulli et al., 2014).

No significant differences were observed among differently cooked carrots for Fmax (Table 1). Focusing on the differences among the cooking practices, the microwave cooked carrots resulted the hardest one, followed by the boiled and steamed ones with 86.5, 90.1 and 93.9 % texture loss if compared to the R sample. These results were supported by the histological observations in which the R_{MW} sample, conversely to the R_B and R_S, did not show any cell separation (Fig.1D). , The compactness of the R_{MW} tissue probably opposed a higher resistance to the probe penetration, resulting in a higher Fmax.

R_B and R_S samples exhibited very similar force-distance curve shapes with lower max force than R_{MW}. It’s possible to hypothesize that the water cooking media, in form of liquid or gas, had an effect on the middle lamella molecular bound hydrolysis.

The area values (Nmm) reveal the amount of energy involved in the cutting of the samples (Bourne, 2002). Area values (Table 1) followed the same Fmax trend with R_{MW} sample showing 39.8% of cutting energy loss followed by R_B and R_S with 91.1 and 92.5% values, respectively, in comparison to R.

The slope of the linear ascendant portion of the curve was also calculated as it gave information about the elastic properties of the sample decreasing when an increase of tissue extensibility was recorded (Kidmose et al. 1999). A significant reduction of the slope values in comparison to R was observed for all samples. In particular, a percentage reduction of 90.8, 93.3 and 91.6 were registered for R_B, R_S, R_{MW} respectively showing a slight opposite trend between max force and slope. Trejo Araya et al. (2007) observed an increasing of force with decreasing the slope These authors explained the observed trend with the presence in carrot tissue of a more deformable material with less cell integrity and hence a rubbery-like texture.

The curves of the frozen samples (Fig.3B) showed elongate profiles and more complex shapes in comparison to the raw group probably because of a more marked tissue damage damaged due to the process steps, as also observed from the histological observations (Fig 2A-2F). Frozen samples, submitted to blanching before freezing and a further storage after freezing, were subjected to both the effects of heat and water crystals growth that damaged the tissues producing dehydration and separation of the cells, as well as the formation of deep oriented fractures in the parenchyma (Fig. 2A), not detectable in the raw samples group, allowing an easy penetration of the probe.

All cooked samples exhibited a marked decrease of Fmax. A consistent reduction of cut force was previously reported for industrially frozen vegetables after cooking (Paciulli et al., 2014). F_{MW} samples resulted the firmest among the frozen cooked vegetables, with a percentage reduction of 84.2% of Fmax (47.6±5.6 N), being F_S samples the softest (94.1%). F_B showed and intermediate Fmax value with 90.8 % of firmness reduction in comparison to F carrots.

Kidmose et al. (1999) observed the same phenomena when carrots slices were subjected to boiling, steam and microwave blanching and consequent freezing. They assume that microwave heating causes some dehydration to the tissue, and that the rapid removal of water may have increased the mechanical strength of the tissue possibly by increasing the crystallinity of the cellulose and the hemicelluloses in the cell wall. Our tissue observations revealed the presence of swollen cell walls with amorphous material in F_{MW} samples (Fig.2D). It’ s possible to hypothesize that the water

present in the tissues was not expelled to the outside, but rather that, due to dehydration, it moved in the matrix phase of the cell wall. Despite the F_S samples appeared more similar to the F_{MW} because of the cell connections (Fig. 2F), it resulted the softest one. It's possible to assume that the cells were apparently connected between them, because of the large swollen cell walls, but they were really only place against each other resulting in an easy probe penetration.

The area values (Nmm) followed the same trend of the Fmax (Tab.1), confirming that great part of the energy involved in cutting is involved in the fracture of the sample. F_{MW} required the highest energy to be cut, followed by F_B and F_S. Comparing frozen and raw samples, significant higher area values were observed for F, F_B and F_S samples. It's possible to hypothesize that the tissue have assumed sticky traits and an higher attachment to the probe because of the greater damages of the frozen tissue in comparison to the raw, and the consequent leakage of intracellular fluids resulting in an a greater friction during the probe penetration and a consequent extra energy amount to cut the samples. Conversely, F_{MW} sample showed lower area value respect to R_{MW}. This was probably related to the drying effect of microwave, which generated a less sticky sample and consequently smaller area.

3.3 Colour

The colour parameters of cooked carrots from raw samples are reported in Table 2. The inner parenchyma of raw carrots resulted to have $L^* \approx 50$, $a^* \approx 25$ and $b^* \approx 30$ while the outer tissue was characterized by a $L^* \approx 56$, $a^* \approx 36$ and $b^* \approx 46$. The colour of carrots has been reported to be largely due to the presence of carotenoid pigments, mainly α- andβ-carotene. The differences between inner and outer parenchyma are mainly due to the larger presence of β-carotene in the external portion that increased all the colour parameters considered.

Cooked samples showed a significantly decrease of all colour parameters (excluding L^* in microwave cooked sample) indicating a general colour loss mainly related to the α- and β-carotene decrease and their isomerisation, as already observed in cooked carrots ((Miglio et al., 2008) and in carrot juice (Chen, Peng, & Chen, 1995).

Different cooking processing did not affect colour parameters in the same way. Boiled carrots resulted to have the lowest L* and C, suggesting that the colour of the inner parenchyma resulted more lost with boiling method if compared with the other cooking procedures. Moreover, as mentioned above, the lightness of microwave cooked sample was found to increase than in other cooking processed samples. No comparable differences were observed in the outer parenchyma of boiled carrots, that, on the contrary, showed smaller changes if compared with steamed and microwave cooked samples. Steaming resulted in lowest L^*, a^* and b^* parameters indicating this cooking method as the more drastic in respect to the colour changes. Few reports deal with changes of carotenoid content in carrots as consequence of the different cooking procedures.

The overall colour changes induced by different cooking methods were evaluated with the ΔE parameter, that, based on the numeric value, may indicate if colour is perceivable by the human eye; higher the value, higher the differences between sample and the reference sample, as indicated by the follow scale (Limbo and Piergiovanni, 2006):

- ΔE< 0.2: not perceptible difference
- 0.2 < ΔE< 0.5: very small difference
- 2<ΔE< 3: fairly perceptible difference
- 3<ΔE < 6: perceptible difference
- 6<ΔE < 12: strong difference
- ΔE> 12: different colours

All carrots (both for inner and outer parenchyma) cooked with the different procedures were characterized by high ΔE values indicating that the colour of samples is perceived as different colour by the human eye if compared with the raw sample. In particular, the external portion colour changed more markedly in steamed carrots with a shift towards yellow tones.

The effect of different cooking methods on frozen carrots is reported in Table 3. Frozen carrots were characterized by $L^* \approx 50$, $a^* \approx 19$ and $b^* \approx 29$ in the inner parenchyma and $L^* \approx 54$, $a^* \approx 31$ and $b^* \approx 41$ in the outer.

The cooking methods applied to process frozen carrots affected colour parameters in a very different way. Colour of the inner parenchyma was not significantly affected when boiling treatment was used to process samples (all parameters of F_B were comparable with F sample). All colour parameters of steamed frozen carrots resulted decreased if compared with frozen samples while microwave cooked frozen carrots (F_{MW}) had a higher lightness and a lower hue angle than frozen samples. The outer parenchyma of the frozen samples was more affected by the heat treatments than the inner portion, as found for raw samples. Cooking induced a decrease of colour parameters with steaming as the more detrimental process. In particular, ΔE values of the inner parenchyma indicated that the colour of boiled and microwave cooked carrots was perceptible different while a different colour was perceived in the case of steamed sample. The same differences between colour of cooked from frozen carrots and frozen carrots were also found for the outer parenchyma. Thus, steaming resulted the cooking method the affected the colour of frozen carrots in a more drastic way.

4. Conclusions

Summarizing obtained results, it seemed that steaming appeared to be detrimental for textural quality of carrots as also shown by the structural observations. If we compare cooked samples obtained from raw and frozen carrots, generally higher values of L^*, a^* and b^* can be observed in the cooked products from frozen than in those cooked from raw ones. A partial retention of colour was found if the cooking was carried out on frozen carrots, and in particular, when boiling and microwaving methods were used to process samples.

The impact of different cooking methods on carrot structure and quality is a topic not deeply in investigated in literature. Thus, the findings of this study could deep this research aspect with the ultimate goal to offer a higher quality final cooked product to the consumers, achieving a greater retention of the original quality of the processed vegetable.

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Captions for figures

Fig. 1 Transverse sections of carrot disks samples stained with PAS–Amido Black and Toluidine Blue: A. raw/uncooked outer parenchyma ; B. raw/uncooked inner parenchyma; C. boiled from raw; D. microwave cooked from raw; E microwave cooked from raw; F steamed from raw.

Fig. 2 Transverse sections of carrot disks samples stained with PAS–Amido Black and Toluidine Blue: A. frozen radial fractures; B. frozen tangential fractures; C. boiled from frozen ; D. microwave cooked from frozen; E microwave cooked from frozen; F steamed from frozen.

Fig. 3 Force (N) vs deformation (mm) curves obtained for cutting test A. Raw group B. Frozen group. Abbreviations: R (raw), R_B (raw boiled), R_S (raw steamed), R_{MW} (raw microwaved), F (frozen); F_B (frozen boiled), F_S (frozen steamed), F_{MW} (frozen microwaved).

Table 1. Texture parameters calculated from force-distance curves of raw, frozen and cooked carrot samples.[a]

	R	R_B	R_S	R_{MW}
Fmax (N)	71.8±9.4 a**	6.7±1.5 b**	4.4±0.8 b**	9.7±2.3 b
Area (Nmm)	163.0±24.7 a**	14.5±3.5 c**	12.2±1.4 c*	64.8±9.8 b**
Slope (Nmm^1)	31.5±9.7 a**	2.9±0.6 b**	2.1±0.2 b**	2.7±0.8 b*
	F	F_B	F_S	F_{MW}
Fmax (N)	47.6±5.6 a	4.4±0.6bc	2.8±0.6c	7.5±1.9b
Area (Nmm)	255.5±39.8a	22.8±2.0bc	15.0±1.9c	45.3±8.2b
Slope (Nmm^1)	16.9±2.6a	1.2±0.2b	1.0±0.3b	1.6±0.5b

[a] n=10, sample size =30. Means in row followed by different letters differed significantly for the same sample ($p < 0.05$). Means in column followed by single ($p<0.05$) or double ($p<0.01$) asterisks differed significantly among raw and frozen samples cooked with the same treatment.

Abbreviations: R (raw); R_B (raw boiled); R_S (raw steamed); R_{MW} (raw microwave cooked) F (frozen), F_B (frozen boiled), F_S (frozen steamed), F_{MW} (frozen microwave cooked).

Table 2. Colour parameters obtained on parenchyma tissue of raw and cooked carrot samples. [a]

Parenchyma	R	R_B	R_S	R_{MW}
Inner				
L*	50.4±2.4b	42.4±1.4d	46.8±1.7c	53.4±3.1a
a*	24.7±3.8a	14.6±1.5b	14.2±1.9b	15.2±2.7b
b*	29.5±3.7a	21.6±2.7b	24.6±3.1b	21.0±3.1b
C	38.5±4.7a	21.5±3.0c	28.9±3.3b	24.7±3.3bc
H°	49.6±2.4c	55.9±1.8ab	57.2±3.4a	53.8±3.4b
ΔE	-	15.5±2.6	11.9±2.6a	4.9±2.6
Outer				
L*	55.9±3.3a	47.0±1.0c	49.1±1.8c	52.1±1.9b
a*	35.7±1.6a	26.6±1.6b	20.6±2.9c	25.2±2.3b
b*	46.2±3.0a	39.8±2.8b	32.8±3.1c	31.0±3.1c
C	58.4±3.2a	47.9±2.8b	38.7±3.8c	40.0±4.3c
H°	52.3±0.9b	56.2±2.0a	57.9±2.7a	50.7±2.2b
ΔE	-	14.5±2.4	21.5±3.6	19.0±3.6

[a] n=3, sample size =15. Means in row followed by different letters differed significantly for the same sample ($p < 0.05$).

Abbreviations: R (raw); R_B (raw boiled); R_S (raw steamed); R_{MW} (raw microwave cooked).

Table 3. Colour parameters obtained on parenchyma tissue of frozen and cooked carrot samples. [a]

Parenchyma	F	F_B	F_S	F_{MW}
Inner				
L*	49.8±1.3b	49.6±1.6b	49.8±1.1b	52.7±2.8a
a*	19.2±4.1a	19.4±2.8a	14.5±2.1b	20.8±4.3a
b*	28.7±3.2a	25.9±4.0a	18.1±2.6b	25.1±4.0a
C	34.6±4.7a	32.4±4.7a	23.3±3.1b	32.3±3.1a
H°	56.5±3.4a	53.1±2.7ab	51.3±2.9b	50.5±2.7b
ΔE	-	5.4±1.6	11.6±2.9	8.1±2.5
Outer				
L*	53.5±0.7a	51.9±0.7b	49.7±1.5c	52.3±1.8ab
a*	30.7±1.3a	27.1±1.5b	23.4±2.6c	27.8±2.1b
b*	40.9±1.5a	36.3±2.6b	31.3±3.6c	35.4±2.3b
C	51.2±1.7a	45.3±2.0b	39.1±4.3c	44.9±3.1b
H°	53.2±1.2a	53.3±2.7a	53.1±1.8a	51.9±1.7a
ΔE	-	6.5±1.9	12.8±4.2	6.8±2.5

[a] n=3, sample size =15. Means in row followed by different letters differed significantly for the same sample ($p < 0.05$).

Abbreviations: F (frozen), F_B (frozen boiled), F_S (frozen steamed), F_{MW} (frozen microwaved).R (raw).

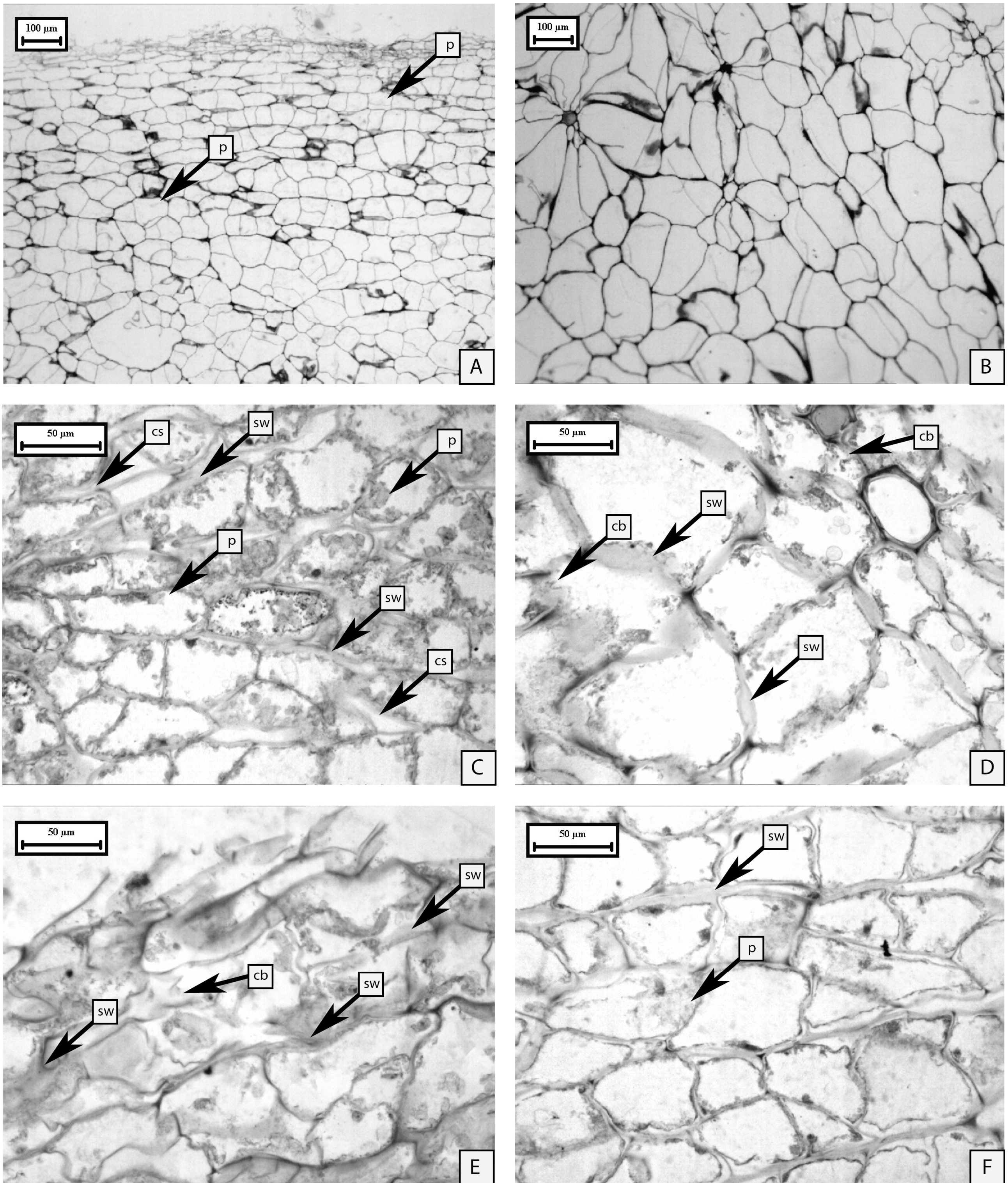

Fig.1

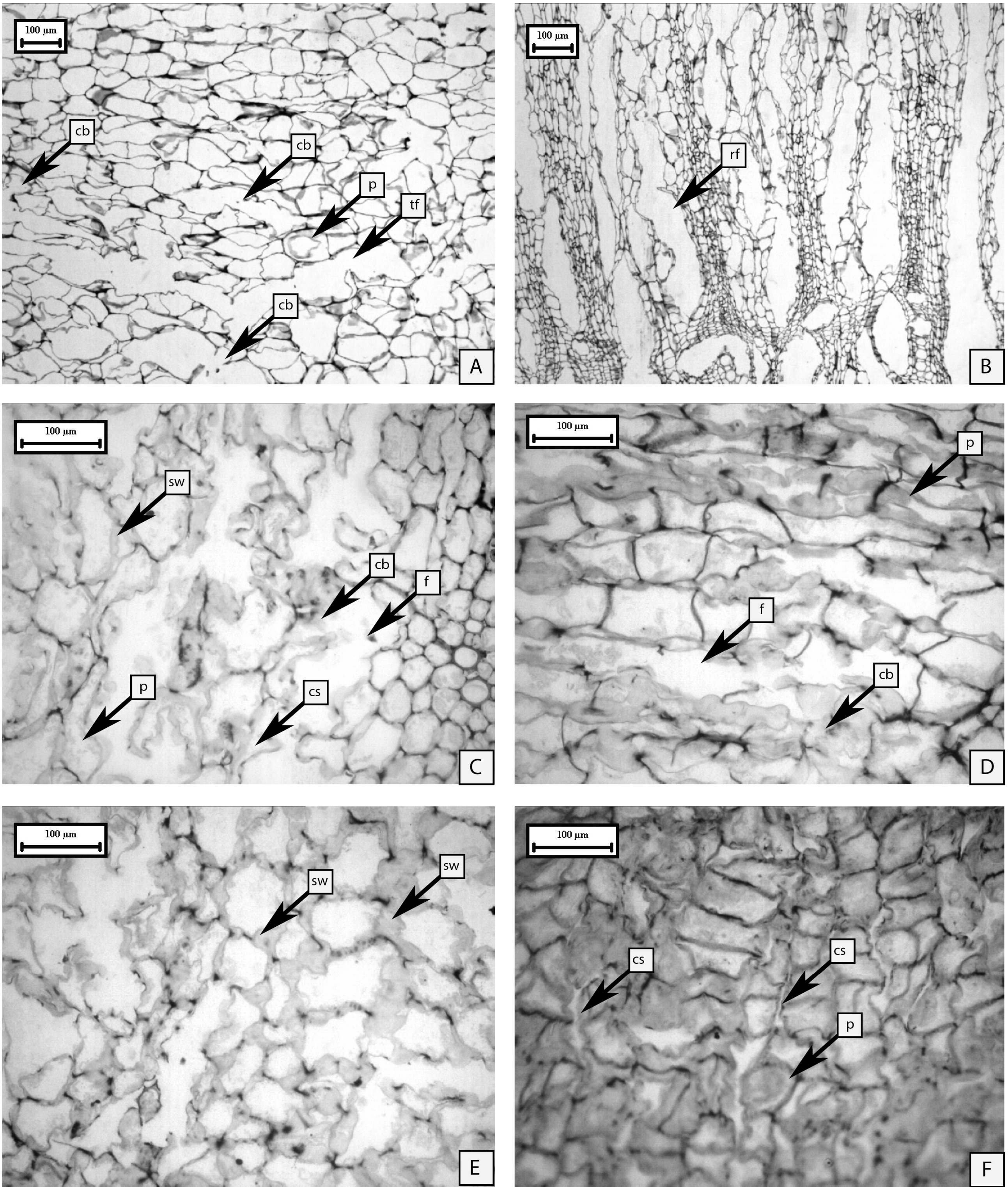

Fig.2

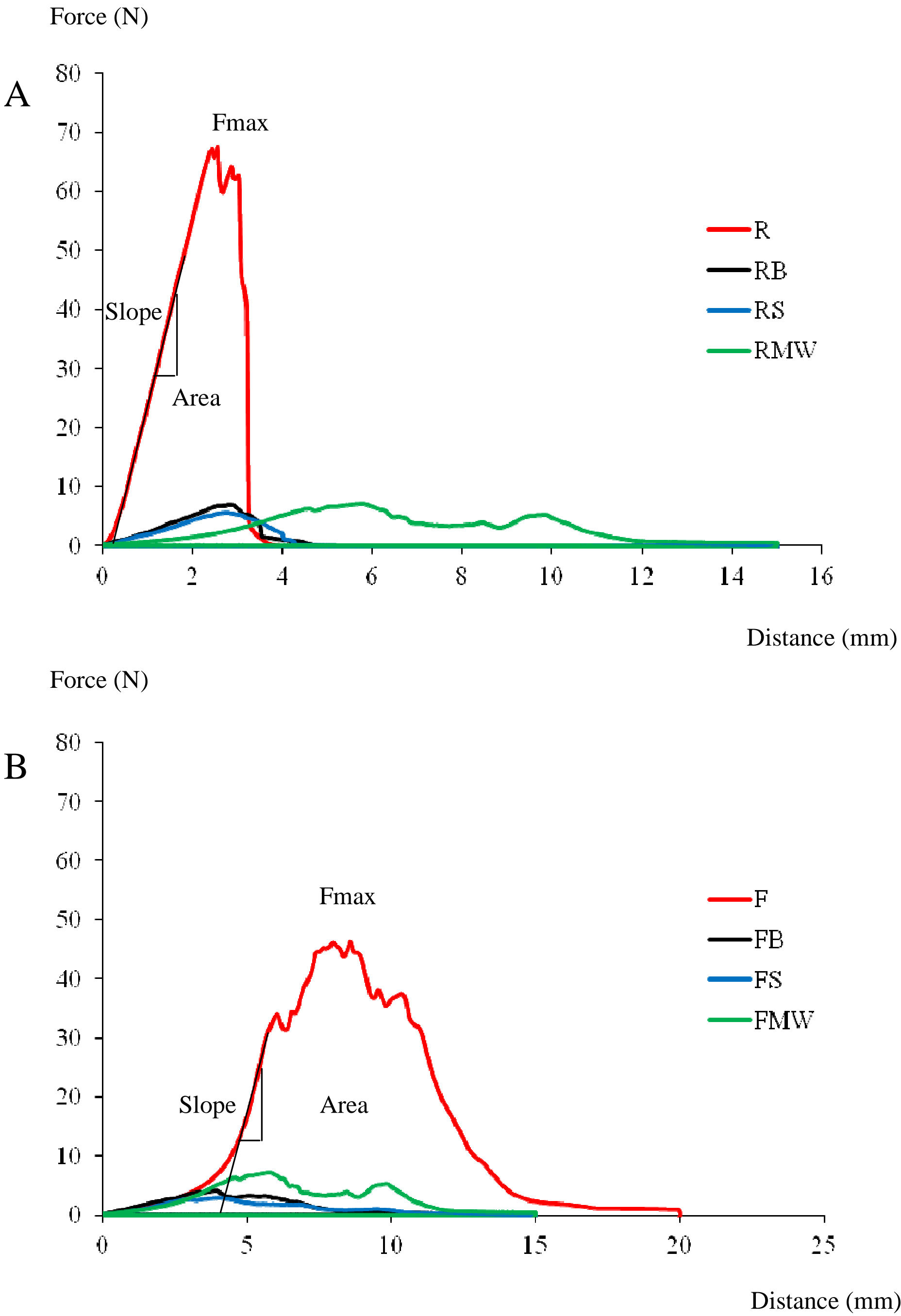

Fig. 3

Application of air/steam cooking on vegetables: impact on quality and nutrition

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Abstract

In this work, pumpkin and Brussels sprouts were subjected to an air/steam cooking under several time/temperature conditions. Changes in texture (cut force), colour indices, humidity and nutritional parameters (total phenols and antioxidant capacity by means of the FRAP test) were compared with a common steaming procedure. A different response to air/steam cooking was obtained for the two selected vegetables. In particular, Brussels sprouts showed higher degree of softening being also internally greener and loosing less polyphenols than steamed product although lower FRAP values were obtained. Otherwise, pumpkin air/steam cooked exhibited an equivalent softening degree and a higher nutritional quality than steamed vegetable but a marked loss of color (redness) was observed. These findings show that the selection of a proper time/temperature combination is essential for obtaining air/steam cooked vegetables with good qualitative and nutritional properties.

KEYWORDS: Cooking treatments; Steam; Vegetables; Texture ; Color; Cook value; Quality

1.Introduction

Vegetable consumption is recommended worldwide (USDA 2010; WHO 2008) as important elements of an healthy and balanced diet. They bring us vitamins, minerals and fiber, some energy (mainly in the form of sugar), as well as certain minor components, often referred to as phytochemicals or secondary plant products, which are potentially beneficial for our health (Krinsky & Johnson, 2005; Verkerk R. et al., 2009). Vegetables are consumed fresh or commonly cooked before being eaten both in the catering and food services industries and in private homes. Cooking is an indispensable prerequisite in order to obtain safe and high-quality food products, and due to chemical reactions during cooking, they are much more digestible. However, cooking can profoundly affect both the organoleptic and nutritional value of vegetables (Canet et al., 2004; Dini et al., 2013).

Several cooking technologies are now available in catering, food service, and domestic kitchens to be coupled with those conventionally applied. A comparison of the influence of cooking methods on the retention of nutrients and phytochemicals help the catering industry and food services, as well as the individual consumer, to select the most suitable cooking method. Several studies were conducted to compare different cooking methods on vegetables, showing advantages and disadvantages of the various techniques. Miglio et al. (2008) founded an overall increase of the antioxidant activity on carrots, courgettes, and broccoli after boiling, steaming, and frying with better texture retention for steamed vegetables and limited discoloration for boiled ones. Pellegrini et al. (2010) reported better retention of phytochemicals and total antioxidant activity for fresh Brassica vegetables than for frozen ones after boiling, microwaving or steaming, founding also in microwave cooking the best methods for the color preservation. De Silva et al. (2013) reported a global discoloration of pumpkins after cooking with an increasing of carotenoids after microwaves or boiling and a reduction after steaming or sous vide cooking. Rinaldi et al. (2012) found firmer sous-vide cooked carrots and Brussels sprouts than those steamed, with better retention of the volatile compounds. Less debated in literature is the use of the combined steam-convection oven for

cooking vegetables. Despite different origins and basic characteristics, this ovens have, according to the manufacturers and users, two main common features: high retention of the food nutritional value and an attractive sensory properties. Additionally, operators reported improved yields, lower consumption of water and energy, and simple and safe cooking operations (Danowska-Oziewicz et al., 2007). Several studies were conducted on the use of the steam-convection oven on meat products (Vittadini et al. 2005, Chiavaro et al.,2009; Mora et al., 2011). According to the best knowledge of the authors a single study in this field was conducted on vegetables (Chiavaro et al., 2006), in which potatoes samples were cooked by four combined air-steamed treatments in comparison with the traditional steaming and force convection.

To improve the knowledge on this technique and provide a scientific support to the food business operators, the aim of this work was the evaluation of the impact of different time-temperature combined air-steam cooking practices on the physicochemical parameters of pumpkins and Brussels sprouts in comparison with a traditional steam cooking.

2. Material and methods

2.1. *Samples*

Brussels sprouts (*Brassica oleracea* cv. gemmifera) and pumpkins (*Cucurbita moschata*) were selected for this investigations on the base of their seasonality, nutritional values and difference in physical characteristics. The two vegetables were purchased from a local market belonging to the same lot. The samples were removed from their non-edible parts and cut in small regular pieces for cooking and next analysis. Brussels sprouts were sheared and private of the stems; pumpkins were cut in 1.5 mm side cubes.

2.2 *Cooking conditions*

The vegetables were cooked by combined air-steam cooking in a Combi-Steam SL (V-Zug, Zurich, CH) oven. The samples steamed in the same oven were used as control.

Preliminary tests were carried out on the two vegetables to select the appropriate time/temperature range. The selected conditions with the appropriate abbreviations used in the remaining of the text are reported in Table 1.

Ten samples were cooked and analyzed for each condition. Three repetitions for each treatments were performed.

2.3 Degree of cooking determination

The degree of cooking (C_{Tref}^{z}) at the thermal centre of each sample was obtained from the integration of the heat penetration curves according to Chiavaro et al. (2006). The temperature in the geometric centre of the samples was measured using wire thermocouples K type (Ni/Cr–Ni/Al) (HF/D-30-KK) connected with a multimeter/data acquisition system (Keithley Instruments Inc., Cleveland, OH, US) and registered every 20 seconds. Taking into consideration only the heating phase of the cooking treatments, the cook value was calculated using the following equation:

$$C_{Tref}^{z} = \int_{0}^{z} 10^{(T-Tref/z)} dt$$

where t=time; Tref=reference temperature, set equal to 100 °C; z=temperature increase that induces a 10-fold increase of the reaction rate of the chemical reaction taken as reference, z was set at 33 °C, as reported by Chiavaro et al. (2005).

2.4 Weight loss determination

Weight loss determination was calculated as the percent weight difference between the raw and cooked samples. The weights of the raw samples were measured immediately before cooking. Three determinations were performed for each cooking treatment.

2.5 Moisture content determination

For moisture determination, 3–4 g of uncooked or cooked homogenised sample (as triplicate) was dried in a convection oven at 105°C for at least 16 h until reaching constant weight according to AOAC method (2002).

2.6 Relative humidity calculation

The % RH of the air within the oven cavity was calculated during the heating phase, as described by Barnes E. in http://www.uswcl.ars.ag.gov/exper/relhum.htm.

2.7 Texture analysis

Texture was measured by means of cut and TPA tests using a TA.XT2i Texture Analyzer equipped with a 245.2 N load cell (Stable Micro Systems, Godalming, UK), a force resolution equal to 0.01 N and an accuracy value of 0.025%. The parameters were quantified using the application software provided (Texture Expert for Windows, version 1.22).

Cut test was performed using a 3 mm thick stainless steel Warner-Bratzler blade driven through vegetable, positioned on a slot surface, at a speed of 1 mm/s. Shearing force (Fmax, N) and shearing work, (Awork, Ns) were obtained from the force-time curves.

Cut tests was conducted for each trial. The data were collected for five replicates.

2.8 Colour analysis

Colour determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65. Both raw and cooked samples were analyzed. For Brussels sprout the assessments were carried out both on the external and internal surfaces, for pumpkin only on the external face. The instrument was calibrated using a white colour tile standard. L* (lightness), a* (redness), b* (yellowness), C (chroma, 0 at the center of the colour sphere), and hue° (hue angle, red = 0°, yellow = 90°, green = 180°, blue = 270°) were quantified on each sample using a 10° position of the standard observer (CIE, Paris, France, 1978). ΔE values were calculated in comparison to the raw samples. A total of 5 determinations was performed for each cooking treatment.

2.9 FRAP and total phenolic content

The method of Benzie & Strain (1996) was used for the antioxidant power determination. The antioxidant activity was expressed as mmol Fe^{2+}/100g dw and calculated plotting the absorbance values in a standard calibration curve of $FeSO_4$ in FRAP solution.

For the extraction of phenols, 1g of each cooked vegetable was finely grounded and mixed with 10 ml of a water/methanol solution in a ratio 70:30 and brought to a boil on a hotplate for 15 min. The suspension was then filtered using whatman filter paper and the filtered solution was used for the analysis. The total phenolic quantification was conducted on the extract, properly diluted with distilled water, by the Folin–Ciocalteu assay, according to the method of Spanos & Wrolstad (1990). The results were expressed as mg of gallic acid/kg dw based on a calibration curve prepared using standard solution of gallic acid (Acros Organics, NJ, USA).

Three replicates were analysed for each sample.

2.12 Statistical analysis

SPSS statistical software (Version 20.0, SPSS Inc., Chicago, IL, USA) was used to perform an one-way analysis of variance (ANOVA). Tukey post hoc test at a 95% confidence level ($p \leq 0.05$) was performed to identify differences among groups. A t-test ($p \leq 0.05$)was performed to compare each air-steam cooked sample and the control. Pearson correlation coefficients were calculated among the measured variables at a 95% confidence ($p \leq 0.05$).

3. Results and Discussion

3.1 Cooking parameters

The values of relative humidity (RH%) measured into the oven cavity, for the different cooking treatments, resulted to be 97.6%±0.8, 95.5%±0.8 and 85.7%±0.9 for 90, 100 and 110°C respectively, showing how, increasing the cooking temperature, the RH% decreased.

Steaming, for Brussels sprouts, showed not only a significant lower weight loss than the other cooked samples, but even an increase of the weight (+3.5%) after cooking because of the absorption of the condensed water. The 90°C air/steamed cooked samples presented also an increase of the weight after cooking, but in a less extent in comparison to the S ones. Chiavaro et al. (2006) reported lower weight loss for steamed potatoes, because of the steam saturation of the oven chamber that minimised evaporative heat loss. For the air-steam cooked samples, the lower % RH

and the mechanical convection of the air in the oven exercised a drying effect. A strong positive correlation was found between weight loss and moisture content ($p \leq 0.01$ R=0.95), showing how the samples weight is largely ascribable to the presence of water.

An high positive correlation was also found between weight loss and degree of cooking ($p \leq 0.01$ R= 0.716). The degree of cooking, allows to assess the extent of damage suffered by the food during a thermal process. The registered values for Brussels sprouts are reported in Fig.1A. It's evident how the values increased approximately linearly with increasing cooking time, resulting in all cases higher at the same time with increasing temperatures. The degree of cooking of the control samples resulted to be an intermediate value between the stronger and milder cooking conditions adopted in this study, even if the cooking time was lower, because of the fast temperature increase induced by the condensation steam in the oven chamber.

A non linear trend of weight loss for pumpkins, after the different cooking treatments, was observed. The lowest values (-2.1%) were however measured for the S sample, as for Brussels sprouts. An high positive correlation ($p \leq 0.01$ R=0.95), also in the case of pumpkins, between weight loss and moisture content was found.

No correlation between weight loss and degree of cooking was found for pumpkins. An high dependence from time and temperature was however observed (Fig.1B). The S control sample resulted to have intermediary degree of cooking between the extreme cooking conditions selected in this study. In comparison to the sample cooked with the same time and temperature conditions with the air/steam method, the steamed one resulted to have slight higher degree of cooking.

3.2 Physical analysis

Texture and color

Cut test results obtained for raw and cooked Brussels sprouts and pumpkin are reported in Table 2. For both the vegetables, a significant decrease of hardness and work were observed in comparison to the raw samples in all the studied conditions. Also the control steamed samples resulted, for both the vegetables, softer than the raw, and according to the time/temperature combinations, also softer

than the air/steam cooked samples. The softening effect of vegetables during cooking was already reported by several authors for different cooking techniques (Miglio et al., 2008; Paciulli et al., 2013) related to the tissue damages occurring because of the heat.

For Brussels sprouts, during the air/steam cooking treatment a significant drop of Fmax was observed increasing the temperature from 90°C to 100°C; no significant differences were observed between 100°C and 1110°C. The texture loss observed with the increasing of the treatment strength could be associated to the enhanced heat mediated tissue damages, which involved the cell walls components (Paciulli et al., 2013).No linear trend were instead observed according to the cooking time. Looking at the Area values (Tab.2), linear changing were observed according to the temperature, but with different trend from the max force. Significant decreases passing from 90°C to 100°C was observed and further increasing passing from 100°C to 110°C, except for the C_40'100C° sample. The increased area value with the increase of temperature, could be associated to an extended dehydration of the samples, as confirmed by the water content (data not shown), due to the lower RH% in the oven chamber. Comparing the air/steamed cooked samples with the steam control, significant reduction of Fmax and Area were observed for the samples treated at 100°C and 110°C probably because of the lower RH% in the oven chamber that allows a cellular dehydration and loss of cellular turgor pressure (Greve et al., 1994). This was confirmed by the water content and weight loss values that showed high correlation with the Fmax ($p \leq 0.01$ R=0.52; R=0.55). Fmax and the degree of cooking were found to be highly correlated ($p \leq 0.01$ R=-0.74), showing how all the three parameters could be used to predict the vegetables texture softening after cooking.

Observing the pumpkins results, more evident changes than Brussels sprouts were observed, because of the different structure. Time and temperature of treatment resulted both significant ($p<0.05$) for the changes of Fmax, a progressive softening was observed increasing the strength of the treatment, showing however a negative correlation with the weight loss ($p \leq 0.05$ R= -0.37). Comparing the air/steam cooking treatment with the control steam one, a significant difference ($p<0.01$) was observed for almost all the studied conditions. Higher hardness were found for all the

three 90°C treatments, because of the lowest temperature, and for the C_5' 100°C, because of the lowest time. All the other samples were softer than the control. Significant differences were also found for almost all the sample in comparison to the control. Higher values than the control were found for all the 90°C cooked pumpkins and the C_5' 100°C; lower values for all the other cooked samples were found. A very similar trend was observed for the Area values.

In Table 3 the color parameters observed in the outer and inner surface of the raw and cooked Brussels sprouts are reported. Focusing on the external surface, the initially negative a* values (greenness) tend to increase for all the conditions. More important changes were observed passing from 25' to 32' except for the samples treated at 110°C, in which it was more consistent the change between 32' and 40'. The reduction of H° values were also observed for all the samples in comparison to the raw in the external surface, showing a shift to the yellow tones. A dependence from both time and temperature was observed in the air/steam cooking samples, showing progressive decrement. This changes, together with the global but non linear reduction of L*, b* and C showed darker color. The control steam sample resulted more green than the air/steam cooked samples as revealed from the significant lower a* and higher H° values. ΔE didn’t gave important information being for the control sample in an intermediate level with the others. It has been reported that green vegetables subjected to a heat treatment often take on a coloration by olive-brown shades. This change, which is undesirable by the consumer, is attributable to the conversion of chlorophyll to pheophytin (Turkmen et al., 2006). Similar changes were stated in a recent paper on the same vegetable (Pellegrini et al., 2010) in relation to the presence of chlorophyll degradation compounds, presenting darker green color.

In the inner surface the opposite phenomenon was observed: an initially positive a* value of the raw sample tends to decrease, showing an intensification of the index of green in a non linear way according to time or temperature. Also the H° shifted to higher values showing an increase greenness. The higher exposition to the oxygen than the internal surface, together with an increased warming, probably resulted in a higher color loss for the external portion of the sample.

In Table 4, the color parameters registered for raw and cooked pumpkins are reported. The orange color of pumpkins is well described from the positive a* parameter (redness). The a* value decreased for all the air/steam cooked samples in comparison to the raw in a linear way according to the time of exposition. This trend indicated a discoloration of the sample. A general but non linear reduction of the L values was also registered. H° was almost unchanged during the treatments, with values around 60, represented orange color. For the steamed samples, significant higher a* values were instead observed in comparison to the air/steamed samples and with a less extent also from the raw. The orange color of pumpkin is the result of the presence of α-carotene and β-carotene (Koch and Goldman, 2005), of which the pumpkin is rich (β-carotene 10-2 g/kg and α-carotene 10-2 g/kg (Silva et al., 2013). The increased a* value for the steam samples could be related to an extended extractability of these molecules. The reduction in the air/steam cooked samples suggest instead an extended oxidation.

3.4 Chemical analysis

FRAP and total phenolic content

The factors that most influence the contents of antioxidants during cooking are substantially two: the disruptive effect of cooking on the plant tissue (Pellegrini et al., 2010) and the oxidation (Loizzo et al., 2013). The first is an important variable that can positively influence the final TAC value. Several studies underline as the thermal treatments can degrade the cell walls polysaccharide fibers with consequent release of intracellular components (Dewanto et al., 2002). In this research the air/steam cooking treatment resulted (Tab.5), on Brussels sprouts, in a total antioxidant activity, measured by the FRAP assay, reduced in comparison to the raw sample. Among the air/ steam cooked samples it resulted enhanced passing from 90 to 100°C for all the three selected cooking times. This phenomena may be attributable to a good release of molecules with antioxidant properties from the damaged cells as the high drop of hardness between 90 and 100°C in the texture analysis demonstrates. A further drop of the antioxidant activity was observed (Tab.5) passing from 100 to 110°C with the exception of the samples cooked at 110°C for 32 minutes. They resulted to

have, together with the C_32'100°C, the highest antioxidant activity (4.8 mmol (Fe^{2+})/100g sample dw). Steaming showed significantly ($p<0.01$) higher antioxidant capacity than the air/steam cooking treatment (7.8 mmol (Fe^{2+})/100g sample dw), and also higher than the raw ones. Similar results were found previously by Pellegrini et al. (2010) and Chiavaro et al. (2012) on steamed Brussels sprouts reporting a total antioxidant activity of 6.4 e 6.18 mmol (Fe^{2+})/100g sample dw respectively.

The analysis of pumpkins reported that the total antioxidant capacity was lower than that found in Brussels sprouts. In comparison to the raw sample, the antioxidant activity of some air/steam cooked samples resulted increased (Tab.6) without any linear correlation with time or temperature. A common trend of increased antioxidant activity was observed passing from 5 to 10 minutes of cooking at the same temperature (Tab.5), with the only further decrement observed at 100°C passing from 10 to 15 minutes cooking. A general, but non linear, trend of increasing antioxidant activity was also observed increasing the temperature of treatment. In the present research the highest antioxidant activity of the combi steamed sample was found in vegetables cooked at 110°C for 15 minutes (1.2 mmol (Fe2+)/100g dw). However, the best results was reported by the steamed control with 1.7 mmol (Fe2+)/100g dw. Increases of the total antioxidant capacity were also shown by Dini et al., (2013) after steaming of pumpkins with a TAC changing from a value of 0.4 to 1.5 mmol (Fe2+)/100g dw for raw and steamed samples respectively. The antioxidant activity in pumpkins may be also related to the presence of carotenoids; the drop of the antioxidant activity The reduction observed in some samples could be justified with the assertion of Dini et al. (2013). They suggested that during baking the production of radical scavenger molecules can occur. Moreover, during cooking the severing of ties between the vegetable matrix and carotenoid-protein increase their availability to the reactions (Kaur and Kapoor, 2001).

Thermal treatments may have a detrimental effect on the bioactive compounds present in vegetables reducing the content of phytochemicals (Roy et al. 2009). In the present research the total phenol content of Brussels sprouts resulted affected from the air/steam cooking in comparison to the raw

samples, even if good retention were observed for some treatments (Tab.5) in a non-linear way with time or temperature. For Brussels sprouts, at the temperature of 90 and 100°C was registered an increase of the total phenols with the increasing of the time of treatment. This trend was more evident at the temperature of 90°C reaching 2452.4 mg gallic acid/kg of sample dw after 40'. This results could be the consequence of the release of phenols from the vegetables matrix (Pellegrini et al., 2010) as also suggested by the texture softening reported above. A correlation between the texture softening and the increased release of antioxidant molecules was already suggested by Paciulli et al., (2014). At temperature of 110°C a general decrease of total phenols was registered (Tab.5) and this could be the consequence of the effect of temperature on the breakdown (Cacace et al., 2003) and oxidation of phenolic substances (Loizzo et al., 2013).

According to Wachtel-Galor et al., (2008), steaming resulted the method that better retained the total phenols in brassica vegetables if compared with the other cooking methods. In this study, instead, steamed Broussels sprouts control reported an intermediate phenol content (1647.2 mg of gallic acid/kg of sample dw) in comparison with the other cooking methods (Table 6).

Focusing on pumpkins, an increase of phenols extraction after cooking in comparison to the raw sample was observed (Tab.5). The increase of temperature and time during the combi air-steam cooking treatments matches the increase of polyphenols reaching values around 170.0 mg/kg at 110°C, both for 10 and 15 minutes. This was presumably due to a greater extraction of the molecules from the damaged cells as suggested before. An exception was observed (Tab.5) for the samples cooked for 5' in which a nonlinear trend was observed with the increasing of temperature. In this study steamed pumpkins showed significant higher ($p<0.01$) values if compared with the combi air-steamed vegetables (198.4 mg gallic acid/kg of sample dw). It could be hypothesized that a lower oxidative polyphenols degradation occurred during the steaming treatment.

Conclusions

The application of air/steam cooking under suitable time/temperature conditions enhanced the

nutritional and textural quality of the tested vegetables. In particular, Brussels sprouts showed a good response at 90° of cooking for texture, with a good enhancement of the total phenol content at 40 min resulting better than the control and being internally greener. A good preservation of the pumpkin color was observed with the air/steam treatment, preserving better the texture when cooked for 5'. Thus, the combined air/steam cooking could represent a valid alternative to the traditional steaming, being faster and producing vegetables with good sensorial and nutritional quality under the correct combination of time and temperature conditions.

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Caption for figures:

Fig.1: Degree of cooking obtained in the different time/ temperature cooking conditions on Brussels sprouts (A) and pumpkin (B) for the air/steam treatment and in comparison to the steamed control.

Tab. 1 Cooking conditions for Brussels sprouts and pumkins

Brussels sprouts	Time (min)	Temperature (°C)
S_17' 100°C	17	100
C_25' 90°C	25	90
C_32' 90°C	32	90
C_40' 90°C	40	90
C_25' 100°C	25	100
C_32' 100°C	32	100
C_40' 100°C	40	100
C_25' 110°C	25	110
C_32' 110°C	32	110
C_40' 110°C	40	110
Pumpkins	Time (min)	Temperature (°C)
S_10' 100°C	10	100
C_5' 90°C	5	90
C_10' 90°C	10	90
C_15' 90°C	15	90
C_5' 100°C	5	100
C_10' 100°C	10	100
C_15' 100°C	15	100
C_5' 110°C	5	110
C_10' 110°C	10	110
C_15' 110°C	15	110

Tab.2 Texture parameters for raw and cooked Brussels sprouts and pumpkin.

Brussels sprouts	Fmax (N)	Area (Ns)
Raw	78.0±9.6	1648.9±39.4
S_17' 100°C	25.8±2.8	487.6±71.0
C_25' 90°C	33.5±4.7aA*	552.5±46.5aA
C_32' 90°C	30.7±5.6abA	552.3±38.4aA
C_40' 90°C	23.6±4.8bA	442.3±52.5bA
C_25' 100°C	12.2±1.7aB**	238.0±22.2aC**
C_32' 100°C	8.9±1.7bB**	172.8±19.9bC**
C_40' 100°C	11.3±1.5abB**	270.3±25.5aB**
C_25' 110°C	13.8±1.9aB**	371.6±74.7aB
C_32' 110°C	9.8±0.9bB**	224.8±13.2bB**
C_40' 110°C	11.9±2.3abB**	271.0±39.2bB**
Pumpkins	Fmax (N)	Area (Ns)
Raw	201.9±1.5	1647.1±4.1
S_10' 100°C	9.3±1.0	34.8±2.0
C_5' 90°C	137.1±2.5aA**	850.2±29.0aA**
C_10' 90°C	14.9±0.5bA**	66.9±2.7bA**
C_15' 90°C	10.8±0.3cA	38.4±0.5bA*
C_5' 100°C	12.6±0.4aB**	67.6±1.2aB**
C_10' 100°C	6.6±0.2bB*	36.5±3.3bB
C_15' 100°C	4.3±0.1cB**	19.7±0.6cB**
C_5' 110°C	9.3±0.7aC	54.1±3.1aB**
C_10' 110°C	4.2±0.6bC**	25.8±1.1bC**
C-15-110°C	2.5±0.3cC**	12.8±0.4cC**

Values are reported as means ± standard deviations of five replicates. Different small letters in the same column and same temperature are statistically different ($p<0.05$). Different capitol letters in the same column and same time are statistically different ($p<0.05$).

Means followed by * or ** are statistically different ($p<0.05$; $p<0.01$) from the control (Steamed).

Tab.3 Brussels sprouts color parameters

External	L	a*	b*	C	h°	ΔE
Raw	58.2±3.6	-9.2±1.6	26.3±5.5	27.9±5.7	109.5±1.5	-
S_17' 100°C	54.0 ± 3.3	-1.6±0.1	14.7±2.9	14.8±2.9	96.2±1.4	14.8±2.8
C_25' 90°C	57.0±1.7aA	-1.0±0.2bB*	10.3±0.6 bC	10.4±0.6cB	95.5±1.2 aA	18.1±0.6
C_32' 90°C	55.3±2.6aB	1.2±0.2aA**	28.9±1.2aA**	28.9±1.2aA**	89.1±1.5bA**	11.3±0.7
C_40' 90°C	47.4±2.4bB*	1.6±0.3aB**	19.2±4.2 bB	19.2±4.2 bB	86.7±2.0 bA**	17.3±1.9
C_25' 100°C	45.2±2.2cC*	0.8±0.1bA**	15.9±3.4 bB	17.9±2.3bA	88.4±3.5aB**	19.6±2.9
C_32' 100°C	61.4±3.2aA*	1.5±0.3aA**	21.4±3.8abB	22.0±3.5abB	87.7±4.2 aA**	12.9±1.8
C_40' 100°C	54.9±3.2bA	1.5±0.3aB**	25.9±2.6 aA*	25.5±2.8 aA*	87.6±2.2 aA**	11.8±1.3
C_25' 110°C	52.7±2.2aB	1.0±0.2bA**	20.3±2.2 aA	20.3±2.2aA	87.2±0.1aB**	13.3±1.1
C_32' 110°C	52.4±2.0aB	1.5±0.3bA**	16.9±1.3abB	16.9±1.3abC	84.9±0.9 aA**	15.5±1.1
C_40' 110°C	54.3±1.0aA	2.5±0.5aA**	16.0±1.9 bB	16.2±1.9 bB	81.1±2.4 bB**	16.1±1.2
Internal	**L**	**a***	**b***	**C**	**h°**	**ΔE**
Raw	81.1±1.1	3.2±0.5	30.6±6.0	30.8±5.9	83.7±2.3	-
S_17' 100°C	67.6±2.9	-2.0±0.4	18.5±3.3	18.6±3.3	96.4±2.0	19.0±3.3
C_25' 90°C	69.5±6.2aA	-0.7±0.1aA**	20.2±3.9aA	20.3±3.9aA	93.6±1.8aA	16.3±6.5
C_32' 90°C	70.3±1.1aA	-1.5±0.1bB	18.9±0.4aB	18.9±0.4aB	94.7±0.4aAB	16.6±0.8
C_40' 90°C	68.2±5.8aA	-1.6±0.3bB	23.4±1.1aA	23.4±1.1aA	94.0±0.8aA	15.9±4.4
C_25' 100°C	69.5±3.7aA	-1.9±0.3bB	19.8±2.6bA	19.8±2.6bA	95.4±0.8aA	16.8±4.0
C_32' 100°C	70.9±2.2aA	-0.5±0.1aA**	25.4±2.0aA*	25.5±2.0aA*	91.9±1.8bB*	12.1±2.4
C_40' 100°C	71.3±2.2aA	-1.5±0.3bB	22.6±2.7abA	22.6±2.7abA	93.7±1.1abA	13.6±2.5
C_25' 110°C	71.8±1.9aA	-2.3±0.3bB	22.2±2.1bA	22.3±2.1aA	95.9±1.1aA	13.8±2.4
C_32' 110°C	69.3±1.3aA	-1.9±0.4bB	18.9±2.4bB	19.0±2.4aB	95.4±2.3abA	17.5±2.1
C_40' 110°C	67.3±3.9aA	-0.9±0.2aA*	19.9±4.3bA	20.0±4.3aA	92.5±0.7bA*	18.0±5.1

Values are reported as means ± standard deviations of five replicates. Same small letters in the same column and same temperature are statistically different ($p<0.05$). Same capitol letters in the same column and same time are statistically different ($p<0.05$). Means followed by * or ** are statistically different ($p<0.05$; $p<0.01$) from the control (Steamed). ΔE is calculated in comparison to the raw sample.

Tab.4 Pumpkin color parameters

	L	a*	b*	C	h°	ΔE
Raw	67.7±1.6	29.9±3.2	41.6±4.6	51.2±5.6	54.3±0.6	-
S_10' 100°C	56.3±1.0	31.3±0.2	49.4±2.1	58.5±1.8	57.6±1.1	14.1±0.5
C_5' 90°C	34.1±2.0C b**	26.2±2.3 aB*	45.4±1.6 aA	52.4±2.5 aA*	60.1±1.5 aA	34.2±2.0
C_10' 90°C	59.5±3.1 aA	22.1±2.3 aA**	36.7±2.7 bA**	42.9±2.3 bA**	58.9±3.7 aA	12.7±3.2
C_15' 90°C	42.5±2.2 bA**	14.8±2.4 bB**	17.0±1.3 cC**	22.6±1.5 cC**	49.1±5.9 bB	38.5±0.7
C_5' 100°C	53.8±3.8 bA	26.6±2.8 aB*	34.6±2.7 aB**	43.7±3.5 aB**	52.5±2.3 bB*	16.0±5.0
C_10' 100°C	61.3±1.4 aA*	25.5±2.5 aA*	41.9±5.8 aA	49.0±6.1 aA	58.6±1.8 abA	9.4±2.4
C_15' 100°C	49.6±1.2 bA**	19.3±1.7 bA**	33.5±7.4 aB*	38.8±7.0 aB*	59.6±4.5 aA	23.3±3.6
C_5' 110°C	57.5±2.1 aA	33.2±1.2 aA	41.9±5.3 aA	53.5±3.7 aA	51.4±4.3 bB	11.7±2.4
C_10' 110°C	58.1±3.0 aA	22.4±3.9 bA*	43.3±4.5 aA	48.9±3.7 aA*	62.5±5.7 aA	13.1±4.1
C-15-110°C	45.6±6.2 bA*	20.9±1.5 bA**	43.5±3.4 aA	48.2±3.5 aA*	64.2±1.3 aA**	24.2±6.1

Values are reported as means ± standard deviations of five replicates. Different small letters in the same column and same temperature are statistically different ($p<0.05$). Different capitol letters in the same column and same time are statistically different ($p<0.05$). Means followed by * or ** are statistically different ($p<0.05$; $p<0.01$) from the control (Steamed). ΔE is calculated in comparison to the raw sample.

Tab.5 Chemical parameters of Brussels sprouts and pumpkin

Brussels sprouts	FRAP	Total phenols
Raw	5.4±0.3	2745.7±2.6
S_17 100°C	7.8±0.3	1647.2±5.4
C_25 90°C	2.9±0.2 a B **	628.0±2.7 c C **
C_32 90°C	2.2±0.2 b B **	1927.3±7.1 bA **
C_40 90°C	2.3±0.2 b B **	2452.4±27.2aA**
C_25 100°C	3.5±0.2 b A **	1246.8±12.3 cB**
C_32 100°C	4.8±0.3 a A **	1311.7±6.6 bB**
C_40 100°C	4.6±0.6 a A **	2098.4±2.5 aB**
C_25 110°C	2.9±0.2 b B **	1854.7±2.3 aA**
C_32 110°C	4.8±0.4 a A**	813.7±2.1 cC**
C_40 110°C	2.8±0.2 b B **	1485.8±3.5 bC**
Pumpkin	FRAP	Total phenols
Raw	0.8±0.0	0.0
S_10' 100°C	1.7±0.0	198.4±2.2
C_5' 90°C	0.5±0.0 b C **	17.6±1.7 b C **
C_10' 90°C	0.9±0.0 a B **	21.8±2.1 b C **
C_15' 90°C	0.8±0.1 a B **	39.6±3.5 a C **
C_5' 100°C	1.0±0.1 a A **	114.2±2.2 c A **
C_10' 100°C	1.0±0.1 a AB **	131.3±2.2 b B **
C_15' 100°C	0.6±0.0 b C **	141.0±2.0 a B **
C_5' 110°C	0.7±0.0 b B **	70.9±1.6 b B **
C_10' 110°C	1.1±0.1 a A **	171.2±1.7 a A**
C-15-110°C	1.2±0.1 a A *	170.8±1.6 a A **

Values are reported as means ± standard deviations of three replicates. Different small letters in the same column fot the same temperature are statistically different ($p<0.05$). Different capitol letters in the same column for the same time statistically different ($p<0.05$). Means followed by * or ** are statistically different ($p<0.05$; $p<0.01$) from the control (Steamed). FRAP is expressed as mmol (Fe^{2+})/100g dry weight of the cooked pumpkins; total phenols are expressed gallic acid equivalent mg/kg of dry weight of the cooked pumpkins.

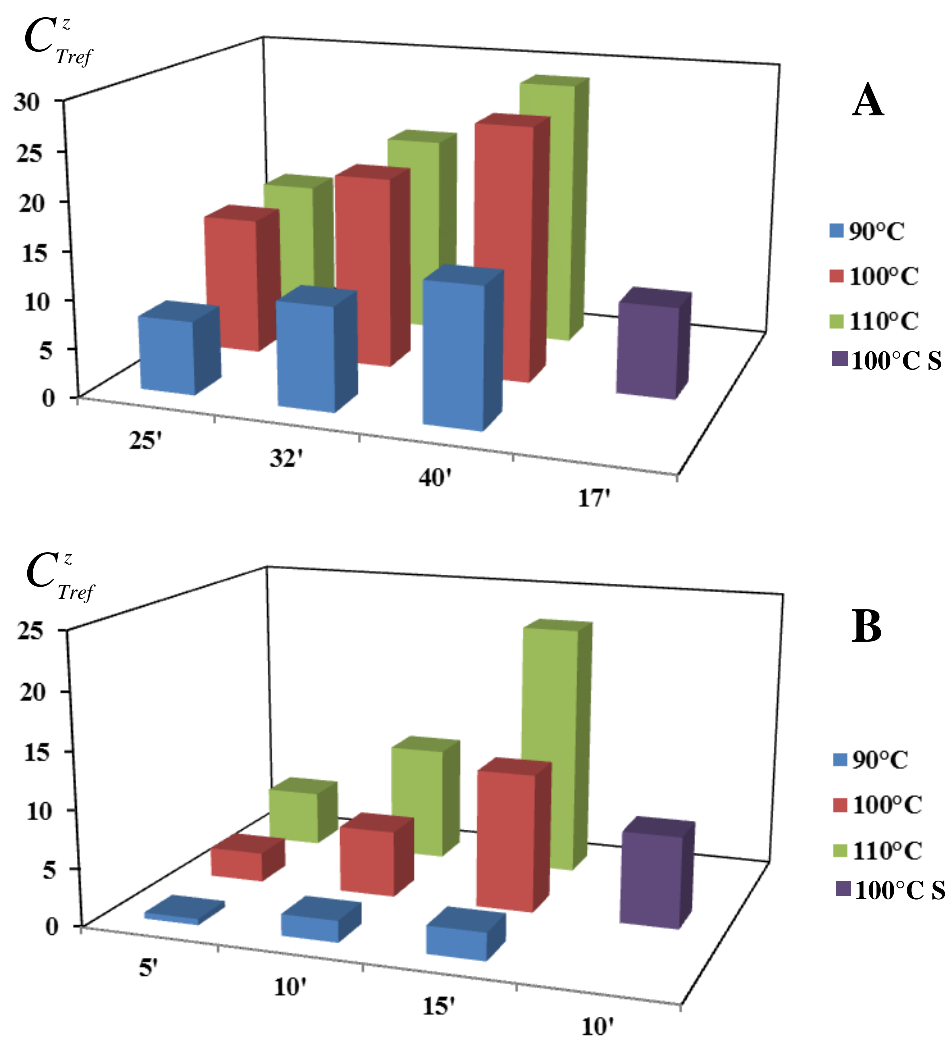

Fig.1

Section III

Effects of high pressure processing on quality parameters of beetroot (*Beta vulgaris L.)*

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Abstract

A pressure treatment of 650 MPa at different processing times (3, 7 15 and 30 min) was applied on beetroot slices (var. *red cloud*) in comparison with thermally treated samples (blanched and canned). Enzymatic activity of polyphenol oxidase (PPO) and peroxidase (POD), physicochemical parameters (texture and colour), bioactive compounds (betalains and total phenols) and total antioxidant activity (FRAP assay) were evaluated. Texture properties were better retained for HHP treated samples than the thermally treated, and in particular increasing the pressure exposure time. a* (redness) decreased in high pressure treated samples in a time dependent way. Total phenol content and FRAP were found to be higher in HPP beetroot slices than canned samples but lower than blanched. Vitamin C was retained especially at the highest times of high pressure treatment. Differently from blanching and canning, high pressure treatments did not reach a sufficient inhibition of PPO and POD enzymes at each tested time. The PCA analysis clustered together the high pressure treated samples without any scattering according to the time.

Keywords: beetroot, high pressure, nutritional quality, physical properties

1. Introduction

Red beet (*Beta vulgaris* L.) is a member of the Chenopodiaceae family, cultivated for its large roots, although leaves are also utilizable. Seeds, roots and leaves of the plant are rich of polyphenols, including betanin, which are a group of water-soluble nitrogen containing pigments. In particular, two classes of betanin compounds are well-known: the red-violet betacyanins (0.04–0.21%) and the yellow-orange betaxanthins, (0.02–0.14%) (Ninfali & Angelino, 2013). Betanins are widely studied for their healthy effect; they are considered as useful cancer preventive agents and presented a high radical scavenging antioxidant activity (Lever & Slow, 2010; Kanner et al., 2001; Ninfali & Angelino, 2013). Beetroot is the only source of betanins approved as food additive (E-162) in the United States and in the European Union, and commercially exempt from batch certification (Moreno et al., 2008). They are commonly consumed as cooked, pickled, canned or as a salad after cooking, other than as fresh in a less extended way. Preservation of foods usually involves thermal treatments that prevent microbial growth and led to the inactivation of heat resistant enzymes like peroxidase (POD) and polyphenoloxidase (PPO). Despite these advantages, thermal processes have been reported to cause some adverse effects on vegetables organoleptic and nutritional quality (Herbach et al., 2004; Lau et al., 2000).

Novel non-thermal technologies are one of the alternatives currently available for the preservation of vegetables as they produce minimal deterioration effects on quality (Barbosa-Canovas et al., 1998). In particular, the application of the high hydrostatic pressure (HHP) within the food industry is encountering a growing interest (San Martin et al., 2002). During HP process of vegetables, food is subjected to pressures between 100 to 1000 MPa for several minutes with temperature ranges from -20°C to 60°C in order to inactivate enzymes, microorganisms or spore (Oey et al., 2008). According to the Le Chatelier's principle, the pressurized system responds to the decrease of volume with reactions that involve an increase in volume. This phenomenon can disrupt large molecules or microbial cell structures, leaving small molecules such as vitamins and flavour components unaffected (Norton et al., 2008).

According to the literature, the colour of the pressurized vegetables is not too much influenced at low or moderate temperature of treatment (Krebbers et al., 2002); sometimes, it is even enhanced because of the extended extraction of the pigments from the broken cells (Krebbers et al., 2002). The same extractive phenomena could be related to the improved antioxidant and nutritional activities often observed on HP treated vegetables (Patras et al., 2009). Despite this colour improvement and the increase of nutritional properties for the HPP treated vegetables, several enzymatic activity like for example PPO, POD or pectinmethylesterase (PME) are reported to be even more enhanced, probably because of the extended release of enzymes from the cells (González-Cebrino et al., 2013) leading to a quick loss of quality during shelf-life (Suthanthanjai et al., 2005). On the other hand, texture was sometimes improved on HPP treated vegetables (Basak et al., 1998), probably because of the stimulated activity of PME (De Roeck et al., 2010). Pressures from 200 to 400 MPa were reported to increase texture damage while pressures greater than 400 MPa led to less apparent damages (Oey et al., 2008).

To the best knowledge of the authors the application of HPP treatment to preserve fresh beetroots from sensory quality deterioration was not still investigated. Thus, the aim of this work is to optimize the HHP preservation of beetroot by means of the evaluation of enzymatic, physical (texture and colour) and nutritional (total phenolic content, antioxidant capacity and ascorbic acid) aspects. A 650 MPa high hydrostatic pressure treatment at four different times was evaluated and compared to two traditionally mild (blanching) and strong (canning) thermal treatments.

2.Materials and Methods

2.1. Sample preparation

Red beet roots (B. vulgaris L. var. red cloud) harvested in Mexico were purchased from a local market (Pullman, WA). Slices of 10 ± 1 mm thickness and 80 mm diameter were obtained. The samples were analysed as raw (R), blanched (B), canned (C) and processed with high hydrostatic pressure at 650 MPa for 3 (HHP3), 7 (HHP7), 15 (HHP15) and 30 min (HHP30).

2.2 Thermal treatments

Blanching treatment was performed according to Latorre et al. (2012) by immersion of the slices (100 g) into a water bath at 90 °C for 7 min in a sample/water ratio 1/5. Commercial canned samples were purchased from a local supermarket.

2.3 High hydrostatic pressure treatment

A pilot plant scale 2 L high hydrostatic press (Engineering Pressure Systems, Inc., Andover, MA, USA) was used to pressurize the red beet slices. Samples (100 g) were vacuum sealed, inside flexible 75 micron thickness, plastic pouches (Ultravac Solutions, Kansas City, MO, USA) to avoid any contact between the pressurization fluid and the samples. A 10% Hydrolubic 123B soluble oil water solution (Houghton Oil Valley Forge, PA) was used as the pressure medium. The plastic pouches were then placed inside the cylindrical pressure vessel (0.1 m internal diameter, 0.25 m internal height) of the high pressure equipment. The unit was operated with a hydraulic intensifier pump (Hochdruck-Systeme GmbH, AP 10-0670-1116, Sigless, Austria) that pressurized the vessel to operating pressure in a few seconds. Come-up time (CUT) was between 0.7 and 0.8 min. Processing conditions were 650 MPa. Experiments were conducted at room temperature (21 °C). The temperature increase due to compression was not higher than 2–3 C/100MPa. Three pouches were processed and analysed for each time of treatment.

2.4 Chemical analysis

The biological material was finely grounded with a domestic blender (Oster® 12-Speed Blender, Osterizer, Milwaukee, WI), then 20 grams were used for each analysis. Three replicates were analysed for each sample.

2.4.1 Enzymatic activity

Raw and treated grounded beetroot slices were used for the evaluation of polyphenol oxidase (PPO) and peroxidase (POD) activities according to Latorre et al. (2012) and Carvajal et al. (2011), respectively. The measurement was done following the change in absorbance at 420 and 470 nm, respectively, with a spectrophotometer Spectronic 20 Genesys (Spectronic Instruments, Inc,

Rochester NY, USA). The absorbance value was registered every 15 seconds and the activity calculated from the slope of a linear segment absorbance-time of at least 120 s duration according to Adams et al. (2003). Percentage variations were calculated in comparison to the raw sample.

2.4.2 Dry matter

For moisture determination, 1-2 g of raw and treated sample was homogenized and dried in a vacuum oven (model MA 30, Edgewood, NY, USA) at 105 °C for at least 45 min until reaching constant weight.

2.4.3 Betanin content

The extraction of betanin from raw and treated tissue was conducted according to the method of von Elbe (2001), with slight modifications. The grounded samples were added to a known volume of water (ratio 1:25) and mixed by vortex for 5 min at 25°C. The suspension was centrifuged (Sorvall RT6000B, DuPont, Delaware, USA) at 9000 rpm for 10 min (7 °C) and the solid part rewashed with distilled water with the same volume for 6 times, to obtain the best discoloration of the sample. The spectrophotometric determination was conducted by a Spectronic 20 Genesys spectrophotometer (Spectronic Instruments,Inc, Rochester NY, USA) on the extract. The extract was suitably diluted with buffer phosphate pH 6.5, up to a value of absorbance of 0.5 at 538 nm. The pigments were quantified by means of a calibration curve obtained by measuring the absorbance at 538 nm of known concentrations of betanin solutions (Sigma Aldrich, St. Louis, MO) in aqueous buffer phosphate at pH 6.5. Values were expressed as mg pigments/ g dry weight.

2.4.4 Total phenolics

For the extraction of polyphenols, the finely grounded biological tissue was mixed with water in a ratio 1:20 and kept under motion in an orbital shaking water bath (Model 3545, Lab-Line Instruments, Inc., Melrose Park, IL, USA) for 60 min at room temperature. The suspension was then filtered under vacuum using whatman filter paper n.5 and the filtered solution was used for the analysis. The total phenol quantification was conducted on the extract, properly diluted with distilled water, by the Folin–Ciocalteu assay, according to the method of Aadil et al., (2013). The

results were expressed as mg of gallic acid/100g dw based on a calibration curve prepared using standard solution of gallic acid (Acros Organics, NJ, USA).

2.4.5 Ascorbic acid

The grounded material was mixed with water in a ratio 1:40 and kept under motion for 1 hour at 25°C. The suspension was then filtered under vacuum using Whatman filter paper n.5 and the filtrate mixed with carbachol (Sigma Aldrich, St. Louis, MO) in a ratio 20:1, for the discoloration. The suspension was maintained under stirring for 30 min, then filtered using Whatman filter paper. 10 ml of the filtered solution were used for the analysis. Ascorbic acid content was determined by direct titration according to the method of Suntornsuk et al., (2002) with slight modifications. 5 ml of 2 N sulfuric acid (Sigma Aldrich, St. Louis, MO) were added to the extract, the solution was mixed and diluted with 50 ml of water and 2 ml of starch was added as an indicator. The solution was directly titrated with 0.01 N iodine (EMD Chemicals, Gibbstown, NJ) previously standardized against ascorbic acid solution.

2.4.6 FRAP assay

For the extraction of antioxidants, grounded red beet samples were mixed with water in a ratio 1:40 and kept under motion on a magnetic stirrer for 60 min at 25°C. The suspension was then filtered under vacuum using whatman filter paper n.5 and the filtered solution, conveniently diluted, was used for the FRAP assay. The method of Benzie & Strain (1996) with some modifications was used. 0.1 ml of diluted sample was added to 3 ml of the FRAP reagent solution and keep at 37°C in a water bath for 30 minutes before reading the absorbance at 593 nm. The FRAP reagent without the sample was used as blank. The FRAP reagent was prepared mixing 200 ml acetate buffer 300 mM pH 3.6; 20 ml of 10 mM TPTZ solution, 20 ml of 20 mM $FeCl_3$ and 24 ml of distilled water. The antioxidant activity was expressed as µmol Fe^{2+}/100g dw and calculated plotting the absorbance values in a standard calibration curve of $FeSO_4$ in FRAP solution.

2.5 Physical analyses

2.5.1 Texture

Texture of raw and treated beetroot slices were analysed using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK). The parameters were quantified using the application software provided (Texture Expert Exceed, version 2.64).

Cutting test was performed using a 3 mm thick stainless steel knife blade driven through vegetable, positioned on a slot surface, at a speed of 3 mm/s. Shearing force (Fmax, N) and shearing work, (Area, Ns) were obtained from the force-time cutting curves.

TPA test was carried out using a 50 mm diameter flat disc aluminium probe and a crosshead speed of 10 mm/min to compress the cylindrical samples (25 mm diameter x 10 mm height) to 50 % strain. The textural parameters considered were hardness, cohesiveness, and chewiness according to the definition of Bourne (1978). The secant modulus (SM), defined by Mohsenin, (1986) as the slope of the secant obtained from the origin to the failure point, was also calculated to measure the capacity of the material to take the elastic and extrapolated from the force-distance curve of the first compression step, considering the max peak force as the fracture point.

Five replicates were analysed for each sample.

2.5.2 *Colour*

Colour determination was carried out using a CR-200 spectrophotometer (Minolta Camera Co., Osaka, Japan) equipped with a standard illuminant D65. The instrument was calibrated using a white colour tile standard. L* (lightness), a* (redness), b* (yellowness), C (chroma, 0 at the center of the colour sphere), and hue° (hue angle, red = 0°, yellow = 90°, green = 180°, blue = 270°) were quantified on each sample using a 10° position of the standard observer (CIE, Paris, France, 1978). Ten measurements were conducted on random points on at least three slices for each sample.

2.6 Statistical analysis

SPSS statistical software (Version 20.0, SPSS Inc., Chicago, IL, USA) was used to perform an one-way analysis of variance (ANOVA) among samples from different treatments. Tukey post hoc test

at a 95% confidence level ($p \leq 0.05$) was performed to further identify differences among groups and the PCA analysis.

3. Results and Discussion

3.1 Enzymatic activity

The percentage variation of the polyphenoloxidase (PPO) and peroxidase (POD) activities of all treated beetroot slices is reported in Fig. 1, in comparison to the raw sample. Blanching and canning had the strongest effect on PPO activity with a reduction of 64.3 and 99.6% respectively, as previously observed on fruit and vegetables (Palou et al., 1999; Keenan et al., 2012). Conversely, HHP treatment seemed to not exercise a strong effect on the PPO activity reduction. In particular, 3 min of HHP treatment leaved the PPO activity unaffected. A partial PPO inactivation was measured for the other HPP samples in comparison to the raw one. HHP7 was the most effective among the high pressure treatments tested showing 21.4% reduction activity as higher PPO activities were registered pressurizing for 15 and 30 minutes (14.3 and 7.1% deactivation, respectively).

The thermal treatments had the most important effect on the reduction of the POD enzymatic activity (Fig.1), too. Blanching treatment showed a 23.3% residual activity, while it was completely lost by applying canning treatment. The high pressure treatment was more effective on POD than on PPO, but a still high activity was detected on the pressurized slices ranged from 56 to 66 % starting from 3 to 30 min of treatment.

To the best knowledge of the authors, no literature data are available on the effect of the high pressure on these enzymes in beetroot, but their high baro-resistance was already studied in other fruits, vegetables and by-products. Guerrero-Beltrán et al. (2005) observed that the application of pressures above 500 MPa for more than 5 minutes were required to improve the inactivation of PPO in peach purees. González-Cebrino et al. (2013) founded that applying pressure in the range 400-600 MPa at different processing times (1-300 sec) did not have a significant effect on PPO activity on red flesh and peel plum purée in comparison to the untreated sample. Woolf et al. (2013) found a significant reduction of POD activity up to 50% on avocado slices, treated in the range 400-600

MPa at different time of time of treatment (3-10 min), and no effect on PPO activity. Garcia Palazon et al. (2004) observed an enhancement of the POD activity for strawberries treated at 400 MPa for 5 and 10 min; enzymatic inactivation was evident only after 15 min of treatment. The same authors reported a POD inactivation in the range of 11–35% under 600 and 800 MPa pressure. Woolf et al. (2013) hypothesized that the cell membrane breakdown due to the pressurization enhanced enzyme release/extractability, which indirectly resulted in an increase of the measured enzyme activity. Another factor, which may have contributed to the observed effect, could be the activation of latent enzymes by the interaction with other constituents in the extract, also counteracting the effect of HP inactivation (Terefe et al., 2010). The delivering of more active enzymatic isoforms or the structural modification of the active site may be other factors explaining the resistance of the enzymatic activity to the high pressure application as previously hypothesized (Guerrero-Beltran et al., 2005).

3. 2 Chemical parameters

3.2.1 Betanin content

The total betanin contents obtained in all samples are reported in Table 1. The pigments content in the raw sample was in agreement with the literature data with slight differences probably due to genetic and agronomical factors (Georgiev et al., 2010; Kujala et al., 2000). Canning appeared to greatly affect betanin, which were found to be significantly lower in C than in all the other samples, probably in relation with its thermal degradation (Herbach et al. 2004). On the other hand, BL sample showed the lowest betanin reduction (14.9%) in comparison to the other samples (Table 1). Similarly, Monteiro Cordeiro de Azeredo et al. (2009) found 19.8% betacyanins reduction in blanched beetroot slices. These authors related its loss to the water leaching of the pigments. The betanin contents were also reduced in all the high pressure treated samples (Table 1). Betalains were reported to show high sensitivity to the effect of oxygen, temperature, pH, light and enzymatic activity (Von Helbe, 2001). It is possible to hypothesize that some of these factors may be enhanced by pressure, other than a slight loss of juice from the broken cells.

It was noteworthy that betanin retention increased rising the time of exposure to the high pressure as losses in the range 44.6-31.2 % were observed for HHP3, HHP7, HHP15 and HHP30, respectively. Patras et al. (2009) found similar results on strawberry and blackberry purées anthocyanins suggesting an increased extractability of the molecules from the vacuoles of the plant cells increasing time of pressure treatment.

3.2.2 Total phenolics, ascorbic acid and FRAP assay

Total phenolics are summarized in Table 1 for all the analysed beetroot and their content in R samples was found to be in agreement with previous data (Kujala et al. 2000). A significant increase was observed in BL sample, in comparison to R, probably in relation with the release of molecules from the cells as a consequence of the thermal degradation of vegetable cell walls (Paciulli et al., 2014). Phenols of C beetroots did not significant change compared to R. Jiratanan & Liu (2004) found even a slight but significant 5% increase of the phenol content in canned beetroots, probably because of the breakage from the covalent bonds with amine functional groups and esterified glycosides, which was the form generally found in fruit and vegetables. Conversely, all the high pressure treated samples showed an increase of phenols when compared to R (and C, of course) but values were also significantly lower in comparison with BL. In addition, total phenolics showed a slight increase, although not significant, rising the time of pressure exposure with the exception of HHP15 that showed the lowest value among HP samples Other authors found that values of pressure treatment comparable to that employed in this study enhanced total phenol content of vegetables (Patras et al., 2009; Zhou et al., 2014). An increase of membrane permeability under the effect of pressure may be responsible for the release of phenols, avoiding the further thermal oxidation probably occurred in canned samples.

Among the thermally treated samples, a better retention of ascorbic acid was observed for BL samples than for C (Table 1), probably because of the different thermal processing conditions.

HHP15 and HHP30 did not show any significant reduction of ascorbic acid in comparison with R while beetroot treated for lower time exhibited a significant loss comparable to those of thermally

treated samples. Several authors observed that pressure application at value higher than 400 MPa preserved vitamin C content in fruits and vegetables more than thermal treatments also for long time of application (Patras et al., 2009¸ Zhou et al. 2014).

Ascorbic acid is well known to be very susceptible to oxidation under certain environmental conditions (i.e. heat, presence of oxygen, heavy metal ions and alkaline pH). Chemical and enzymatic reactions occurring in food samples may be enhanced by pressure, as previously hypothesized (Zhou et al., 2014), leading to its loss after HP processing at short time of application. When pressure was applied for longer time, it was possible to hypothesize a sort of regeneration of the oxidized vitamin C molecules, probably because of the extended contact with the extracted antioxidant molecules.

FRAP assay results (Table 1) were in agreement with those previously described for total phenolics as BL and C showed the highest and lowest values, respectively, among all samples. The different intensity degree of the thermal treatment probably led to the release of the antioxidant molecules from the cells during blanching and to their consistent thermos-oxidative damage during canning.

High pressure treatment resulted in a significant increase of the antioxidant activity in comparison to R and C samples, with a trend that did not appear to be time dependant as for total phenolic determination. Opposing results were reported in literature about the HP influence on the antioxidant activity of fruits and vegetables as it seemed to be strictly dependent on the vegetable matrix (McInerney et al. (2007). Pressure processing was found to either increase or not influence the antioxidant activity (Patras et al., 2009). Higher FRAP values for HP treated pumpkin compared to thermally treated samples were recently reported by Zhou et al. (2014), too, probably in relation with a different extraction yield and breakdown of some bioactive compounds.

3.3 Physical analyses

3.3.1 Texture

Textural parameters are shown in Table 2. BL and C lost 46.4 and 94.5% of the original cut force (Fmax), respectively. Likewise, Area values, which represented the work done for cutting samples,

showed losses of 47.5 and 97% for BL and C, respectively (Table 1). Thermal treatment is well known to greatly influence vegetable structure and texture causing an initial loss of instrumental firmness due to the disruption of the plasmalemma and subsequently, to an increase of the ease of cell separation often accompanied by the swelling of the cell walls (Waldron et al., 2003).

Cut hardness of HPP samples was found to be not statistically different from R. Several authors (Oey et al., 2008; De Roeck et al., 2010) suggested that the disruption of the tissue, after the high pressure process, liberated PME, which contacted the substrate (the highly methylated pectin), leading to the demethylation; the so de-esterified pectin formed a gel-network with divalent ions resulting in increased hardness.

HHP30 exhibited similar cut force values to R. Otherwise, the others HPP samples showed slight cut force reductions (13.9, 23.6 and 3.6% for HHP3, HHP7, HHP15 respectively), in comparison to R. A similar trend was shown by the Area values: increasing the pressure exposition time, a higher recovery of consistence was observed. Trejo Araya et al. (2007) founded a slight hardness increase on pressurized carrot disks by compression test (from 6 to 10%) extending time of treatment from 2 to 30 min at pressure 300-500 MPa. This phenomenon was explained by the authors as a tissue recovery during the holding time. Microscopic observations of carrot tissue damage induced by pressure also showed that long-time application (20 min) of high pressure treatment (400 MPa) just decreased cells cubage, which was probably restored when the pressure was released, inducing little change on texture (Xu & Han, 2006).

TPA hardness and chewiness values were in agreement with Fmax values obtained with cutting test (Table 2). R exhibited the highest hardness and chewiness, comparable to those obtained applying high pressure processing for long time (15 and 30 min). On the other hand, HHP3 and HHP7 showed 24.4 and 26.4% hardness reduction, respectively, if compared to R. Cohesiveness was also significantly higher for all HPP samples than R, BL and C. It could be hypothesized that beetroot tissue little changed during the application of high pressure and/or probably recovered its original structure, especially at the highest times of treatment. The Secant modulus values, reported in Table

2, confirmed the trend shown by hardness values. HHP15 and HHP30 samples had values similar to R, while HHP3 and HHP7 showed about 20% reduction of this value. Thermally treated samples (BL and C) exhibited the lowest values with a high decrease of secant modulus. A decrease of the secant modulus could be attributed to a general increase of the tissue extensibility (Cybulska et al., 2011). Thus, the obtained results gave effort to the hypothesis that the application of high pressure for the longest times of treatment (15 and 30 min) induced less change of beetroot tissue.

3.3.2 Colour

Colour parameters of R and treated beetroot are reported in Table 3. Blanched beetroots showed a decrease of L*, a* and b* parameters, resulting in a darker appearance. Similar colour changes were reported by Latorre et al. (2012) under the same blanching conditions used in this study. These authors related the changes to the pigment decompartmentalization consequent to the cell membrane thermal denaturalization.

Colour of the commercial canned sample was extremely different from the other samples, showing the highest lightness (L*), and a shift of colour towards greenness (- a*, H° value closer to 180). Herbach et al. (2004) similarly founded an increase of L* for thermally treated beetroot juice (85°C for 8 h), relating this change to the molecular degradation of betalains.

High pressure treated beetroots retained colour better than C samples, but less than BL (Table 3), as resulted by the ΔE values. In particular, L* and a* values of HPP samples were significantly lower than R and BL, resulting in a darker colour and in accordance with the lower content of betanin found in pressurized samples (Table 1). Besides the instability of colour pigments, browning was reported to play an important role in the discoloration of HP-treated food products (Oey et al., 2008). The darkening of strawberry purees after high pressure treatments was also reported (Patras et al. 2009) and ascribed to a still high oxidative enzymatic activity after the treatment. Higher POD and PPO activities for HP samples in comparison with BL were found in this study (Fig. 1). On the other hand, H° values did not change among R, BL and HP samples, as founded by Patras et al (2009), except for the highest time of pressure treatment.

These colour changes resulted in an increase of ΔE values increasing time of pressure exposition. In particular, the growing release of betanin resulted in a less red colour (lower a*)and a decrease of saturation (lower C) increasing pressurizing time

3.4. PCA

PCA analysis was performed to discriminate according to the different treatment conditions among samples, as shown in Fig. 2, with the two first principal components (PC1 and PC2) that explained the 81.4% of the total variance.

The samples were grouped into three different clusters: R and BL that presented positive loadings on PC1 and PC2; all the HHP samples that obtained positive loadings on PC1 and those negative on PC2 and canned beetroot (C), which showed negative and positive loadings on PC1 and PC2, respectively. Thus, BL appeared to be more similar than the others processed samples to R according to the betanin content, and such colour parameters as a*, b* and C (Fig. 2B). All the pressurized samples, grouped together, appeared to be well described by the textural parameters (Fig.2B) and total phenolics but the time of pressure application did not scatter the samples. On the other hand, C, resulted very different from all the other samples, in view of such colour parameters as L, H° and ΔE values.

5. Conclusions

In this work, the effect of different treatment times of HHP application was examined for the first time on beetroot, which presents a well-known high nutritional value. These preliminary findings suggested that HHP could be used as valid alternative for the preservation of beetroots, being less invasive than canning and quite comparable with blanching, as also shown by the PCA results. In particular, HPP samples appeared to be similar to BL considering both nutritional and textural quality, despite the different enzymatic resistances observed. On the other hand, colour parameters were less retained than BL, probably in relation with the high PPO activity shown by the HPP samples. Thus, the improved quality of HPP beetroots could probably change during storage due to

the incomplete inactivation of enzymes, which may result in undesired chemical reactions of the food matrix.

Examining the time of treatment, it should be preliminary concluded that a better extraction of the healthy molecules and a better retention of the textural parameters could be obtained extending the 650 MPa pressurizing holding time from 5 to 30 min. These results should be better explained and understood carrying out microstructural observation of the pressurized beetroot tissues.

Finally, further studies should be carried out on beetroot, improving the high pressure processing conditions applied in this study with the application of different time-temperature and pressure-temperature combinations, finding a correct compromise between the spent energy and the final quality.

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Caption for figures

Fig.1 Percentage (%) variation of the PPO and POD enzymatic activities of all samples in comparison to raw. Abbreviations: BL, blanched; C, canned; HHP3, 3 min of high hydrostatic pressure treatment; HHP7, 7 min of high hydrostatic pressure treatment; HHP15, 15 min of high hydrostatic pressure treatment; HHP30, 30 min of high hydrostatic pressure treatment. Values of the raw samples were considered as equal to 100 %.

Fig. 2 PCA results obtained for the two principal components: (A) Projection of the cases on the factor plane (B) Projection of the variables on the factor plane. Abbreviations: HHP3, 3 min of high hydrostatic pressure treatment; HHP7, 7 min of high hydrostatic pressure treatment; HHP15, 15 min of high hydrostatic pressure treatment; HHP30, 30 min of high hydrostatic pressure treatment.

Table1. Chemical parameters of beetroot slices.

	R	BL	C	HHP3	HHP7	HHP15	HHP30
Betanin (mg/g dw)	81.7±0.1 a	69.5±1.6 b	23.0±0.5 e	45.3±1.8 d	48.2±4.9 d	52.3±3.8 cd	56.2±1.4 c
Total phenolics (mg gallic acid/100g dw)	588.3±5.0 d	1026.8±9.1 a	526.8±16.3 d	831.8±58.8 b	871.3±46.7 b	723.0±31.1 c	906.1±7.5 b
Ascorbic acid (mg/100g dw)	0.166±0.009a	0.134±0.01abc	0.128±0.019bc	0.135±0.007abc	0.114±0.006c	0.165±0.015a	0.160±0.015ab
FRAP (mg Fe^{2+}/100g dw)	1870.9±13.8d	2751.9±116.9a	1337.2±89.3e	2405.3±164.9b	2298.3±54.7b	1944.5±42.4d	2264.0±43.0b

Means in row followed by different letters are significantly different according to Tukey test ($p<0.05$). Three replicates were performed for each measurement. Abbreviations: R, raw; BL, blanched; C, canned; HHP3, 3 min of high hydrostatic pressure treatment; HHP7, 7 min of high hydrostatic pressure treatment; HHP15, 15 min of high hydrostatic pressure treatment; HHP30, 30 min of high hydrostatic pressure treatment.

Table2. Texture parameters on beetroot slices.

Test		R	BL	C	HHP3	HHP7	HHP15	HHP30
Cut	Fmax (N)	308.3±33.9a	165.3±34.0c	17.0±2.4d	265.4±12.1ab	235.5±21.7b	272.1±34.2ab	325.0±30.8a
	Area (Ns)	623.1±68.1a	347.2±65.9c	19.9±4.0d	364.5±34.9c	460.7±22.0bc	490.2±72.0b	488.7±50.6b
TPA	Hardness (N)	408.8±19.1a	161.6±28.2c	20.0±5.4d	309.2±6.3b	300.7±14.1b	387.3±28.2a	385.1±22.4a
	Cohesiveness (-)	0.26±0.05 b	0.15±0.02 c	0.15±0.01 c	0.42±0.02 a	0.46±0.04 a	0.51±0.05 a	0.48±0.02 a
	Chewiness (N)	82.4±12.6b	10.1±3.4c	1.5±0.3c	81.3±4.1b	84.0±8.9b	120.3±4.2a	113.4±12.8a
	Secant modulus (N/mm)	77.7±4.1a	31.1±3.3c	6.0±1.5d	61.3±3.3b	61.8±4.3b	76.2±6.9a	76.9±5.1a

Means in row follow by different letters are significantly different according to Tukey test ($p<0.05$). Ten replicates were performed for each measurement. Abbreviations: R, raw; BL, blanched; C, canned; HHP3, 3 min of high hydrostatic pressure treatment; HHP7, 7 min of high hydrostatic pressure treatment; HHP15, 15 min of high hydrostatic pressure treatment; HHP30, 30 min of high hydrostatic pressure treatment.

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Table3. Colour parameters of raw and beetroot slices.

	R	BL	C	HHP3	HHP7	HHP15	HHP30
L	23.3±2.0 b	20.7±0.7 c	99.3±0.8 a	20.0±0.8 c	20.7±0.2 c	20.6±0.5 c	20.3±0.4 c
a*	31.1±3.3 a	17.5±0.6 b	-5.1±1.5 f	9.9±0.3 c	7.3±0.6 cd	4.0±0.9 e	4.8±0.6 de
b*	5.2±1.5 a	2.1±0.4 b	1.1±0.6 bc	1.0±0.0 bc	0.6±0.1 c	0.4±0.2 c	0.3±0.1 c
C	31.5±3.5 a	17.6±0.6 b	5.2±1.3 de	10.0±0.3 c	7.4±0.6 cd	4.0±0.9 e	4.8±0.6 de
H°	6.2±1.1 c	8.4±1.6 c	173.3±4.1 a	10.0±0.5 c	12.8±0.6 bc	9.8±3.0 c	16.8±4.0 b
ΔE	-	14.2±0.6 e	84.3±0.5 a	21.8±0.3 d	24.3±2.3 c	27.6±0.9 b	26.9±0.6 b

Means in row followed by different letters are significantly different according to Tukey test ($p<0.05$). Ten replicates were performed for each measurement. Abbreviations: R, raw; BL, blanched; C, canned; HHP3, 3 min of high hydrostatic pressure treatment; HHP7, 7 min of high hydrostatic pressure treatment; HHP15, 15 min of high hydrostatic pressure treatment; HHP30, 30 min of high hydrostatic pressure treatment.

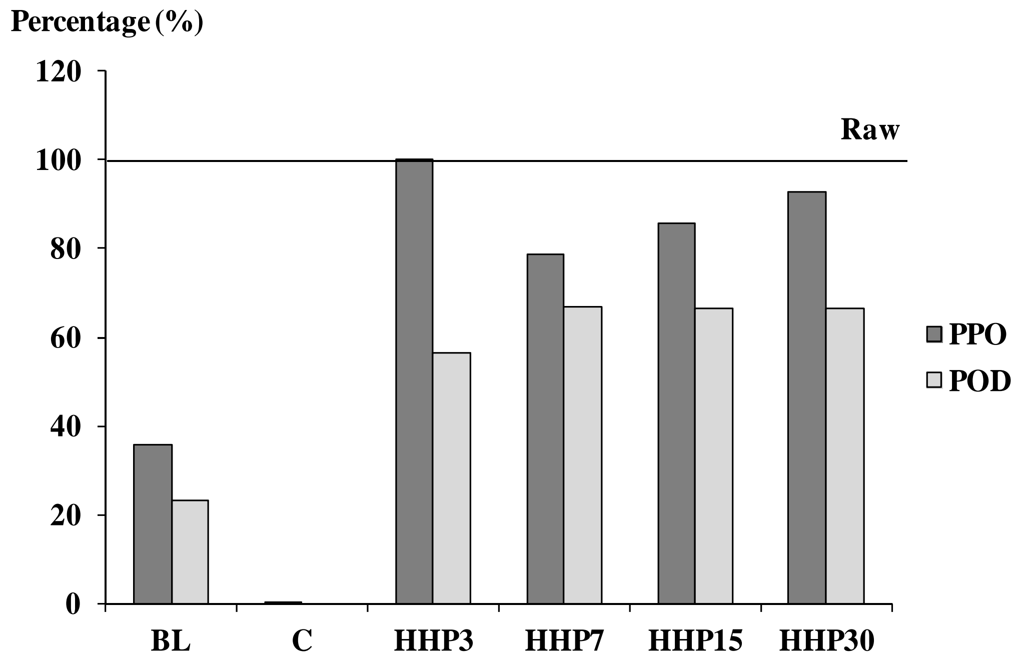

Fig. 1

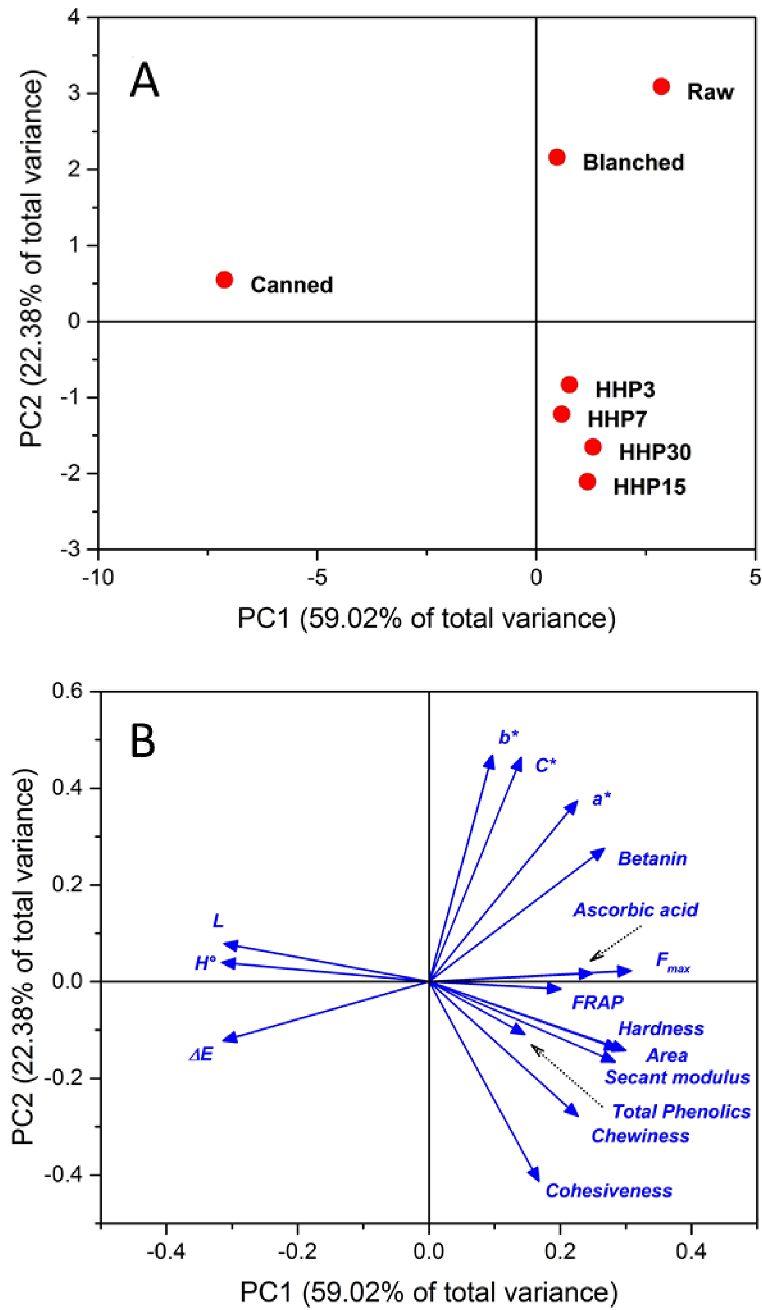

Fig.2

Section IV

Raman spectroscopy application in frozen carrot cooked in different ways and the relationship with carotenoids

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Published in Journal of the Science of Food and Agriculture

DOI 10.1002/jsfa.7009

Abstract

Background: Raman spectroscopy, in its confocal micro-Raman declination, has been recently proposed as a spatially resolved method to identify carotenoids in various food matrices being faster, non-destructive, and avoiding the sample extraction, but no data are present in literature about its application to the evaluation of carotenoid pattern changes after thermal treatments on carrots.

Results: The effect of three cooking methods (i.e. boiling, steaming and microwaving) was evaluated on frozen carrot comparing changes on carotenoid profiles measured by means of Raman spectroscopy with their HPLC determinations and colour. A more pronounced detrimental effect on carotenoids was detected in steamed carrots, in accordance with colour data. Differently, the boiling and, to a lesser extent, microwaving caused an increase of carotenoid concentration. Cooking procedures affected the Raman spectral features of carotenoids causing the shift of vibration frequencies toward a higher energy, the increase of the spectral baseline and of the peak intensities as well as the broadening of their width, probably in relation with the thermal degradation of longer carotenoids (i.e., the all *trans* form) and the isomerisation process. In particular, steamed samples showed a significantly higher increase of centre frequency in accordance with a more pronounced isomerisation and changes of colour parameters.

Conclusions: This work showed that the evolution of Raman spectral parameters could give information on carotenoid bioaccessibility for carrots differently cooked. This paves the way for a future use of this technique to monitor and optimize the cooking processes aimed at maximize carotenoids bioaccessibility and bioavailability.

Keywords: carrot, carotenoids, cooking, Raman spectroscopy, freezing.

1. Introduction

Currently, freezing is one of the most widely used food preservation techniques. Considering vegetables, this practice permits to preserve seasonal foods during prolonged storage periods, maintaining their quality parameters, such as sensory attributes and nutritional properties. In effect, freezing retards chemical and biochemical reactions and microbial growth, and also maintains colour due to the blanching treatment.[1]

Carrots are one of the most consumed vegetables in both fresh and frozen forms.[2,3] However, frozen carrots need to undergo a cooking process before their consumption. Consumers generally believe that fresh vegetables are healthy, whereas cooking and freezing processes are generally considered negative to nutritional compounds.[4] Recent studies have highlighted that carrots maintain nutritional benefits after cooking not only processing fresh carrots[5,6] but also frozen material in relation with several pre-and post-harvesting factors,[6,7] because carotenoids and other beneficial compounds remain in relevant amount in the cooked products.

Spectral techniques have been recently tested and proposed to analyse food in cooperation with standard analytical methods being faster, non-destructive, avoiding sample extraction[8] and offering in addition space-resolved information, such as spatial distribution of nutrients. Among them, Raman spectroscopy was suggested for the identification and quantification of the main constituents and nutrients from plants.[9] In this context, this technique has been recently explored as a spatially resolved method to identify carotenoids in various food matrices [10-12] by a detailed comparison of frequency shifts in the main observed bands, which are related to the molecular conjugation length and/or to the presence of specific end-groups.[13] In particular, the Raman spectroscopy was applied on carrots to visualize the proportions of individual carotenoids accumulated in various root tissues.[14] More recently, quantitative measurements of carotenoids have been obtained on several carrot lines

comparing Raman spectra with their content determined by means of visible spectrophotometry.[15]

Raman spectroscopy could be applied to the study of carotenoids both in the Fourier Transform Near Infrared configuration (FT-NIR)[14] and in the Raman Resonance (RR) mode.[16] In the FT-NIR Raman case, the sample is excited in the infrared region in order to avoid the ubiquitous fluorescence emission in plant materials, while in RR, the laser excitation is provided inside the carotenoids spectral absorption band to induce the Raman resonance effect. The FT-NIR configuration enables to obtain a chemical map of the carotenoids together with the other plant constituents, while RR configuration is particularly suited for fast or low levels of detection, since the resonance enhances the signal by factors of 10^2-10^6.

To the best knowledge of the authors, no data are present in literature about the application of Raman spectroscopy to the evaluation of carotenoid pattern changes after thermal treatments on carrots. Thus, in this preliminary work, the effect of three common cooking methods (i.e. boiling, steaming and microwaving) is evaluated on frozen carrot slices comparing changes on carotenoid profiles measured by means of Raman spectroscopy with their quantitative determinations acquired by the high-performance liquid chromatography (HPLC) and the colour parameters.

2. Material and methods

2.1 Samples and processing

Carrot (*Daucus carota* L., Napoli variety) slices (8 mm thickness) were obtained by a local manufacturer, being industrially blanched and frozen within 24 h from harvesting.

All samples were stored for two months at –18 °C in a thermostat to better mimic the common storage conditions applied prior to the commercialization. Then, they were processed within 24 h from the end of storage.

Three cooking conditions were applied by optimizing time of treatment according to the judgment of a large group of semi-trained panellists, as previously reported[5]. Vegetables were not defrosted before cooking. Boiling was performed by adding carrot slices to boiling tap water in a covered stainless steel pot (1:5, food/water) cooking on a moderate flame. Cooking time, measured when the vegetable was put into boiled water, was 20 min. Steaming treatment was carried out at 100 °C under atmospheric pressure in a Combi-Steam SL oven (V-Zug, Zurich, Switzerland) that presented an internal volume of 0.032 m^3, an air speed of 0.5 m s^{-1} and a steam injection rate of 0.03 kg min^{-1}. Oven was pre-heated at the set temperature before inserting samples for each cooking trial. Cooking time was 30 min. Microwave treatment was carried out in a MW651 domestic microwave oven (De Longhi, Treviso, Italy), where frozen carrot slices, placed in a plastic microwave steamer (1:2, food/water) not in the direct contact with water on the rotating turntable plate of the oven, were exposed at a frequency of 2450 Hz at low power (450 W). Cooking time was 10 min. Microwave cooking was also carried out at different time from 0 to 12 min under the same condition

All cooking procedures were performed in triplicate.

2.2 Carotenoid analysis

The determination of carotenoids was carried out by HPLC analysis, as previously described by Leonardi et al.[17] Briefly, carotenoids were extracted with tetrahydrofuran in the presence of 0.1 g kg^{-1} butylated hydroxytoluene. The extract was dried under nitrogen flow in dark tubes and resuspended in 5 mL chloroform. Ethyl-β-apo-8'-carotenoate was used as standard with a calculated recovery of 98%. HPLC separation was carried out at a flow rate of 0.8 mL min^{-1} using a HPLC (Model LC 10, Shimadzu, Osaka, Japan), with diode array detector and a Supelcosil C18 (250×4.6 mm; 5 μm, 100 Å particle size) column (Supelco, Bellefonte, Pa., USA). Carotenoid elution was achieved using the following linear gradient: starting condition, 82% A, 18% B; 20 min, 76% A, 24% B; 30 min, 58% A, 42% B; 40 min, 39% A,

61% B, 45 min, back to 82% A, 18% B. A and B were acetonitrile and a methanol-hexane-methylene chloride (1:1:1, v/v/v) mixture, respectively. Extraction was repeated twice with duplicate analyses each time; thus four results were obtained for each sample.

The quantification of α-carotene, all *trans*- and *cis*- isomers of β-carotene was carried out by comparison with a calibration curve of standard all *trans* β-carotene, in the concentration range of 3-100 mg L^{-1}. Data were expressed as g kg^{-1} on dry weight basis. Three frozen and cooked samples were analysed.

For the determination of the moisture, 3–4 g of raw or cooked homogenized sample (as triplicate) was dried in a convection oven at 105 °C for at least 16 h until reaching a constant weight, according to the AOAC method.[18]

2.3 Colour analysis

Colour determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D_{65}. L* (lightness; black = 0, white = 100), a* (redness > 0, greenness < 0) and b* (yellowness > 0, blue < 0) were quantified using a 10° position of the standard observer.[19] The individual differences in L*, a* and b* values of each cooking treatment with respect to the colour of the frozen uncooked samples were evaluated using ΔE calculation, as previously reported.[7]

The assessments were carried out at room temperature (25 °C) on three pre-selected positions of each slice picking approximately the same points for the peripheral and central points of the specimens. Five slices for frozen and cooked samples were analysed for a total of 15 determinations for each trial.

2.4 Raman spectroscopy

The Raman spectra were collected with a Jobin Yvon T64000 triple monochromator spectrometer (Horiba, Kyoto, Japan) coupled to a confocal microscope equipped with a liquid nitrogen-cooled CCD detector and a temperature-controlled stage. The spectrometer was used in its backscattering, single monochromator, micro configuration, using as excitation the

488.8 nm line of an Ar/Kr - ion laser source (Innova 70 C, Coherent Inc., Santa Clara CA, USA). In these conditions the overall energy resolution is $\Delta\nu<0.15$ cm^{-1}. The laser wavelength is well inside the carotenoid optical absorption region,[20] ensuring the condition for the resonance Raman enhancement. To reduce radiation damage effects, the incoming laser beam, of 10 mW power, was focused by a 10X microscope objective to probe an area of 10 μm diameter. Due to the low damage threshold of carotenoids, some photoinduced degradation (photobleaching) is unavoidable. This laser power density was selected- after a series of preliminary measurements- to provide a good steady state regime, whose Raman emission intensity was estimated -by fitting the exponential decay of the Raman- signal to be about 80% of the theoretical maximum signal. Furthermore, an accurate analysis of initial and steady state spectra evidenced no selective photobleacing, therefore the Raman spectra obtained were representative of the total carotenoid content and of their composition.

The Raman spectral features were extrapolated by the analysis of the characteristic carotenoid peaks [8-12] by a standard peak fitting procedure using the Matlab© computing environment (Mathworks, Natik Massachusetts, USA). The experimental peaks were modelled by a Gaussian on a background:

$$f(\nu)=F+I\frac{1}{\Delta\sqrt{2\pi}}e^{\frac{-(\nu-\nu_0)^2}{2\Delta^2}}$$

Where:

ν is the abscissa of the Raman spectrum, namely the Raman energy shift measured in cm^{-1}. The parameter F is the background signal due to fluorescence, while I, ν_0 and Δ are the intensity (or area), the centre frequency and the half width at half maximum of the peak, respectively. The parameter F is a direct measure of the sample fluorescence.

In order to achieve a statistical accuracy, ten slices of frozen and cooked samples were analysed and each carrot slice, placed on the micro-motorized microscope stage, was probed in about hundreds of different points uniformly distributed of the surface.

2.5 Statistical analysis

SPSS (Version 19.0, SPSS Inc., Chicago, IL, USA) statistical software was used to perform one-way analysis of variance (ANOVA) and a Tukey test at a 95% confidence level ($P \leq 0.05$) to identify differences among samples. Pearson correlation coefficients were calculated among all variables at a 95% and 99 % confidence levels ($p < 0.05$ and $p < 0.01$).

3. Results and discussion

3.1 Carotenoid analysis

The amount of the identified carotenoids is reported in Table 1 for frozen and cooked samples. The β-carotene was almost completely present in the all *trans* form in frozen uncooked carrots, with a double concentration with respect to α-carotene (see at α/all trans β carotene ratio). Minor amounts of phytoene and phytofluene were quantified in all the samples. The concentration of the identified carotenoids resulted slightly higher than that found in previous studies on the same vegetable,[7,21] being these differences probably related to several factors such as different genotype, growing condition, season, maturity as well as processing conditions.

Considering the effect of cooking on the total carotenoid content, a detrimental effect was detected in carrots cooked in steam oven, in agreement with our previous studies[5,7], with a decrease of 34 % of the initial amount of total carotenoids. Differently, the boiling and, to a lesser extent, microwaving processes caused an increase of the carotenoid concentration. A positive effect of cooking on carotenoid accessibility of carrot has been already reported[5,22-23] and it has been ascribed to the breakdown of the cellulose structure of the plant cell, which allows a more effective and complete extraction of these compounds.[24]

All the cooking methods determined a significant increase of *cis*-β-carotene forms, while the *cis* forms of α-carotene was not quantifiable. The isomerisation does not occur in crystalline carotene; thus its dissolution, for instance due to the thermal treatment, is a prerequisite for the formation of *cis*-isomers.[25] Moreover, it has been shown that thermal treatments above 100 °C induce a considerable isomerisation of β-carotene.[25] To better estimate the effect of the different cooking methods on the all-*trans*- and *cis*-β-carotene content, the *cis*-/all-*trans*-ratio was calculated and shown in Table 1. An increase of such ratio with all the cooking methods was observed. This increase could be caused by the release of the already existing *cis*-forms from cooked carrots, especially in those boiled, as already suggested.[23] Conversely, in the steamed carrots, where a decrease of *trans* β-carotene was observed, the increase of *cis*/all *trans* ratio could be resulted from the conversion of all-*trans*- β-carotene in its *cis*-forms. This could not have a negative nutritional consequence, as the carotenoid isomerization, decreasing the length of the molecule, favour their inclusion in the chilomicrons and consequently their bioavailability.[26] However, as already observed,[5] the longer time requested to reach an appropriate tenderness in steaming may have exposed the carotenoids to oxygen and light determining their oxidation[27] and explaining the low carotenoid recovery observed after this procedure with respect to the others.

3.2 Colour analysis

Colour parameters are summarized in Table 2 for all samples. Cooking greatly affected colour of carrots, despite the stabilizing effect of blanching. Carrots became significantly darker (L*), less red (a*) and yellow (b*) than uncooked samples. Similar changes of colour parameters after heating have been found to be highly related with the decrease of α- and β-carotene content as well as their *trans-cis* isomerisation.[28]

It is well known that the colour of carrots is associated with the presence of carotenoids, α- and β-carotene in particular.[28] Conversely, phytoene and phytofluene, which were both found in frozen and cooked samples, are well known to be colourless molecules.[29] All colour

parameters were found to be positively correlated with α carotene (R= 0.649, 0.660 and 0.656 for L*, a* and b*; respectively, $p<0.05$). Accordingly, b* value was previously found to be positively correlated to α-carotene in carrot slices under different frying conditions.[30] The authors of that study also referred to a high statistical correlation between a* and *trans* β-carotene, which was not found under the experimental conditions applied in our study. In addition, high correlation coefficients were found among all colour parameters and the ratio *cis*/all *trans* β-carotene. In particular, the decrease of lightness (L*), redness (a*) and yellowness (b*) displayed R values of -0.666 ($p<0.05$), - 0.760 ($p<0.01$) and – 0.745 ($p<0.01$), respectively, with this ratio. A high statistical correlation value was previously found between Hunter b* parameter and the isomerisation of β-carotene in carotenoid powder from carrot pulp waste under storage.[31]

In this study, changes appeared to be more consistent after steaming, in accordance with carotenoid loss, and as observed also by the ΔE values. This was also in agreement with previous data on fresh[5] and frozen carrots.[7] On the contrary, the colour of boiled and microwaved samples did not show differences, although losses and isomerisation of carotenoids were quite different between these two treatments. This may be related to the influence of other carotenoids influencing carrot colour not quantified in this study (i.e. lutein, lycopene).

3.3 Raman spectroscopy analysis

3.3.1 Effect of different cooking methods

The Raman spectra of frozen and cooked carrots, shown in Figure 1, were characterized by the three main carotenoids peaks, centred at the frequencies ν_1= 1525 cm^{-1}, ν_2= 1155 cm^{-1} and ν_3=1008 cm^{-1}. Such modes originate from the carbon-carbon double-bond stretch vibrations (C=C) of the polyene backbone, from the carbon-carbon single-bond stretch vibrations (C–C),

and from the rocking motions of the molecules methyl components ($C–CH_3$), respectively. Cooking procedures affected the Raman spectral features of carotenoids as follows:

- Shift of the ν_1 and ν_2 vibration frequencies toward a higher energy;
- Increase of the peak intensities (I);
- Broadening of the peak width (Δ, frequency distribution);
- Increase of the spectral baseline (F, fluorescence background).

Table 3 showed the extrapolated Raman spectral parameters from ν_1 in frozen and cooked carrots by the three cooking procedures. Looking at the value of peak frequency (ν), steaming produced more pronounced changes, while boiling seemed to be the less invasive cooking procedure: namely, the steamed carrots displayed a marked shift of the ν_1 vibration towards a higher energy.

It is well established in literature[20,32] that the centre frequencies of this vibration in carotenoids has an inverse relationship with the conjugation length N, where N is the number of carbon double bounds. Moreover, an increase of this frequency may also be induced by *trans cis* isomerisation process.[28] Therefore, the measured shift toward higher energies of the ν_1 molecular vibrations in the Raman spectra of the cooked carrots could be attributed either to a higher thermal degradation of longer carotenoids (i.e., the *trans* form) or to an isomerisation process. The HPLC data suggested that both effects could be present in our case; in particular, after cooking, the relative α/ all *trans* β-carotene ratio decreased and at the same time, the *cis/*all *trans* ratio of β-carotene increased, as shown in Table 1. The variation of Raman frequency (ν) displayed a positive statistical correlation ($R= 0.787$, $p< 0.01$) with the increase of the ratio cis/all trans β-carotene, in agreement with this observation. In addition, loss of redness (a*) and yellowness (b*), and a darkening effect (L*) were observed after cooking (Table 2) and negative correlations were found among ν and these colour

indices ($R= -0.632$, $p< 0.05$ with L*; $R= -0.673$, $p<0.05$ and -0-733, $p<0.01$ with a* and b*, respectively).

Among the cooking samples, steamed carrots showed a significantly higher increase of centre frequency, in accordance with a more marked isomerisation effect (Table 1) and colour changes (Table 2).

The thermal treatment has also changed the width of Raman peaks (Δ), which reflects the frequency distribution of the relative molecular vibration. Such parameter, very sensitive to the molecular environment, measures the degree of disorder in the molecular packing: narrow peaks characterize a crystalline structure, while broaden peaks a disordered environments, such is the case of amorphous matrices.[33] In this study, we observe a comparable broadening of the peak after any of the cooking procedures. This can be ascribed to the dissolution of β-carotene crystals[34] consequent to the rupture of cellular ultrastructures (i.e., chromoplasts), a process that took place in a similar fashion with all the cooking procedures. The loss of crystalline form and their dissolution, as a consequence of the thermal treatment, has been recognised as a major factor leading to carotenoid isomerisation.[35] The peak broadening (Δ) was found to be highly correlated to the increase of the ratio cis/all trans β-carotene ($R= 0.857$, $p<0.01$) and the decrease of α– carotene ($R= -0.767$, $p<0.01$), respectively. Such evidence may suggest that the crystal dissolution and the variation of the carotenoid pattern undergoes to the same dynamic during the cooking process.

In extracting the information from the intensity of the Raman peaks (I), a parameter that has been used in literature to evaluate the total carotenoids content,[15, 36] more caution has to be used. As a matter of fact, the Raman detection efficiency is different for each carotenoid. In particular, it is higher for shorter or *cis*-carotenoids. An enhancement of the Raman signal was measured in all the cooked samples in comparison to the frozen ones and it was found to be correlated with the increase of the *cis* β-carotene ($R= 0.718$, $p<0.01$), and with the decrease of

α– carotene ($R= -0.600$, $p<0.05$). This enhancement was due to an improvement of Raman detection efficiency of carotenoids. This effect was probably linked to the well-known increase of bio-accessibility of carotenoids after cooking,[24] due to the cooking induced changes in the vegetable microstructure. As a matter of fact, the same change reduced also the self-screening effects present when carotenoids were confined in crystalline form inside the chromoplasts.

Another indication of the occurrence of carotenoid isomerisation came from the analysis of the background always present in these Raman spectra, which has to be attributed to fluorescence (F). The intensity of this fluorescence is known from the literature to increase with the concentration of *cis* isomers of the β-carotene, which are characterized by a higher fluorescence quantum yield with respect to the all *trans* isomer.[37] As reported in Table 3, the fluorescence background intensity (F) increased significantly after cooking, in agreement with the increase of both *cis* β-carotene ($R = 0.718$, $p <0.01$) measured by HPLC and *cis/*all *trans* ratio ($R = 0.646$, $p <0.05$). Based on this parameter, the steamed carrots presented the most modified carotenoids, while the boiled carrots retain the highest concentration of pristine carotenoids, in agreement with that stated in the above paragraph. Moreover, the *cis-trans* isomerisation of carotenoids has been associated with a decrease of the red colour intensity of carrot [28] as confirmed by the colour analysis (see at a* and ΔE values of Table 2) and by the high correlation found between the a* decrease and the increase of cis/all trans β-carotene ratio ($R = -0.760$, $p< 0.01$).

3.3.2 Effect of the cooking time

In order to exploring the potentiality of Raman spectroscopy on the characterization of the carotenoid dynamic during thermal treatment, the microwaving method was selected in view of the short time of cooking as well its potential application as blanching treatment.

For this purpose, the carrot samples were cooked in the microwave oven, as previously described, and extracted at different times until 12 min. The Raman spectra were acquired in about 1000 points along the disk diameter at a 10 μm resolution.

The mean values of the parameters extracted for each spectrum, such as intensity, frequency, width, and fluorescence baseline, relative to the ν_1 vibration were plotted as a function of cooking time in the graphs of Figure 2.

The peak frequency, width and fluorescence increased in average with cooking time, in accordance with the carotenoid pattern changes previously discussed. An effect of heating time of Raman spectra of carotenoids was previously described comparing conventional and microwave heating on olive oil.[11] In our study, it appears noteworthy that the trend of parameters (i.e. ν and I) of Raman frequency reached a steady state around 10 min of cooking time.

4. Conclusions

The findings of this preliminary work showed that changes of carotenoid pattern can be evaluated by studying the evolution of the Raman spectral features on frozen and cooked carrots, as a function of cooking procedure applied. The trend of spectral parameters revealed good statistical correlations with those measured by traditional techniques, in particular with the quantitative data obtained by HPLC and the qualitative information given by colourimetry.

In addition, the evaluation of the trend of such spectral parameters with heating time could offer a fast, non-destructive, real time information on chemical transformation of carotenoids to optimize protocols during industrial heating treatments, like blanching. This may be useful to realize a process improvement aimed at the maximum carotenoids bioaccessibility and bioavailability by modulating the isomerisation degree.

Finally, the spatial resolution capability of Raman spectroscopy may be applied to study the evolution of the spatial distribution of carotenoids in carrots and other carotenoid-rich vegetables undergone to thermal treatments.

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Captions of figures

Figure 1 Average Raman spectra of frozen carrots before and after cooking. The three main peaks centered at the frequencies ν_1= 1530 cm^{-1}, ν_2= 1159 cm^{-1} and ν_3= 1008 cm^{-1}correspond to the C=C, C-C and $C\text{-}CH_3$ molecular vibrations of carotenoids.

Figure 2. Extrapolated Raman spectral parameters from ν_1 (C=C stretch) peak as a function of microwaving time of frozen carrot. The parameter is reported in the four panels: A) peak intensity I, B) center frequency ν, C) peak width Δ and D) fluorescence intensity F. The points correspond to the average values, while the bars indicate the distribution variance.

Table 1. Carotenoid content (g kg^{-1}) in frozen and cooked carrots. †

Samples	α-carotene	all *trans* β-carotene	*cis* β-carotene	α/all trans β carotene	cis/ all trans carotene	phytoene	phytofluene	total carotenoids
Frozen	1.07 a	2.19 c	0.12 d	).49	0.05	0.24 b	0.28 b	3.90 c
Boiled	1.15 a	2.88 a	0.73 a	).40	0.25	0.34 a	0.43 a	5.53 a
Steamed	0.54 c	1.28 d	0.37 c	).42	0.29	0.17 c	0.21 c	2.57 d
Microwaved	0.91 b	2.33 b	0.51 b	).39	0.22	0.27 b	0.30 b	4.32 b

† n=3, sample size = 1. Means in column followed by different letters are significantly different ($P \leq 0.05$). RSD < 3 %.

Table 2. Colour parameters for frozen and cooked carrots. †

	Frozen	Boiled	Steamed	Microwaved
L*	53.6 a	51.9 b	49.7 c	52.3 ab
a*	30.7 a	27.1 b	23.5 c	27.7 b
b*	40.9 a	36.3 b	31.3 c	35.4 b
ΔE	-	6.5 b	12.8 a	6.9 b

† n=3, sample size = 15. Means in row followed by different letters are significantly different ($P \leq 0.05$). RSD < 5 %.

Table 3. Extrapolated Raman spectral parameters from ν_1 (C=C stretch vibration) in frozen and cooked carrots. The reported parameters in columns are: ν center frequency, I peak area, Δ peak width and F fluorescence background.

	Frozen	Boiled	Steamed	Microwaved
ν (cm^{-1})	1525.55 d	1526.28 c	1527.15 a	1526.76 b
Δ (cm^{-1})	9.72 b	10.82 a	10.83 a	10.77 a
I (cps)	1617 b	2365 a	1912 a	2176 a
F (cps)	2240 c	3007 b	3147 a	3046 b

† n=3, sample size = 10. Means in row followed by different letters are significantly different ($P \leq 0.05$). RSD of ν and Δ is < 0.1 and 3 %, respectively. RSD of I and F is < 15 %.

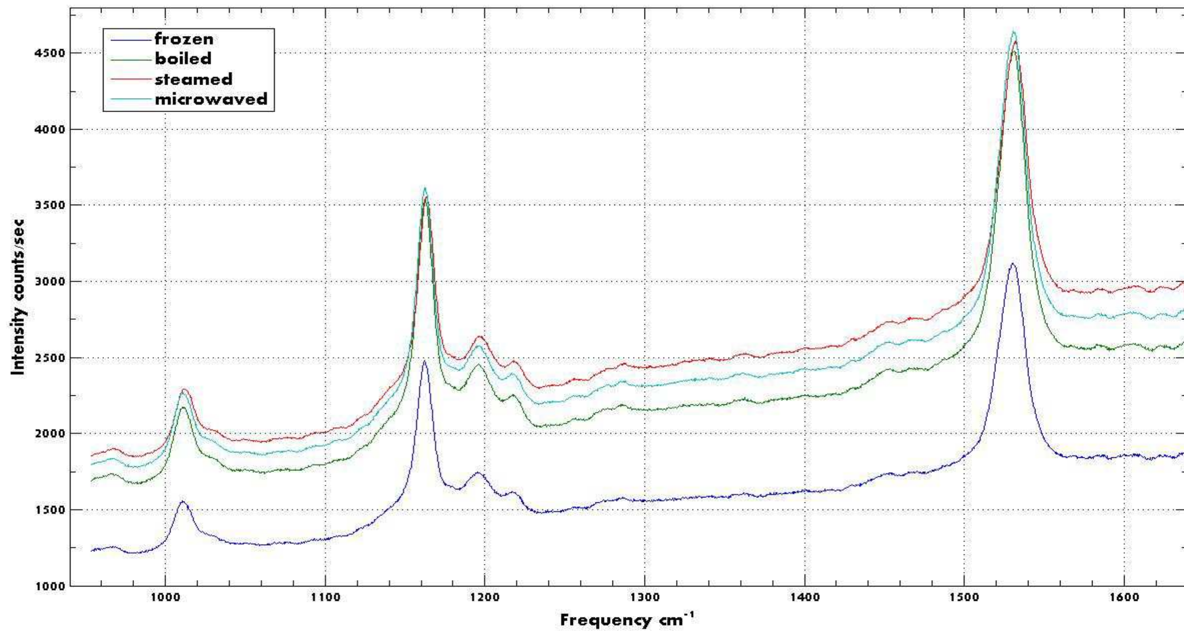

Figure 1

Conclusions

Conclusions

The growing demand of vegetable products represents a big challenge for the food industry because of the seasonal and perishable nature of such products. The industrial vegetable preservation is traditionally based on the use of temperature as process variable both at high (blanching, canning etc.) or low temperature (freezing etc.). Despite the effectiveness of such treatments, they have a strong impact on the physico-chemical properties of the products.

New technologies, called nonthermal, are based on the use of process variables different from temperature such as pressure (high pressure), electricity (pulsed electric fields) or electromagnetic wavelength (ultrasounds etc.). These technologies have the advantage of getting the same goals of the traditional treatments preserving sensory and nutritional properties. Also academia is called from the governments to contribute in the improvement of the traditional technologies or in the development of the novel ones to better preserve vegetable quality parameters.

A lack of literature is however still present, because of the fragmented information on the effect of the conventional heating treatments and the few data on the novel ones. In this context, this PhD thesis dealt with the evaluation of the effects of traditional thermal and innovative nonthermal preservation technologies on the quality parameters of vegetables.

In the first section of the thesis (Study 1, 2), the effect of a complete industrial freezing process was evaluated on asparagus, green beans and zucchini. The first goal of this study was the better comprehension of the existing relations between the macroscopical textural properties and the histological the structure obtained by means of the optical microscopy. Being texture one of the most considered vegetable characteristics for consumer choice, understanding the phenomena at the base of its modifications may help the manufacturers to better control the processes. Samples were examined in all the stages of production “from farm to fork”: raw/uncooked, as control test; blanched; boiled from the raw samples; and after industrial freezing and domestic storage. Important relations were found between structural and textural modifications under processing. Cells dehydration, separation and swelling of the cell walls, occurring with the increasing of the

treatment strength, was correlated with an extended softening degree observed by the texture analysis. Frozen boiled samples resulted the more damaged because of the formation of large fissures in the tissue due to the ice formation, resulting in extended texture loss. The nature of the vegetables also influenced the responses, visible in the increased asparagus cutting force after blanching, in relation with their fibrousness linked to the presence of fenolic acid in the cell walls; or in the higher cutting and penetration forces observed for the frozen boiled green beans in comparison to the cooked from the boiled ones, because of the extended swollen of the cell walls; or the impossibility to perform the penetration test on zucchini, because of the extended softening related to absence of a mechanical tissue. A correlation between these first observations and the extraction of some bioactive compounds was also found. The antioxidant molecules resulted better extracted during blanching and boiling, being instead leaching out in the samples boiled after freezing, as revealed by the FRAP assay. The blanching treatment retained phytochemicals in all green vegetables studied. This was particularly true for green beans where a good retention of ascorbic acid and an increase of rutin were found. The frozen storage did not negatively affect phytochemicals, with increasing of lutein and rutin, particularly for green beans, suggesting the effectiveness of blanching on the peroxidase inactivation.

The reduction of chlorophyll during the thermal treatments was related with the increasing of pheophytin, but only partially in agreement with the color changes, resulting particularly evident during blanching and frozen storage. A good retention of phytochemicals and even an extraction of lutein were observed in the frozen boiled samples, in particular for asparagus and zucchini. The antioxidant activity results were in disagreement with the lutein trend, but in agreement with the quantity of total phenols. Color changes were comparable for green beans and zucchini, but less with asparagus that resulted more affected from the frozen treatment.

In conclusion, this study filled a gap of knowledge about the correlation between texture and histology and their modification under process. The texture analysis results a useful tool to understand the structural modifications, also in accordance with the nature of the product. The

different physical and nutritional responses given by the three vegetables to the process encourages manufacturers and researchers to join together in order to develop industrial freezing process conditions according to the matrix of vegetable, to provide texturally and nutritionally higher quality frozen product.

In the second section (Study 3,4), the effect of different cooking methods was evaluated on the quality of selected vegetables. The study 3 deals with the evaluation of the effect of boiling, steaming or microwave cooking on raw and frozen carrots. The steamed samples resulted more affected from the treatment among all, revealing an extended reduction in both texture and color parameters. A good relation between textural and microstructure was obtained, as also confirmed by the observation of the microwave cooked sample, resulted the hardest among the cooked samples and with a good compactness of the cells. A high impact of the freezing process was observed on carrot structure, resulting instead in a better preservation of color after cooking.

The impact that different cooking methods have on the quality of carrots is of great interest both for consumers and manufacturers, being interested in the preservation of the sensorial and nutritional quality of the products.

In support of this evidence, the study 4 provides important information, being poor debated in literature. It focused on the effect of cooking in a combined air/steam oven, with different time/temperature combinations, in comparison with a steam one, on Brussels sprouts and pumpkin. The results showed that, a better texture retention than the control was observed for Brussels sprouts cooked at 90°C and for pumpkin cooked for 5'. For Brussels sprouts the external green loss was opposite to the improved internal color, better also than the steamed sample. A good preservation of pumpkin orange color was observed, resulted instead enhanced in the steamed samples.

A good retention of the total phenols was observed for both the air/steam cooked vegetables, being even better for Brussels sprouts, in comparison to the control, when cooked for 40'. A better preservation of the total antioxidant activity was instead observed for the steam cooked samples.

These findings show that the selection of a proper time/temperature combination is essential for obtaining air/steam cooked vegetables with good qualitative and nutritional properties.

In the third part of the thesis (Study 5), the effect of high hydrostatic pressure treatment was studied for the first time on beetroot, known healthy products. The comparison of a pressure treatment (650 MPa; 21°C) performed in four different holding times (3,7,15,30 min) in comparison to the traditional thermal blanching and canning was performed. A better retention of the physical and nutritional properties was observed for the HHP treatment in comparison to the canning one. In comparison to blanching, the high pressure treatment better retain the textural properties, being instead more invasive on color, probably because of the still high enzymatic activity that may affect the pigments and in general the product during its shelf-life. A good retention of the bioactive compounds was observed for the high pressure treated samples, resulted even in an enhancement for the phenol content and the total antioxidant activity, in a time independent way, as also revealed from the PCA analysis that clustered together the four HHP treatments without any scatter according to the holding time.

Increasing the pressure holding time, an improvement of the textural parameters and a better extraction of the betanin pigment was observed, in relation to the reducing of the redness (a*).

Finally the result of this study, encourage the use of the high pressure for the beetroot treatment, resulting in a good and retention of all the quality studied parameters despite the strong conditions used. Further improvements of the process are request, although it’s already possible to suggest the use of smaller treatment time, being the performances independent from it (as also confirmed by the PCA analysis), which would involve also in an improvement of the energetic cost.

The last study (Study 6) focused on the changing observed in the carotenoid pattern of frozen carrots differently cooked by means of Raman spectroscopy in comparison to the classical chemical or colour analysis. The modifications of the Raman spectra under cooking gave information about the carotenoid degradation or isomerisation, the increase of their bio-accessibility, the modification of their chemical status together to the increasing of their molecule disorder. A good correlation was

found between the three analytical methods, according to the cooking procedure. In addition, the evaluation of the trend of such spectral parameters with heating time could offer a good method to optimize protocols during industrial heating treatments. Raman technique can give furthermore information about the molecule distribution in the sample and without any pre-treatment, representing a more complete, faster and ecological alternative to the traditional methods.

In conclusion, while the application of the traditional thermal (freezing or cooking) practices resulted in an extended modification of qualitative and nutritional parameters, the application of the HHP process showed their better retention despite the strong treatment conditions. The extent of the modifications observed was related to the type of vegetable and to the process conditions, therefore this finding should encourage to research and plan specific processing conditions. The application of Raman spectroscopy and verification of its effectiveness underlines the continuing trend of research towards more specific methods of investigation.

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Acknowledgements

La realizzazione di questo lavoro di tesi non sarebbe stata possibile senza l'importante contributo di brillanti menti impegnate nella ricerca scientifica.

Ringrazio in primis la mia insostituibile guida, nonchè tutor, Prof.ssa Emma Chiavaro. I miei diretti collaboratori, Prof. Gustavo Victor Barbosa-Canovas, Dott. Paolo Camorani, Dott.ssa Martina Cirlini, Prof. Luigi Cristofolini, Prof.ssa Chiara Dall'Asta, Prof. Vincenzo Fogliano, Dr. Tommaso Ganino, Dott.ssa Teresa Mazzeo, Dott.ssa Ilce Gabriela Medina-Meza, Prof.ssa Nicoletta Pellegrini, Dr. Massimiliano Rinaldi, Dott.ssa Maria Zaupa. E tutta l'area di Tecnologie del dipartimento di Scienze degli Alimenti dell'Università di Parma, Prof. Davide Barbanti, Dott. Giampaolo Betta, Dott.ssa Eleonora Carini, Dott.ssa Elena Curti, Dott. Matteo Cordioli, Dott. Joseph Agoura Diantom, Prof. Roberto Massini, Prof. Germano Mucchetti, Dott. Alessandro Pugliese, Prof.ssa Elena Vittadini.