



Thermal versus high pressure processing of carrots: A comparative pilot-scale study on equivalent basis

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ABSTRACT

This report describes the first study comparing different high pressure (HP) and thermal treatments at intensities ranging from mild pasteurization to sterilization conditions. To allow a fair comparison, the processing conditions were selected based on the principles of equivalence. Moreover, pilot- and industrial-scale equipment were opted for, supporting conditions close to industrial application. The overall impact on carrot quality was characterized by analyzing a wide range of quality attributes, including specific (micro)nutrients (carotenoids and sugars), process-induced contaminants (furfural and 5-hydroxymethylfurfural), enzyme activities (pectin methylesterase and peroxidase) and other relevant quality aspects (texture, dry matter content and color). This study demonstrated that the potential benefit of HP over thermal processing of carrots is largely dependent on the processing intensity applied. Thermal sterilization affected carrot quality the most, while mild and severe thermal pasteurization, mild and severe HP pasteurization and HP sterilization resulted in a comparable overall quality.

Industrial relevance: The extensive nature of this investigation and the corresponding results can be considered of key importance for further implementation of HP technology in the food industry, since a correct and complete assessment of process-induced changes is of major importance in the context of legislative aspects of novel processing technologies.

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1. Introduction

Ever since the invention of thermal processing as a method for food preservation in the 19th century, there has been a relentless search to reduce the degree of thermal damage to the quality of food products. Traditional in-pack thermal processing of conductively heating products relies on extensive heat treatment to ensure a prolonged shelf life and food safety, because of slow heat penetration to the core of the product and subsequent slow cooling. This relatively long exposure to high temperatures is commonly known to induce detrimental quality changes, affecting nutritional as well as organoleptic attributes. In fact, a number of foods exist that cannot even be converted into shelf-stable products by means of retort processing, because of the non-acceptable or low-quality values obtained after long exposure to heat (Barbosa-Cánovas & Juliano, 2008). This knowledge, together with the growing consumer awareness of the relation between health and diet, has stimulated the food industry to take up

the challenge of exploring the potential of alternative novel preservation technologies. The extensive research on high pressure (HP) processing has created new opportunities to improve the balance between the safety and quality of current food products. During the last decades, this technology has emerged as an industrially adopted method for food pasteurization of rare and cooked meat, fish and seafood, dairy and vegetable products, and ready-to-eat meals. In this application, HP processing is essentially a non-thermal decontamination process, in which the food is typically subjected to pressures of 400 to 600 MPa at ambient or cooled temperature for 1 to 15 min. These conditions inactivate vegetative microorganisms, providing safety and prolonged shelf life to chilled or high-acid (e.g. fruit juices, guacamole) foods (Patterson, 2005; Smelt, 1998). Unfortunately, bacterial spores are extremely resistant to commercially attainable pressure levels, and therefore low-acid shelf-stable products cannot be achieved by elevated pressure only (Black et al., 2007; Heinz & Knorr, 2005; Smelt, 1998). To reach commercial sterility, an additional inactivating factor is necessary. In HP sterilization, HP is combined with elevated temperature (de Heij, van den Berg, van Schepdael, Hoogland, & Bijmolt, 2005; Heinz & Knorr, 2005; Wilson & Baker, 1997; Wilson, Dabrowski, Stringer, Moezelaar, & Brocklehurst, 2008). By taking advantage of the compression heating associated

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with pressure build-up, an initial product temperature of 70–90 °C can be increased to 110–125 °C in less than a minute. After a holding time of about 5 min or less, a fast cooling is obtained at pressure release. This instantaneous volumetric heating and cooling results in much shorter processing times, compared to conventional thermal processing, which is recently claimed to be the key benefit of HP sterilization over retort sterilization (Barbosa-Cánovas & Juliano, 2008; de Heij et al., 2005; Matser, Krebbers, van den Berg, & Bartels, 2004). Although HP pasteurized products have already been successfully introduced to the market, implementation of HP sterilization in the food industry still remains a challenge.

There is a wealth of scientific publications on the impact of HP processing on food quality attributes. In the majority of them, HP-treated foods are generally claimed to have superior quality compared to their thermally treated counterparts (Knorr et al., 2011; Patterson, Linton, & Doona, 2008), including a better retention of nutritional value, flavor, texture and color. This statement is usually explained by the reduced thermal load and the limited effect of HP on covalent bonds. Although a few authors have reported possible adverse effects of HP, whether or not in comparison to thermal processing (Gimenez, Kajda, Margomenou, Piggott, & Zabetakis, 2001; Krebbers, Matser, Koets, & van den Berg, 2002; Lambert, Demazeau, Largeteau, & Bouvier, 1999; Porretta, Birzi, Ghizzoni, & Vicini, 1995; Verbeyst, Bogaerts, & der Plancken, in press), the general conviction remains that HP processing is usually preferable over thermal processing. However, among the studies in which a comparison is made of the impact of HP and thermal processing on food quality attributes, one general inadequacy persists: HP and thermal processing conditions are usually not selected on equivalent basis. Often irrelevant comparisons are made, such as comparing a mild HP pasteurization with a thermal cooking process (20 min in boiling water), two processes with completely different aims. To make a fair comparison, the impact on quality must be evaluated with regard to an equivalent starting point. The most obvious foundation to base this comparison on is equivalent microbial safety. Until today, very few studies have taken this key factor into account: the study on HP and pulsed electric field (PEF) processing of orange juice, described in two complementary publications by Timmermans et al. (2011) and Vervoort et al. (2011), and the study on HP processing of carrots by Knockaert et al. (2011).

The objective of the present work was to compare the impact of HP and thermal processing on a fair basis, by selecting processing conditions that result in equivalent microbial safety, and to extensively characterize this impact by analyzing a wide range of quality attributes. For this comparison, carrot, a vegetable widely consumed worldwide with important dietary value, was selected as relevant vegetable-based model food product. Carrots were often used in previous studies to investigate the potential of HP processing. In this context, Trejo Araya et al. (2009) have conducted a comprehensive study, comparing sensory and other quality attributes of HP and thermally treated carrots, though unfortunately no equivalent processing conditions were used. Knockaert et al. (2011) on the other hand, did make a comparison on equivalent basis; however, this study was limited to the effects on structural and carotenoid properties. Moreover, as for most studies, treatments were performed on lab-scale HP equipment, which often results in conditions deviating from those in industrial-scale equipment. In the present work, pilot- and industrial-scale equipment were opted for, supporting conditions closer to industrial application. In addition, the effect of increasing processing intensity was investigated by comparing thermal and HP processing impact at three intensity levels: mild pasteurization, severe pasteurization and sterilization. After making a well-considered selection of processing conditions for each intensity level, the impact of all treatments was investigated on a wide range of carrot quality attributes, providing an overall characterization of the treated carrots.

2. Materials and methods

2.1. Sample handling

A single batch of fresh carrots (*Daucus carota* cv. Nerac) was bought wholesale and stored at 4 °C. All treatments were done within 10 days, in which thermal and HP treatments of the same intensity were carried out on the same day. On the day before processing, carrots were manually cut into pieces as homogeneously as possible, packed and stored again at 4 °C. Packing was done in plastic bags for HP treatment (15/75 oriented polyamide/polypropylene, inner dimensions 8 × 28 cm for mild pasteurization and 5.5 × 28 cm for severe pasteurization and sterilization, filled with 140 ± 0.5 g and 100 ± 0.5 g carrot pieces respectively) and glass jars for thermal treatment (99 mm height and 80 mm diameter, filled with 190 ± 0.5 g carrot pieces). In each bag and jar, two to six carrot pieces with calibrated dimensions (discs of 10 mm height and 10 mm diameter) were included (in total, 12 per treatment repetition) for texture measurements (cfr. Section 2.3). On the day of processing, a brine of deionized water was added to the packed carrots in the morning, after which the package was sealed. All transportations were done at a maximum temperature of 4 °C.

After processing, the bags and jars were stored overnight at 4 °C and opened the following day, after which the carrot pieces were separated from the brine. Texture analysis was done immediately after on the calibrated pieces, which were also used for color analysis the same day. The non-calibrated pieces for the remaining analyses were stored at –40 °C until the day of analysis. At this time, a certain amount of frozen carrot pieces was cooled in liquid nitrogen and mixed for 20 s at 7500 rpm (Grindomix GM 200, Retsch, Haan, Germany) resulting in a fine powder.

2.2. Processing

A selection of thermal and HP processing conditions was made to result in equivalent microbial safety, for three different intensity levels: mild pasteurization, severe pasteurization and sterilization. In Table 1, an overview of all processing conditions is given, with the corresponding target pathogen, per intensity level. A discussion on the selection of these conditions is given in Section 3.1. Each treatment was repeated six times.

2.2.1. Thermal treatments

Thermal treatments were performed in a pilot-scale water-cascading retort (Barriquand Steriflow retort, Paris, France). During the heating and holding phase, the external heat exchanger was

Table 1

Equivalent thermal and HP processing conditions, with regard to microbial safety, per intensity level. For thermal treatments, *P* and *F* indicate process values, T_p the process temperature of the retort and *HT* the holding time. The coming up time of the retort was 8.5 min. For HP treatments, T_i indicates the initial temperature and T_m the calculated maximum temperature after pressure build-up (cfr. Section 3.1).

Intensity level	Processing conditions		Target pathogen
	Thermal	HP	
Mild pasteurization	$P_{70}^{10} = 2$ min $T_p = 70$ °C HT = 7.5 min	600 MPa $T_i = \sim 10$ °C HT = 10 min	<i>Listeria monocytogenes</i>
Severe pasteurization	$P_{90}^{10} = 10$ min $T_p = 90$ °C HT = 19.6 min	700 MPa $T_i = 38$ °C $T_m = 61.3$ °C HT = 5 min	Non-proteolytic <i>Clostridium botulinum</i> type E spores
Sterilization	$F_0 = 5$ min $T_p = 117$ °C HT = 23 min	700 MPa $T_i = 90$ °C $T_m = 124.8$ °C HT = 3 min	Proteolytic <i>C. botulinum</i> type A spores

supplied with steam, while cold water was used for the cooling phase. In a first step, the samples were equilibrated within the retort at 40 °C (30 min). The retort coming-up time was programmed at 8.5 min, and was followed by a predetermined holding time at the process temperature (Table 1). The product temperature was registered in the coldest spot in 2 different glass jars, using thermocouples (type T) connected to a thermocouple box (TR9216, Ellab) and a CMC-92 data acquisition system (Ellab). The coldest spot in the jars and the required processing times corresponding to a certain process value and temperature were previously determined by Lemmens et al. (in press).

2.2.2. High pressure treatments

HP treatments were conducted in 2 different HP units. Mild HP pasteurizations were performed in an industrial Wave 6000/55 unit (55 l, 20 cm inner diameter, NC Hyperbaric, Burgos, Spain). The equipment allows controlled pressure build-up at a rate of 150 MPa/min. Carrot samples were loaded in two perforated cylindrical food baskets (polyethylene, 18 cm outer diameter, 85 cm outer length), designed by the manufacturer to optimize the vessel filling ratio. The HP unit was located in a cooled working area at 10 °C, which was assumed to be the initial temperature before pressure build-up, and tap water was used as pressure medium.

For severe HP pasteurizations and HP sterilizations, a pilot-scale unit (developed by Resato, Solico, Unilever and Wageningen UR Food and Biobased Research, The Netherlands) was used. This unit consists of a vertically oriented vessel (2.5 l, 10 cm inner diameter), in which pressure can be built up at a rate of ~30 MPa/s. Carrot samples were first loaded in a perforated cylindrical sample holder (polyoxymethylene acetale polymer (POM), 8.4 cm outer diameter, 29.4 cm outer length) and preheated in a water bath for a predetermined time at the initial temperature (10 min at 38 °C for severe HP pasteurization and 10 min at 90 °C for HP sterilization), to ensure a homogeneous temperature distribution in the carrot samples. In a next step, the sample holder was transferred to an isolating sample container (POM, 9.5 cm outer diameter, 36 cm outer length, with movable stopper), also equilibrated at the same temperature and containing water of the preheating water bath. The whole was immediately transferred to the HP vessel, which was equilibrated at the initial temperature by an outer heating jacket and bottom heater. During processing, the heat, generated by pressure build-up, was aimed to be maximally retained by an isolating POM liner at the inner vessel wall and the isolating POM sample container, which was designed to optimally fill the vessel. After the selected holding time, pressure was released and the sample container was transferred to a cooling bath (10 °C), where container and holder were separated again to enable fast cooling.

2.3. Texture measurement

Carrot tissue hardness was evaluated by a compression test, using a TA-XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). The following parameters were used: load cell 25 kg, probe 25-mm diameter aluminum cylinder, and test speed 1 mm/s. The hardness of a carrot cylinder was defined as the maximum force needed to compress the carrot cylinder to 70% of its original thickness (De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008). For each treatment repetition, the mean value of the compression forces of 12 carrot cylinders was considered as a single data point.

2.4. Dry matter measurement

The total dry matter content was determined gravimetrically as the residue remaining after drying. 3 g of the mixed carrot powder was weighed in a porcelain crucible and dried in a vacuum oven at 70 °C.

2.5. Pectin methylesterase (PME) activity measurement

PME was extracted from the carrots by mixing 2 g carrot powder with 0.2 M Tris (hydroxymethyl-amino methane)-HCl buffer containing 1 M NaCl (pH 8.0, 1:1.3 w/v) and stirring this mixture for 2 h at 4 °C. The crude PME extract was obtained by filtration, using a cheese cloth.

The PME activity in the crude extracts was determined by monitoring the release of acid during pectin hydrolysis as a function of time at pH 7.0 and 25 °C. The reaction mixture consisted of 0.5 ml crude extract and 30 ml of a 0.35% (w/v) apple pectin solution (DM 70–75%, Fluka, Buchs, Switzerland) containing 0.117 M NaCl. During pectin hydrolysis, the pH was maintained constant by addition of 0.01 N NaOH using an automatic pH-stat titrator (Metrohm, Herisau, Switzerland) and the enzyme activity was related directly to the amount of NaOH added per minute (De Roeck et al., 2008).

2.6. Peroxidase (POD) activity measurement

POD was first extracted from the carrots by adding 5 ml of 0.2 M sodium phosphate buffer pH 6.5, containing 1 M NaCl, to 1 g of carrot powder and stirring this for 10 min at 4 °C. The mixture was filtered over a cheese cloth and the filtrate was further centrifuged for 30 min at 22,000 ×g and 4 °C.

The POD activity of the enzyme extract was measured spectrophotometrically by adding 200 µl supernatant and 2800 µl substrate solution (3 g/l o-phenylenediamine solution and 1.11 g/l hydrogen peroxide in 0.2 M sodium phosphate buffer pH 6.5) to a 1 cm path cuvette. The formation of the colored oxidation product (2,3-diaminophenazine) was measured as the change in absorbance at 485 nm and 25 °C for 10 min. The POD activity of each extract was measured in duplicate.

2.7. Color measurement

Color measurements (CIE $L^*a^*b^*$ values) were conducted using a Hunterlab ColorQuest colorimeter (45°/0° geometry, Illuminant D65, Reston, VA, USA). The instrument was calibrated daily with a black and green ceramic tile. The carrot cylinders, previously used for texture measurement, were mixed (Waring Blender 7010 G, Torrington, CT, USA) and applied to calibrated glass Petri dishes (three replicates). At a 10° angle, the CIE color space coordinates were determined in triplicate and averaged: L^* , indicating the lightness (varying from 0, black, to 100, white), a^* , a measure for the redness (varying from -60, green, to +60, red) and b^* , the yellowness (varying from -60, blue, to +60, yellow).

In addition, total color differences (ΔE) were calculated using Hunter-Scotfield's equation:

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

2.8. Analysis of the carotenoid content

Carotenoids were extracted from the carrot samples according to the method described by Sadler, Davis, and Dezman (1990). 1 g of carrot powder was homogenized with 50 ml extraction solvent (50% hexane, 25% acetone, 25% ethanol, containing 0.1% butylated hydroxytoluene) by stirring for 20 min at 4 °C. After addition of 15 ml milli-Q water, the solution was stirred for another 10 min at 4 °C. This mixture was transferred to a separatory funnel, in which the water layer was separated from the organic phase. The latter, containing the carotenoids, was filtered through a 0.20 µm syringe filter (Chromafil PET-20/25, Macherey-Nagel, Düren, Germany), resulting in the eventual extract.

Of this extract, both the total carotenoid content and the carotenoid profile were analyzed. The total content was measured spectrophotometrically at 450 nm, the maximum absorbance wavelength of β -carotene (the major carotenoid in carrots) in hexane. Hexane with 0.1% butylated hydroxytoluene was used as a blank. The total carotenoid concentration was calculated applying Beer's law, with the extinction coefficient of β -carotene in hexane, $E_{1\%}^{1\text{ cm}} = 2560$ (Hart & Scott, 1995).

The carotenoid profile was analyzed by RP-HPLC. The apparatus consisted of an Agilent 1200 Series HPLC system (Agilent technologies, Diegem, Belgium) equipped with a UV-DAD detector (G1315B, Agilent technologies, Diegem, Belgium). For all HPLC analyses, the autosampler was cooled to 4 °C. The carotenoids were separated on a YMC C30 column (150×4.6 mm, 3 μm particle size, Alltech, GRACE, Deerfield, IL, USA), coupled to a corresponding C30 guard cartridge and equilibrated at 25 °C. Linear gradient elution was applied, at a flow rate of 1 ml/min. The gradient was built up in 13 min from 81% methanol, 15% methyl-*t*-butyl ether and 4% milli-Q water to 41% methanol, 55% methyl-*t*-butyl ether and 4% milli-Q and held there for 5 min. The carotenoids were detected at 450 nm. Calibration curves of α -carotene, β -carotene, 9-*cis*- β -carotene, 13-*cis*- β -carotene, 15-*cis*- β -carotene (CaroteNature, Lupsingen, Switzerland), dissolved in hexane, were used for quantification. All solvents used for HPLC analyses were HPLC grade.

During all steps of the carotenoid analysis, light exposure was avoided (darkroom, amber recipients) to prevent carotenoid degradation during analysis. For each treatment repetition, three carrot extracts were prepared, of which the total carotenoid content and the carotenoid profile were analyzed once.

2.9. HPLC analysis of sugars

Sugars were extracted according to the method of O'Donoghue et al. (2004), which was indicated as the most efficient procedure by Davis, Terry, Chope, and Faul (2007). 0.5 g of carrot powder was combined with 10 ml of 62.5% (v/v) methanol and mixed well. This slurry was placed in a shaking water bath at 55 °C for 15 min, with vortex mixing for 20 s every 5 min. The samples were cooled on ice and centrifuged for 10 min at 16,000 $\times g$ and 4 °C. The supernatant was filtered through a 0.45 μm syringe filter and 5 μl of the filtrate was injected into the HPLC system.

Separation was carried out on a Prevail carbohydrate ES column (250×4.6 mm, 5 μm particle size, Alltech, Grace, Deerfield, IL, USA), protected with a Prevail C₁₈ guard cartridge (7.5×4.6, 5 μm particle size, Alltech, Grace, Deerfield, USA), by isocratic elution using 75% (v/v) acetonitrile/water at a flow rate of 1 ml/min and 30 °C. Sugars were determined by evaporative light scattering detection (Alltech 3300 ELSD, Grace, Deerfield, IL, USA). The drift tube temperature was set at 38 °C and nitrogen was used as nebulizer gas at a flow rate of 1.5 ml/min. Identification and quantification of the sugars was performed respectively by comparison with retention times and by using calibration curves based on peak area. For this, standard solutions of different sugars (glucose, fructose and sucrose) were prepared in 62.5% (v/v) methanol.

2.10. HPLC analysis of furfural and 5-hydroxymethylfurfural

Extraction and RP-HPLC analysis of furfural and HMF was carried out as described by Lee, Rouseff, and Nagy (1986), with some modifications. First, 10 ml juice was clarified with 0.5 ml Carrez I and II. After 30 min, the mixture was centrifuged at 24,000 $\times g$ and 4 °C for 15 min. The supernatant was filtered through a 0.45 μm syringe filter and 1 ml of the filtrate was applied on a C18 SPE pre-column (Sep-PAK Waters, Milford, MA, USA), preconditioned with 2 ml methanol and 5 ml 0.5% acetic acid. After washing the SPE column with 2 ml of milli-Q water, furfural and HMF were selectively eluted with

4.5 ml of ethyl acetate, and dried with anhydrous sodium sulfate. The eluate was again filtered through a 0.45 μm syringe filter before injection (5 μl).

The chromatographic separation was performed using a Zorbax Eclipse XDB C18 column (150×4.6 mm, 5 μm particle size, Agilent technologies, Diegem, Belgium), coupled to a Prevail C18 guard cartridge, at 25 °C. A mixture of 15/85 (v/v) acetonitrile/water was used as mobile phase at a flow rate of 1 ml/min. The effluent was monitored at 280 nm.

Detection limits (LOD) were determined as the lowest concentration of a standard solution that yields a signal-to-noise ratio (S/N) of 3, with noise level defined as the peak-to-peak noise of the baseline measured over a period of 5 min. Stock solutions of furfural and HMF were prepared and diluted to the appropriate concentrations in 10% methanol.

2.11. Statistical data analysis

As mentioned in Section 2.2, each processing condition was repeated six times. Data reported are the mean and standard error values of the analyzed quality properties, calculated from the replicates. All data were subjected to one-way analysis of variance (ANOVA) using SigmaPlot (version 11.0, Systat Software Inc, Chicago, IL, USA). Differences between the means were tested with Tukey's multiple comparison test and were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Selection of microbially equivalent processing conditions

As pointed out in the Introduction, a fair comparison of novel and conventional processing can only be achieved when processing conditions are selected on equivalent basis. Therefore, a selection of thermal and HP processing conditions was made to result in products with equivalent microbial safety (Table 1). This was done for three levels of processing intensity, to gain insight in the effect of increasing processing intensity on the differences between thermal and HP processing impact.

For mild pasteurization of low-acid foods, such as carrots, traditionally a 6 log reduction of *Listeria monocytogenes* is aimed at, ensuring a shelf life of maximally 10 days at 5 °C. In terms of thermal processing, this corresponds to a process value of $P_{10}^{10} = 2$ min (ECFF, 2006; CSIRO Food and Nutritional Sciences, 2010). According to the kinetic data gathered by Dogan and Erkmén (2004), a 6 log reduction of *L. monocytogenes* is also attained after HP treatment of brain heart infusion broth for 10 min at 600 MPa and 25 °C. Of all reported matrices in which kinetic studies on HP inactivation of *L. monocytogenes* were conducted, brain heart infusion broth (pH 6.65) was considered most similar to carrots (pH 5.5–6.5; (Belitz, Grosch, & Schieberle, 2004)). Since the HP unit used for mild HP pasteurization was located in a cooled working area at 10 °C (cfr. Section 2.2.2), and the machine was not equipped with a thermal control unit, it was impossible to conduct the treatments at 25 °C. However, treatment at an initial temperature of 10 °C was considered at least as safe, since the pressure stability of vegetative microorganisms is usually found to decrease at lower temperatures (Heinz & Buckow, 2010).

For low-acid products intended to have a longer shelf life (up to 6 weeks at 5 °C), severe pasteurization is necessary, which provides a 6 log reduction of psychrotrophic non-proteolytic *Clostridium botulinum* type E spores. It is generally agreed that this is achieved after a thermal treatment with process value $P_{10}^{10} = 10$ min (ECFF, 2006; CSIRO Food and Nutritional Sciences, 2010). Until today, literature data on HP inactivation of non-proteolytic *C. botulinum* spores are extremely scarce. The single study on HP inactivation of *C. botulinum* type E spores in phosphate buffer (pH 7.0) by Reddy et al. (1999) reports a complete inactivation (>5 log reduction) after 5 min at 827 MPa and 55–60 °C.

Therefore, an initial temperature was chosen, which would result in a process temperature of 60 °C after pressure build-up. Given the fast pressure build-up and the isolating properties of the sample container and liner of the equipment used for severe HP pasteurization and HP sterilization, assuming adiabatic heating during pressure build-up seems admissible. Based on this assumption and the databases of the thermodynamic and thermophysical properties of water under pressure (National Institute of Standards and Technology (NIST) and International Association for Properties of Water and Steam (IAPWS)) the temperature increase was calculated from:

$$\frac{dT}{dp} = \frac{T\alpha(T,p)}{\rho(T,p)C_p(T,p)} \quad (2)$$

with α the volumetric thermal expansion coefficient (K^{-1}), ρ the density (kg/m^3), C_p the specific heat ($J/kg.K$) at a particular T temperature (K) and p pressure (Pa) (Barbosa-Cánovas & Rodríguez, 2005; Denys, Van Loey, & Hendrickx, 2000). Taking a safety margin for potential heat loss during processing into account, 38 °C was selected as initial temperature, which would result in a maximum temperature of 61.3 °C after pressure build-up till 700 MPa, the maximum pressure of the equipment. Pressure was released after 5 min treatment time.

For commercial sterilization of low-acid foods, a general consensus was established that a minimum process should at least result in a 12 log reduction of proteolytic *C. botulinum* type A spores. Therefore, a thermal treatment with a process value F_0 of 3 min has been adopted as the minimum standard for a 'botulinum cook'. However, in practice, most low-acid foods are processed beyond this minimal value, e.g. for controlling spoilage organisms (Holdsworth, 2009; Ramaswamy & Markakis, 2006). Therefore, a thermal sterilization process was applied resulting in a more common F_0 -value of 5 min. Until today, the current research state of HP sterilization is still insufficient to allow commercial applications. Extensive inactivation of *C. botulinum* by commercial HP treatment is most likely only possible in combination with initial temperatures that exceed 70 °C (Bull, Olivier, van Diepenbeek, Kormelink, & Chapman, 2009; Wilson et al., 2008). Although researchers often concluded that pressure and heat act synergistically in inactivating *C. botulinum*, this synergy was not consistently observed among different strains and products by Bull et al. (2009). To err on the side of caution, a complete lack of synergy was assumed, and HP sterilization conditions were chosen to be at least thermally equivalent to the thermal treatment with an F_0 -value of 5 min. Analogous as for severe HP pasteurization, an initial temperature of 90 °C was calculated to result in a maximum temperature of 124.8 °C after pressure build-up till 700 MPa. Taking into account a small potential heat loss during processing, a holding time of 3 min should correspond to a F_0 -value of at least 5 min.

3.2. Texture

Texture is a vital component of the organoleptically perceived quality of carrots. During thermal processing, this texture is known to be critically affected, since the subjection to elevated temperatures entails turgor loss due to mechanical damage and loss of cell adhesion attributed to solubilization and depolymerization of pectic polymers, predominantly by β -elimination (Greve, Mcardle, Gohlke, & Labavitch, 1994; Greve, Shackel, et al., 1994; Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006; Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009). HP processing, on the other hand, has been found to result in only minimal β -eliminative pectin solubilization and depolymerization (De Roeck et al., 2008; De Roeck et al., 2009; Van Buggenhout et al., 2009).

Texture is a multi-parameter attribute. In this study, the carrot hardness or firmness was assessed by a compression test, widely applied for objective texture measurement (Lu & Abbott, 2004). In Fig. 1, the hardness of the processed carrots, relative to the hardness of untreated carrots is plotted. All processing conditions caused a

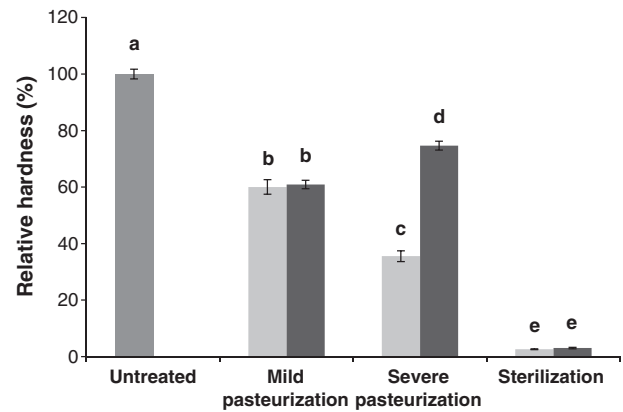


Fig. 1. Relative hardness of untreated (■), thermally processed (□) and HP processed (■) carrots, per processing intensity. Significant differences ($p < 0.05$) are indicated with different letters.

significant decrease in hardness. With the exception of severe HP pasteurization, hardness decreased with increasing processing intensity. Mild thermal and mild HP pasteurization had a similar impact on carrot hardness (40 and 39% reduction respectively). For thermal processing, traditionally the main contributing factor to tissue softening is indicated to be pectin depolymerization by β -elimination (Sila et al., 2006). The occurrence of this reaction is, however, not considerable at the low temperatures applied for mild pasteurization. Also for HP pasteurization, β -elimination is negligible, as this reaction requires relatively high temperatures to take place (De Roeck et al., 2009). Therefore, texture decrease of both the thermally processed as well as the HP processed carrots can probably be mainly attributed to mechanical membrane damage and the associated turgor loss, which resulted in the same hardness loss.

At severe pasteurization intensity on the other hand, a much better retention of carrot hardness was found after HP treatment (75%), compared to thermal treatment (36%). This retention was even significantly higher than after mild pasteurization. A likely explanation for the relatively larger hardness in comparison to mild HP pasteurization can probably be found in the action of PME during the preheating phase. At the mild temperatures used for preheating (38 °C), prior to severe HP pasteurization, carrot PME is activated (Jolie et al., 2009; Sila et al., 2007) and can demethoxylate pectin, creating a large number of free carboxyl groups on the pectin chains. In this way, crosslinking with naturally present divalent cations, mostly calcium ions, can occur, enhancing cell adhesion (Smout, Sila, Vu, Van Loey, & Hendrickx, 2005). For mild HP pasteurization, no preheating was necessary, thus PME could not cause any notable demethoxylation. The thermal pasteurizations, on the other hand, were also preceded by a preheating phase at 40 °C, which supported PME action. However, the subsequent thermal load during processing was much larger than for HP pasteurization. Furthermore, severe thermal pasteurization conditions can in their turn induce β -elimination, which provides an additional softening, compared to the mild pasteurizations and severe HP pasteurization. Knockaert et al. (2011) reported comparable results for mild and severe pasteurization, although texture retention after severe HP pasteurization was not higher than after mild pasteurizations, but similar. However, this previous study was conducted at lab-scale, without taking into account a preheating phase of the carrots. Severe HP pasteurization in the present study, on the other hand, was performed in pilot-scale equipment with a much larger carrot load, which requires a preheating phase to ensure all carrots are at the required initial temperature before pressure build-up (cfr. Section 2.2.2), which bears more resemblance to industrial applications. This apparent contradiction can be seen as a confirmation of the aforementioned hypothesis of PME action and pectin crosslinking during the preheating phase.

Sterilization caused a substantial texture loss, both for thermal and HP treatment (both 97% reduction). The similar impact of thermal and HP sterilization on carrot texture contradicts previous research by Nguyen, Rastogi, and Balasubramaniam (2007), De Roeck, Mols, Duvetter, Van Loey, and Hendrickx (2010), De Roeck et al. (2008) and Knockaert et al. (2011). These studies indicate a clear benefit of HP sterilization over thermal sterilization in texture preservation. Nguyen et al. (2007) and De Roeck et al. (2010, 2008) performed kinetic experiments in which the processing temperature and time were matched for thermal and HP processing. The temperature history during the pre-process phase, preceding the actual process holding time, however, was clearly different for both types of treatment. This pre-process phase can have a substantial additional impact to the overall process impact, especially at high process temperatures. Due to the fast compression heating in HP processing, the integrated temperature-time profile or thermal load was smaller for HP treatment, compared to thermal treatment. These differences in pre-process phase can aid in explaining the observed improved texture preservation during HP sterilization. Knockaert et al. (2011), on the other hand, did choose processing conditions with an equivalent thermal load. They observed a slightly better hardness after HP sterilization; however, this difference was not significant. Just as for severe HP pasteurization, in all previous studies lab-scale HP equipment was used, with a small carrot load and associated negligible preheating phase. Although a better texture preservation was demonstrated for HP sterilization at lab-scale, apparently this advantage is largely reduced by the slow preheating phase at pilot- and industrial scale. These results indicate that care should be taken in extrapolating conclusions from lab-scale experiments to industrial scale.

3.3. Dry matter content

The impact of thermal and HP processing on the dry matter content of carrots is depicted in Fig. 2. All treatments resulted in a significant decrease in dry matter. However, this decrease was more pronounced after thermal processing, compared to HP processing. A reduction in dry matter content can indicate leaching out of water-soluble components (e.g. sugars and degraded pectins) into the brine on the one-hand, and/or water absorption from the brine in between or into denatured cells on the other hand (De Belie, Laustsen, Martens, Bro, & De Baerdemaeker, 2002; Nielsen & Martens, 1997). A possible explanation for the fact that this effect was less pronounced during HP treatments could be that a product under pressure comprises a more compact structure, allowing less freedom of movement to water and/or water-soluble molecules (reduced diffusion). An increased processing intensity did not cause any significant changes in dry matter content.

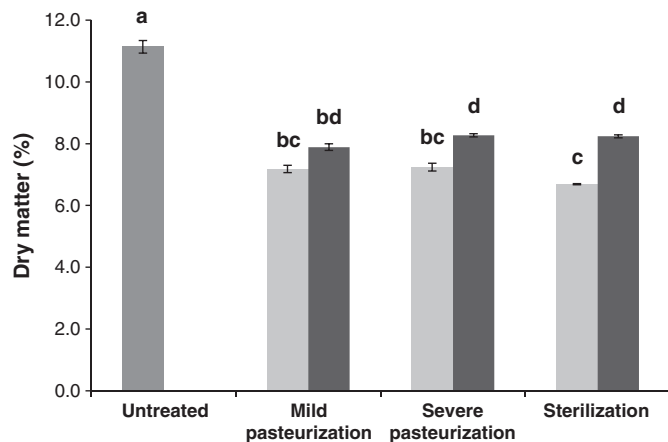


Fig. 2. Dry matter content of untreated (■), thermally processed (■) and HP processed (■) carrots, per processing intensity. Significant differences ($p < 0.05$) are indicated with different letters.

3.4. Pectin methylesterase (PME) activity

The texture of carrots is largely dependent on pectic polymers present in the cell walls and middle lamella, contributing to tissue firmness and elasticity. The enzyme pectin methylesterase (PME) catalyzes the removal of methyl esters from pectin, resulting in a number of free carboxyl groups on its backbone. This reaction can play a key role in stabilizing the cell wall structure. On the one hand, the reduction of methylester groups hampers the rate and extent of β -eliminative pectin solubilization and depolymerization, occurring at high processing temperatures ($> 80^\circ\text{C}$). On the other hand, the free carboxyl groups allow ionic crosslinking of the pectin chains with divalent cations, such as calcium ions, which results in a strengthening of the middle lamella, cementing the cells together (Jolie, Duvetter, Van Loey, & Hendrickx, 2010; Sila et al., 2009; Van Buggenhout et al., 2009).

The thermal stability of PME from different plant sources is well documented by kinetic inactivation studies in different media (Duvetter et al., 2009). Generally, PMEs are rather thermolabile, although the carrot tissue can offer a considerable protection against denaturation (Balogh, Smout, Nguyen, Van Loey, & Hendrickx, 2004). Mild thermal pasteurization of the carrot pieces at 70°C could only induce an inactivation of 18.6% (Fig. 3). Likewise, Balogh et al. (2004) determined a slow inactivation rate ($D_{70^\circ\text{C}} = 64.8$ min) at this temperature. Severe thermal pasteurization at 90°C and thermal sterilization, on the other hand, caused a complete activity loss. Considering the large temperature dependence ($z_T = 4.1^\circ\text{C}$) found by Balogh et al. (2004), this result could be expected.

In contrast to the temperature sensitivity, most PMEs are rather barotolerant (Duvetter et al., 2009). PME inactivation during mild and severe HP pasteurization of the carrot pieces was significantly smaller (12.2 and 32.7% respectively), compared to the corresponding thermal treatments. Accordingly, Balogh et al. (2004) and De Roeck et al. (2008) reported a stabilizing effect of pressure against PME inactivation in carrots at 40 and 80°C respectively. The preheating phase, preceding severe HP pasteurization, was conducted at rather mild temperatures (38°C), which cannot cause PME inactivation. Moreover, at this temperature, PME exhibits an increased activity towards pectin (Jolie et al., 2009; Sila et al., 2007), eventually resulting in increased firmness, and offering protection against texture loss during further processing (cf. Section 3.2). The preheating phase for HP sterilization, however, was performed at 90°C , at which most PME activity will be easily lost. In combination with the HP treatment at high temperature, a residual activity of only 3.1% remained.

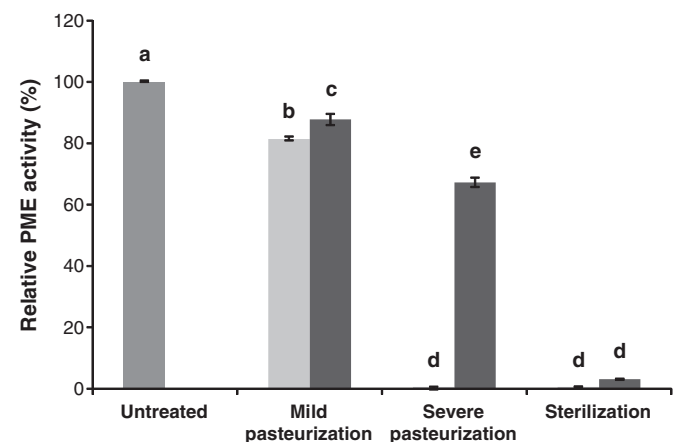


Fig. 3. Relative PME activity in untreated (■), thermally processed (■) and HP processed (■) carrots, per processing intensity. Significant differences ($p < 0.05$) are indicated with different letters.

3.5. Peroxidase (POD) activity

Peroxidase (POD), an oxidoreductase enzyme occurring in almost all plants, is considered since many years to have an empirical relationship to off-flavors and off-colors during storage of fruit and vegetable products (Burnette, 1977). However, until today its role in causing quality problems remains controversial (Anthon & Barrett, 2002; Barrett & Theerakulkait, 1995). Despite this dispute, the degree of POD inactivation is often assayed to monitor the effectiveness of heat treatments. The choice of POD as indicator enzyme can be attributed to its high concentration in most plant tissues, its high thermal stability and its ease of activity assay (Anthon & Barrett, 2002; Burnette, 1977; Gökmen et al., 2005; Günes & Bayindirli, 1993). It is generally considered to be the most thermostable enzyme in plants; consequently a heat treatment sufficient to inactivate POD is assumed to eliminate all other quality-affecting enzymes as well (Burnette, 1977). Nonetheless, in this study, mild thermal pasteurization already caused an activity loss of 77.1%, while after severe thermal pasteurization and thermal sterilization POD was completely inactivated (Fig. 4). Previous studies on POD inactivation in carrots by Günes and Bayindirli (1993) and Goncalves, Pinheiro, Abreu, Brandao, and Silva (2010) reported higher stabilities at 70 °C, although Goncalves et al. (2010) also found a complete inactivation after only 2 min at 90 °C. In contrast to the general conviction of POD being the most heat stable enzyme in plants, in the current study, PME was found to be more resistant to the applied heat treatments (cf. Section 2.5). This discrepancy was also reported for carrot juice by Vora, Kyle, and Small (1999), who suggested that PME might be a more suitable indicator for thermal treatment of carrots.

PODs from various plant sources are generally rather pressure stable (Fang, Jiang, & Zhang, 2008; Garcia-Palazon, Suthanthangjai, Kajda, & Zabetakis, 2004; Krebbers et al., 2002; Rastogi, Eshtiaghi, & Knorr, 1999; Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). HP pasteurization had a significantly smaller impact on POD activity (58.5% and 19.4% residual activity after mild and severe pasteurization respectively) than the corresponding thermal treatments. HP sterilization, though, caused a complete activity loss, as for thermal sterilization. This inactivation was presumably already largely achieved during the necessary preheating phase at 90 °C, before the actual HP treatment. Only one previous study on POD inactivation during HP treatment of carrots was found in literature (Akyol, Alpas, & Bayindirli, 2006); however, these authors applied lower pressure levels (300 to 450 MPa) and longer treatment times (15 to 60 min), making a comparison not sensible. Anese, Nicoli, Dall'aglio, and Lerici (1995) and Soysal, Soylemez, and Bozoglu (2004) investigated the effect of pressure on carrot POD extracts. According to

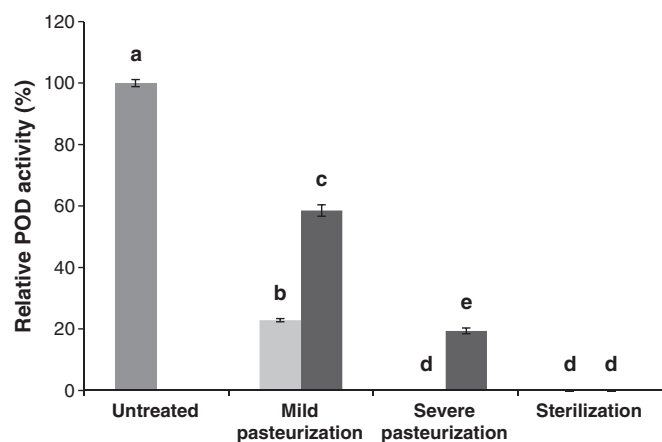


Fig. 4. Relative POD activity in untreated (■), thermally processed (□) and HP processed (■) carrots, per processing intensity. Significant differences ($p < 0.05$) are indicated with different letters.

Soysal et al. (2004), a treatment of crude extract for 15 min at 600 MPa, room temperature, resulted in 44.7% residual activity, in line with the degree of inactivation found in this study after 10 min treatment. At the same pressure–temperature conditions, Anese et al. (1995) applied shorter treatments of only 1 min on cell-free extracts, which caused inactivation or activation of POD (about 50 to 120% residual activity), depending on the pH of the extract (5.0, 6.0 or 7.0). Increasing the pressure to 900 MPa entailed a complete inactivation for all extracts.

3.6. Color

Although orange carrots are the norm in the West, their color may vary from cultivar to cultivar, from place to place and from season to season, depending upon numerous factors. Nevertheless, color can serve as a useful criterion of quality and can be an indication of various types of deteriorative changes undergone by carrots. Fig. 5 shows that all treatments had a significant impact on the CIE color parameters. The changes in L^* , a^* and b^* values indicate a deterioration of the initial intense orange color of the untreated carrots. At the level of mild pasteurization, no significant differences in this color degradation were found between thermal and HP treatment. However, when the process intensity was forced up, thermal and HP processing impact diverged. Severe HP pasteurization and HP sterilization retained significantly more redness (a^*), compared to their thermal counterparts. Thermal sterilization, for its part, resulted in a large increase in yellowness (b^*), which was not observed for HP sterilization.

Color degradation of carrots by thermal and HP processing has been reported frequently. Nguyen et al. (2007), Patras, Tiwari, Brunton, and Butler (2009), Trejo Araya et al. (2009), Goncalves et al. (2010), Nguyen et al. (2010) and Patras, Brunton, and Butler (2010) perceived a decrease in L^* and a^* values. The impact on b^* values, however, seems to be dependent of the processing conditions applied. Trejo Araya et al. (2009) and Nguyen et al. (2010) reported a significant decrease after thermal and HP processing at pasteurization intensities, while after more severe processes, applied by Nguyen et al. (2010), the b^* value remained unaffected. Increasing the intensity of a thermal process to a sterilization intensity of $F_0 = 3$ min, Patras, Brunton, Da Pieve, Butler, and Downey (2009) detected an increase in b^* , consistent with the present results. However, when F_0 reached 15 min and higher, b^* decreased again. An increase in b^* values after HP treatment has not been reported till today.

Some authors have compared the impact of thermal processing with that of HP processing on carrot color and all report a better retention after HP treatment (Nguyen et al., 2007, 2010; Trejo Araya et al., 2009). However, caution is recommended for these conclusions, since the processing conditions used were not selected keeping in mind the principles of equivalence (cf. Section 1). The current study has demonstrated that improved color retention by HP treatment is largely dependent on the processing intensity.

Because significant differences in individual color parameters and separations in PCA biplots cannot indicate whether a difference in color is perceptible by humans or not, Hunter-Scottfield's total color differences (ΔE) were calculated between each type of carrots (Table 2). In theory, a ΔE of 1 represents a just noticeable color difference to the human eye, under ideal viewing conditions. However, in less than ideal lighting, samples with values between 2 and 3 can be considered equivalent by some viewers. Therefore, ΔE values of at least 3, which indicate a color difference perceptible by most people, are marked in bold. Although all values in Table 2 are larger than 1, denoting differences that can be considered noticeable, not all of them are larger than 3. The differences in between the two types of mild pasteurized carrots, and with severe pasteurized carrots are possibly not perceptible by all people, while this was the case for the differences between all remaining types of

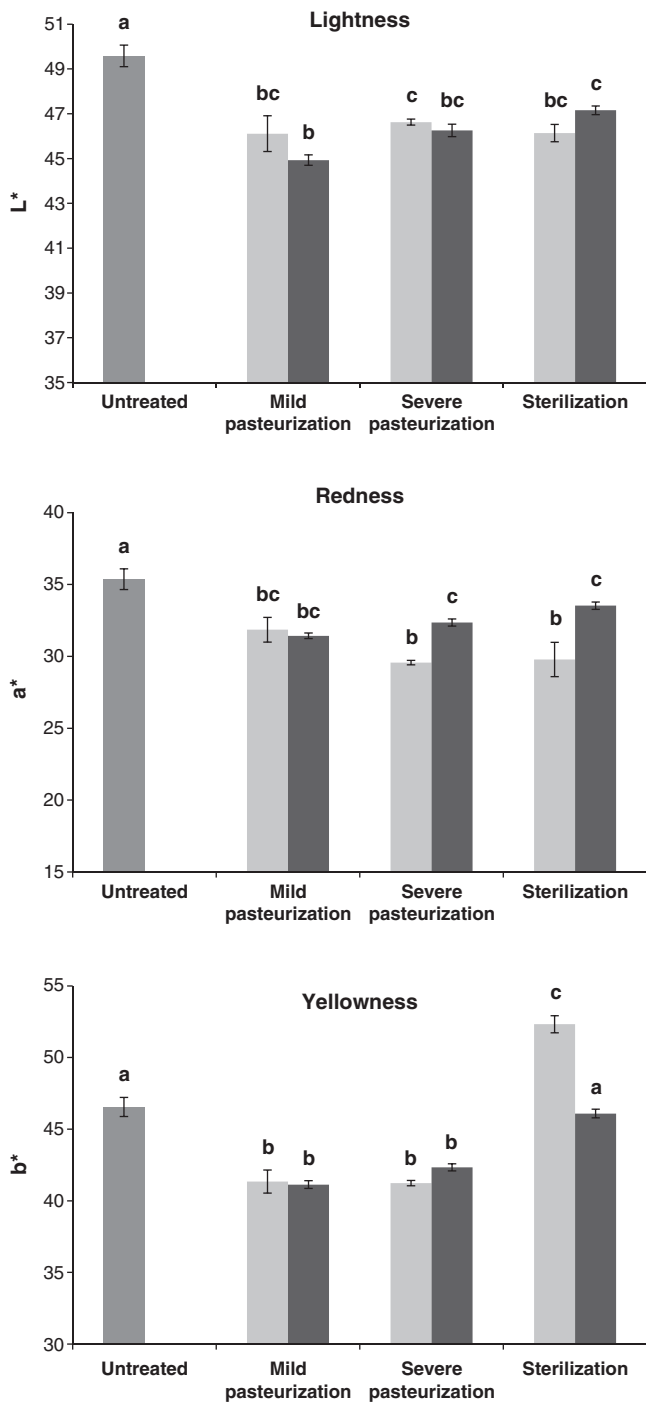


Fig. 5. Color parameters (CIE $L^*a^*b^*$ values) of untreated (■), thermally processed (▨) and HP processed (■) carrots, per processing intensity. For each parameter, significant differences ($p < 0.05$) are indicated with different letters.

carrots. Furthermore, all processing conditions caused a clearly noticeable change in color from raw carrots, with HP sterilization preserving the original color the most. The largest differences were found between the thermally sterilized carrots and all other carrots.

3.7. Carotenoids

The natural orange color of carrots is mainly due to their rich content in carotenoid pigments, predominantly α - and β -carotene. Nonetheless, apart from being responsible for the color of carrots, the most valuable role of these components lies in their provitamin

Table 2

Total color differences (ΔE) between the different treatments, calculated according to Eq. (1). UT indicates untreated, MTP mild thermal pasteurization, MHPP mild HP pasteurization, STP severe thermal pasteurization, SHPP severe HP pasteurization, TS thermal sterilization and HPS HP sterilization. Differences larger than 3, perceptible by most people, are marked in bold.

	UT	MTP	MHPP	STP	SHPP	TS	HPS
UT		7.18	8.15	8.40	6.15	8.74	3.08
MTP			1.27	2.34	1.13	11.18	5.15
MHPP				2.52	2.01	11.38	5.82
STP					3.02	11.11	6.29
SHPP						10.31	4.03
TS							7.35
HPS							

A activity, important for several vital systematic functions in humans. Additionally, they are also known for their antioxidant capacity, protecting cells and tissues from damaging free radicals (Maiani et al., 2009). Carrots are the richest source of α - and β -carotene of the common fruits and vegetables consumed in Europe and the U.S. Furthermore, they serve as the major dietary source of carotenoids (Holden et al., 1999; O'Neill et al., 2001). The raw carrots used for this study contained on average 2.65 mg/100 g α -carotene and 8.26 mg/100 g β -carotene (Table 3), which falls within the concentration range reported elsewhere (Holden et al., 1999; Maiani et al., 2009; O'Neill et al., 2001). In addition, a small quantity of 13-*cis*- β -carotene was found. A limited amount of β -carotene *cis*-isomers in raw carrots was also found by Hart and Scott (1995), Lemmens, Van Buggenhout, Oey, Van Loey, and Hendrickx (2009) and Knockaert et al. (2011).

The impact of thermal and HP processing on the carotenoid content of carrots was largely dependent on the processing intensity applied. After pasteurization, no considerable differences occurred. The total carotenoid content, determined by spectrophotometry, remained constant after mild and severe pasteurization, both for thermal and HP processing (Fig. 6). Also no significant changes occurred in the individual α - and β -carotene concentrations. Only the initial small amount of 13-*cis*- β -carotene significantly decreased during mild pasteurization (Table 3). This *cis*-isomer is presumably more sensitive to oxidation than the all-*trans*-carotenes (Rodríguez & Rodríguez-Amaya, 2007).

It was only at the level of sterilization that substantial changes were detected. At this intensity, thermal processing caused a decrease of 28.3% in α -carotene and 31.0% in β -carotene. This was accompanied by a generation of β -carotene *cis*-isomers (9-*cis*- β -carotene, 13-*cis*- β -carotene and 15-*cis*- β -carotene), indicating isomerization of β -carotene. However, the overall increase in *cis*-isomers could not account for the loss in β -carotene concentration. In other words, besides isomerization, also oxidation of α - and β -carotene must have occurred. This is confirmed in Fig. 6, in which a significant decrease of 15.6% in the total carotenoid content can be seen. HP sterilization, on the other hand, did not result in a significant decrease in the total carotenoid content, nor in the individual α - and β -carotene concentrations. Only a minor increase in 13-*cis*- β -carotene was found.

Carotenes are said to be very sensitive to isomerization and oxidation, due to their highly unsaturated structure (Rodríguez-Amaya & Kimura, 2004). In carrots, however, the carotenes are enclosed in a protective matrix, which can preserve them from degradation. This could explain the stability during pasteurization; these conditions are not severe enough to cause a notable isomerization and/or oxidation. For HP sterilization, though, the thermal load was at least as severe as for thermal sterilization (*cf.* Section 3.1), so one could expect a significant change in carotenoid content, just as for thermal sterilization. However, the kinetic study performed by Lemmens et al. (2010) revealed low activation energies for isomerization and

Table 3

Carotenoid profile of untreated, thermally processed and HP processed carrots, per processing intensity. For each carotenoid, significant differences ($p < 0.05$) are indicated with different letters in superscript.

Concentration in carrots (mg/100 g)				
Carotenoid	Process intensity	Raw	Thermally processed	HP processed
α -carotene	Untreated	2.65 \pm 0.03 ^{ab}		
	Mild pasteurization		2.94 \pm 0.07 ^a	2.76 \pm 0.08 ^{ab}
	Severe pasteurization		2.71 \pm 0.12 ^{ab}	2.58 \pm 0.03 ^b
	Sterilization		1.90 \pm 0.05 ^c	2.53 \pm 0.03 ^b
β -carotene	Untreated	8.26 \pm 0.11 ^{ab}		
	Mild pasteurization		8.58 \pm 0.15 ^a	8.13 \pm 0.16 ^{ab}
	Severe pasteurization		8.08 \pm 0.18 ^{ab}	7.74 \pm 0.07 ^b
	Sterilization		5.70 \pm 0.21 ^c	7.98 \pm 0.09 ^{ab}
9- <i>cis</i> - β -carotene	Untreated	nd*		
	Mild pasteurization		nd	nd
	Severe pasteurization		nd	nd
	Sterilization		0.220 \pm 0.005	nd
13- <i>cis</i> - β -carotene	Untreated	0.286 \pm 0.021 ^{ac}		
	Mild pasteurization		nd	0.209 \pm 0.007 ^b
	Severe pasteurization		0.347 \pm 0.012 ^{ce}	0.251 \pm 0.008 ^{ab}
	Sterilization		0.963 \pm 0.019 ^d	0.364 \pm 0.010 ^e
15- <i>cis</i> - β -carotene	Untreated	nd		
	Mild pasteurization		nd	nd
	Severe pasteurization		nd	nd
	Sterilization		0.310 \pm 0.004	nd

* nd = not detected.

degradation of β -carotene, indicating a low temperature sensitivity of the isomerization and degradation rate constants. The fact that HP sterilization was performed at a higher temperature but with a much shorter process time, compared to thermal sterilization, can therefore explain the difference in impact.

In accordance with the results of this study, Lemmens et al. (in press) found only a significant degradation of β -carotene in thermally sterilized carrots, but not after thermal pasteurization. Knockaert et al. (2011), on the other hand, observed an increase in the total carotenoid content after thermal sterilization, which could be explained by an improved extractability due to matrix disruption. The amount of carotenoids detected after processing is always the net effect of possible degradation and increased extractability (Rodriguez-Amaya & Kimura, 2004). In the current study though, more severe sterilization conditions were chosen, with a more industrially relevant F_0 -value of 5 min, instead of the 3 min applied by Knockaert et al. (2011) (cf. Section 3.1). During this more severe process, degradation can take the upper hand of the enhanced extractability.

The impact of HP pasteurization and sterilization on the carotenoid content of carrots was also reported to be insignificant by McInerney, Seccafen, Stewart, and Bird (2007) and Knockaert et al.

(2011). On the other hand, Chen, Peng, and Chen (1995) and Kim, Park, Cho, and Park (2001) found a considerable amount of isomerization and degradation after less severe thermal and HP processing of carrot juice. However, in carrot juice the protective matrix of intact carrots or carrot pieces is lost, which can explain the reduced stability of the carotenoids.

Changes in the carotenoid profile and total content inevitably affect carrot color. The strongest correlation was found for carrot yellowness. All individual carotenoids as well as the total content were significantly correlated with b^* values ($p < 0.05$), with positive correlation coefficients for α - and β -carotene and the total carotenoid content ($r = -0.79$, -0.76 and -0.72 respectively) and negative correlation coefficients for the three β -carotene *cis*-isomers ($r = 0.82$, 0.85 and 0.82 respectively). Carrot redness (a^*), on the other hand, was only significantly correlated with the three *cis*-isomers ($r = -0.40$, -0.33 and -0.39 respectively). Their increase in concentration, upon isomerization of β -carotene, was accompanied by a significant loss in redness. Although the lightness (L^*) of the carrots significantly decreased during processing, no significant correlations were found with the evolution of the carotenoid profile. After all, for this color dimension no significant differences were found in impact of the different processing conditions, which was the case for the carotenoid profile.

Notwithstanding color being an important quality aspect of (processed) carrots, the strongest concern in carotenoid degradation is the associated loss of their nutritional function. In this comparative study, it was found that only thermal sterilization caused a significant loss in carotenoids. However, as stressed by Lemmens et al. (2010), Lemmens et al. (in press), Lemmens, Colle, Van Buggenhout, Van Loey, and Hendrickx (2011), the carotenoid degradation during severe thermal processing is of minor importance, compared to the increased bioaccessibility. Processing induces fracture of plant cell walls and membranes, which allows carotenoids to be released more easily from the tissue. This bioaccessibility is in fact more relevant for the nutritional value of carrots, rather than the concentration itself. Lemmens et al. (in press); Lemmens et al., 2011) have demonstrated that the relative increase in β -carotene bioaccessibility, due to thermal processing, is considerably larger than the decrease in β -carotene concentration. By contrast, HP sterilization did not result in a significant loss in carotenoids, but in addition, Knockaert et al. (2011) found no increase in bioaccessibility. This points out that,

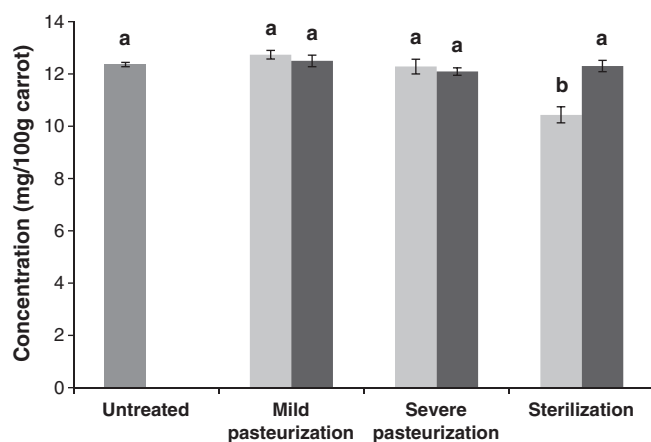


Fig. 6. Total carotenoid content (by spectrophotometry) of untreated (■), thermally processed (■) and HP processed (■) carrots, per processing intensity. Significant differences ($p < 0.05$) are indicated with different letters.

despite their decreased carotenoid content, thermally sterilized carrots might possess the highest nutritional value after all.

3.8. Sugar profile

Besides providing a source of energy, sugars are indispensable for the flavor of carrots. According to Simon, Peterson, and Lindsay (1980), they are one of the most important sensory indicators for consumer perception, since the sweetness and overall preference of carrots are enhanced by their sugar content. The initial total sugar concentration of the untreated carrots was 5.24 g/100 g carrot, of which sucrose was the predominant contributor (representing 54.8% of the total content), followed by glucose (25.2%) and fructose (20.0%). Comparison with previously reported data on various carrot varieties shows that these amounts can be considered average values (Kreutzmann, Christensen, & Edelenbos, 2008; Nyman, Svanberg, Andersson, & Nilsson, 2005).

Processing caused a significant reduction in all sugar concentrations, with comparable percentage losses for the three different sugars (Fig. 7). Conversely, Pither (2003) stated that the sugar content of fruits and vegetables remains largely unaffected during canning. It is very likely that the losses found in the present study are mainly a result of leaching out, rather than a breakdown of the sugars. This hypothesis is confirmed by the strong correlation coefficients found between the dry matter content and sugar contents (0.87, 0.93 and 0.97% for glucose, fructose and sucrose respectively, $p < 0.05$). Comparable conclusions were made by Rodríguez-Sevilla, Villanueva-Suárez, and Redondo-Cuenca (1999) for the impact of thermal sterilization on carrot sugar content. In accordance with the results on dry matter content, the decrease was significantly more pronounced for thermal treatments, irrespective of the intensity level, which can again be related to reduced sugar mobility due to the compressed carrot structure under HP (cf. Section 3.3).

3.9. Furfural and 5-hydroxymethylfurfural (HMF)

Browning reactions in food, like the Maillard reaction and ascorbic acid oxidation, are a widespread phenomenon during preservation processing, which affects their flavor, appearance and nutritional value. Care must be taken to keep these reactions under control, since excessive browning could lead to detrimental changes of the product (Eskin, 1990; Nursten, 2008). Of particular concern is the toxicity and potential mutagenicity of some of the intermediates formed (Capuano & Fogliano, 2011; Skog & Alexander, 2006). Furfural and 5-hydroxymethylfurfural (HMF) are two furanic compounds formed during these browning reactions, which have gained interest last decades due to their potential

cytotoxic, genotoxic, mutagenic and carcinogenic risks (Glatt & Sommer, 2006). However, until today, the toxicological relevance of their human exposure has not yet been clarified. Nevertheless, because of their correlation with browning reactions, they are commonly measured as indicators of quality deterioration as a result of excessive heating in a wide range of foods (Delgado-Andrade, Seiquer, Haro, Castellano, & Navarro, 2010; Gaspar & Lucena, 2009; Soria, Olano, Frias, Penas, & Villamiel, 2009).

The method of analysis used ensured a detection limit of 0.1 mg/kg carrot for both components. However, only the thermally sterilized carrots contained a detectable amount of HMF, namely 0.27 ± 0.01 mg/kg carrot or 4.05 ± 0.11 mg/kg dry matter. Furfural, though, was not detected in any of the carrot samples. Likewise, Soria et al. (2009) detected HMF quantities in the same range after convective air drying of carrots.

At first sight, these results are in favor of HP processing, instead of thermal processing, for sterilization of carrots. However, in comparison to concentrations detected in some other food products, like chicory, dried fruits, breakfast cereals, coffee, etc. (Capuano & Fogliano, 2011), the amount detected in the thermally sterilized carrots of this study can be considered negligible. The estimates for human daily intake range from 2 to 150 mg per person, of which bread and coffee are regarded to deliver the most important contribution (Capuano & Fogliano, 2011). Consequently, considering an average carrot meal portion, the small amount detected in thermally sterilized carrots can hardly affect the overall daily intake and is not relevant for potential toxicological risks involved. The HMF concentration detected can only offer an indication of occurring browning reactions, which was also confirmed by a significant correlation with the carrot yellowness ($r = 0.82$, $p < 0.05$). This relation with b^* value was reported by Delgado-Andrade et al. (2010) as well.

The reduced HMF production during HP sterilization, in comparison to thermal sterilization, is an affirmation of the retarding effect of HP on the overall Maillard reaction, demonstrated by De Vleeschouwer, Van der Plancken, Van Loey, and Hendrickx (2010). Although this benefit of HP sterilization over thermal sterilization is not of toxicological relevance for processing of carrots, it might be of significant importance for other food products eligible for HP sterilization. Further investigation can elucidate this matter.

3.10. Principal component analysis (PCA)

To visualize the similarities and differences in overall impact of the different processes on carrot quality, all analyzed parameters were brought together in a principal component analysis (PCA). The biplot for the first two principal components, explaining 76.3% of the total variance, is shown in Fig. 8. In this plot, three clusters of samples can be distinguished, indicating a comparable overall quality within these groups. The first group consists of the untreated carrots. The large distance between this group and the processed carrots indicates that all treatments induced a significant change in quality. However, this change was not the same for each treatment. The second cluster comprises the thermally pasteurized carrots and all HP treated carrots. Since these five types of samples are located in close proximity of each other, their overall quality can be considered comparable. In other words, the differences in impact of mild and severe thermal pasteurization, mild and severe HP pasteurization and HP sterilization were rather limited. On the other hand, the thermally sterilized carrots, the third cluster, were located far from the rest of the treated carrots, pointing out substantial differences in impact between thermal sterilization and all other treatments, including HP sterilization.

To determine which quality parameters are responsible for the separation of the three clusters, the loadings of each parameter on the principal components of the biplot were examined (Table 4). A distinction between untreated and processed carrots can be made according to the direction of the second principal component

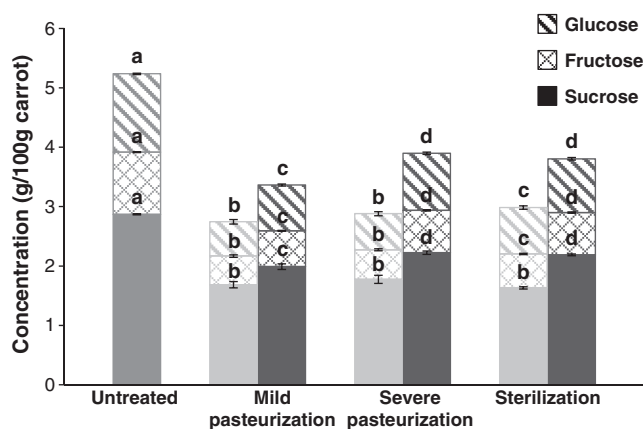


Fig. 7. Sugar profile of untreated (▨), thermally processed (▩) and HP processed (■) carrots, per processing intensity. For each sugar, significant differences in concentration ($p < 0.05$) are indicated with different letters.

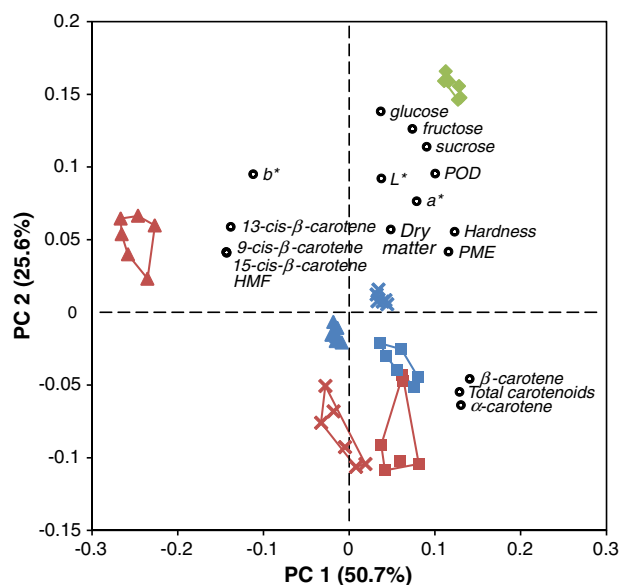


Fig. 8. Biplot of the principal component analysis (PCA) of carrot quality parameters (○) for untreated (green), thermally processed (red) and HP processed (blue) carrots, per processing intensity: untreated (◆), mild pasteurization (■), severe pasteurization (×) and sterilization (▲).

(PC 2). Among the loadings on this principal component, the three sugars displayed the largest values, indicating that these have contributed the most to the second principal component. In other words, the largest quality differences between the untreated and processed carrots are reflected in their sugar content. Indeed, as described in Section 3.8, a large reduction in sugar concentration was induced by all processing conditions, which was probably mainly a result of leaching out to the brine.

The first principal component (PC 1) accounted for the separation between the thermally sterilized carrots and all other carrots. The loadings in Table 4 indicate that HMF and carotenoids delivered the largest contribution to this principal component. Therefore, the considerable difference between the impact of thermal sterilization and that of all other treatments on carrot quality must be most reflected in these parameters. HMF and the *cis*-isomers of β -carotene are located on the same side of the biplot as the thermally sterilized carrots (negative loadings), indicating an increased concentration, while the opposite applies to α - and β -carotene and the total carotenoid content, all in line with the results presented in Sections 3.7 and

Table 4
Loadings on PC 1 and PC 2 for the principal component analysis (PCA) of carrot quality parameters.

Variable	Loading on PC 1	Loading on PC 2
Hardness	0.27	0.17
Dry matter	0.11	0.17
PME	0.25	0.13
POD	0.22	0.29
Lightness	0.08	0.28
Redness	0.17	0.23
Yellowness	−0.24	0.29
Total carotenoids	0.28	−0.17
α -carotene	0.28	−0.20
β -carotene	0.31	−0.14
9- <i>cis</i> - β -carotene	−0.31	0.13
13- <i>cis</i> - β -carotene	−0.30	0.18
15- <i>cis</i> - β -carotene	−0.31	0.13
Glucose	0.08	0.42
Fructose	0.16	0.39
Sucrose	0.20	0.35
HMF	−0.31	0.13

3.9. From these findings, it can be concluded that among all reactions occurring during thermal sterilization, browning reactions and carotenoid degradation and isomerization are the ones affecting the overall carrot quality the most, and that these reactions are of less importance in case of HP sterilization.

4. Conclusion

In contrast to most previous comparative studies on HP and traditional thermal processing, in this work a fair comparison was made, starting from processing conditions that lead to an equivalent microbial safety. This study demonstrated that, for the case of carrot processing, the potential benefit of HP over thermal processing is largely dependent on the processing intensity applied. At the level of mild pasteurization, the enzymes PME and POD were more inactivated by thermal treatment, although for PME this difference was rather small. At severe pasteurization intensity, enzyme inactivation was still more pronounced after thermal treatment, but at this level the difference for PME was patently obvious. On the other hand, HP treatment resulted in a better texture preservation and red color retention. Finally, at sterilization intensity, the impact of thermal and HP treatment on enzyme activities and carrot texture became equally large, but the difference in color retention only grew. Besides the loss in redness, thermal sterilization resulted in a marked increase in yellowness. This was also reflected in a significant carotenoid degradation and isomerization, which was not detected after HP treatment. Furthermore, thermal sterilization was the only treatment during which browning reactions occurred to the extent that detectable amounts of HMF were formed.

A principal component analysis (PCA) of all analyzed quality parameters provided a picture of the overall impact of all treatments. Three clusters of samples could be distinguished, with a comparable quality: (i) untreated carrots, (ii) thermally pasteurized carrots and all HP treated carrots, and (iii) thermally sterilized carrots. From these results, it can be concluded that thermal sterilization had the largest impact on carrot quality, while all other treatments resulted in a comparable overall quality. In this thermal sterilization impact, browning reactions and carotenoid degradation and isomerization affected the overall carrot quality the most.

To support conditions close to industrial application, pilot- and industrial-scale equipment was used in this study. Contradictions with previous research demonstrated that care should be taken in extrapolating results from lab-scale experiments to industrial scale. Optimization of the preprocessing conditions, necessary for industrial-scale HP treatments at elevated temperatures and virtually absent for the corresponding lab-scale treatments, will be crucial for implementation of HP sterilization in the food industry.

In the current work, the starting point of equivalence was microbial safety. It is obvious that safety should always take first place. However, when equivalence in shelf life is also considered, similar microbial inactivation is not the sole factor that should be taken into account. For products in which enzymatic deterioration is important, inactivation of enzymes by processing can be of crucial importance. As shown in this work, and confirmed by many others, HP processing is often less effective in inactivating enzymes. Therefore, when equivalent shelf life is put forward for these products, enzymatic inactivation might become a more relevant starting point for comparison.

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References

- Akyol, C., Alpas, H., & Bayindirli, A. (2006). Inactivation of peroxidase and lipoxygenase in carrots, green beans, and green peas by combination of high hydrostatic pressure and mild heat treatment. *European Food Research and Technology*, *224*, 171–176.
- Anese, M., Nicoli, M. C., Dall'aglio, G., & Lerici, C. R. (1995). Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *Journal of Food Biochemistry*, *18*, 285–293.
- Anthon, G. E., & Barrett, D. M. (2002). Kinetic parameters for the thermal inactivation of quality-related enzymes in carrots and potatoes. *Journal of Agricultural and Food Chemistry*, *50*, 4119–4125.
- Balogh, T., Smout, C., Nguyen, B. L., Van Loey, A. M., & Hendrickx, M. E. (2004). Thermal and high-pressure inactivation kinetics of carrot pectinmethylesterase: From model system to real foods. *Innovative Food Science and Emerging Technologies*, *5*, 429–436.
- Barbosa-Cánovas, G. V., & Juliano, P. (2008). Food sterilization by combining high pressure and thermal energy. In G. F. Gutiérrez-Lopez, G. V. Barbosa-Cánovas, J. Welti-Chanes, & E. Parada-Arias (Eds.), *Food engineering: Integrated approaches* (pp. 9–46). New York: Springer.
- Barbosa-Cánovas, G. V., & Rodríguez, J. J. (2005). Thermodynamic aspects of high hydrostatic pressure food processing. In G. V. Barbosa-Cánovas, M. S. Tapia, & M. P. Cano (Eds.), *Novel food processing technologies* (pp. 183–205). Boca Raton, FL: CRC Press.
- Barrett, D. M., & Theerakulkait, C. (1995). Quality indicators in blanched, frozen, stored vegetables. *Food Technology*, *49*, 62–65.
- Belitz, H. D., Grosch, W., & Schieberle, P. (2004). *Food chemistry* (3rd ed.). Berlin, Germany: Springer Verlag.
- Black, E. P., Setlow, P., Hocking, A. D., Stewart, C. M., Kelly, A. L., & Hoover, D. G. (2007). Response of spores to high-pressure processing. *Comprehensive Reviews in Food Science and Food Safety*, *6*, 103–119.
- Bull, M. K., Olivier, S. A., van Diepenbeek, R. J., Kormelink, F., & Chapman, B. (2009). Synergistic inactivation of spores of proteolytic *Clostridium botulinum* strains by high pressure and heat is strain and product dependent. *Applied and Environmental Microbiology*, *75*, 434–445.
- Burnette, F. S. (1977). Peroxidase and its relationship to food flavor and quality: a review. *Journal of Food Science*, *42*, 1–6.
- Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *LWT-Food Science and Technology*, *44*, 793–810.
- Chen, B. H., Peng, H. Y., & Chen, H. E. (1995). Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. *Journal of Agricultural and Food Chemistry*, *43*, 1912–1918.
- CSIRO Food and Nutritional Sciences (2010). *Make it safe: A guide to food safety*. Collingwood: CSIRO Publishing.
- Davis, F., Terry, L. A., Chope, G. A., & Faul, C. F. J. (2007). Effect of extraction procedure on measured sugar concentrations in onion (*Allium cepa* L.) bulbs. *Journal of Agricultural and Food Chemistry*, *55*, 4299–4306.
- De Belie, N., Laustsen, A. M., Martens, M., Bro, R., & De Baerdemaeker, J. (2002). Use of physico-chemical methods for assessment of sensory changes in carrot texture and sweetness during cooking. *Journal of Texture Studies*, *33*, 367–388.
- de Heij, W., van den Berg, R., van Schepdael, L., Hoogland, H., & Bijmolt, H. (2005). Sterilisation – Only better. *New Food*, *2*, 56–61.
- De Roeck, A., Duvetter, T., Fraeye, I., Van der Plancken, I., Sila, D. N., Van Loey, A., et al. (2009). Effect of high-pressure/high-temperature processing on chemical pectin conversions in relation to fruit and vegetable texture. *Food Chemistry*, *115*, 207–213.
- De Roeck, A., Mols, J., Duvetter, T., Van Loey, A., & Hendrickx, M. (2010). Carrot texture degradation kinetics and pectin changes during thermal versus high-pressure/high-temperature processing: A comparative study. *Food Chemistry*, *120*, 1104–1112.
- De Roeck, A., Sila, D. N., Duvetter, T., Van Loey, A., & Hendrickx, M. (2008). Effect of high pressure/high temperature processing on cell wall pectic substances in relation to firmness of carrot tissue. *Food Chemistry*, *107*, 1225–1235.
- De Vleeschouwer, K., Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2010). The effect of high pressure-high temperature processing conditions on acrylamide formation and other Maillard reaction compounds. *Journal of Agricultural and Food Chemistry*, *58*, 11740–11748.
- Delgado-Andrade, C., Seiquer, I., Haro, A., Castellano, R., & Navarro, M. P. (2010). Development of the Maillard reaction in foods cooked by different techniques. Intake of Maillard-derived compounds. *Food Chemistry*, *122*, 145–153.
- Denys, S., Van Loey, A. M., & Hendrickx, M. E. (1–3-2000). A modeling approach for evaluating process uniformity during batch high hydrostatic pressure processing: Combination of a numerical heat transfer model and enzyme inactivation kinetics. *Innovative Food Science and Emerging Technologies*, *1*, 5–19.
- Dogan, C., & Erkmen, O. (2004). High pressure inactivation kinetics of *Listeria monocytogenes* inactivation in broth, milk, and peach and orange juices. *Journal of Food Engineering*, *62*, 47–52.
- Duvetter, T., Sila, D. N., Van Buggenhout, S., Jolie, R., Van Loey, A., & Hendrickx, M. (2009). Pectins in processed fruit and vegetables: Part I – Stability and catalytic activity of pectinases. *Comprehensive Reviews in Food Science and Food Safety*, *8*, 75–85.
- ECFF (European Chilled Food Federation) (2006). Recommendations for the production of prepacked chilled food [online]. http://www.chilledfood.org/Resources/Chilled%20Food%20Association/Public%20Resources/ECFF_Recommendations_2nd_ed_18_12_06.pdf [Last accessed on 25/07/2011]
- Eskin, N. A. (1990). Biochemistry of food processing: Browning reactions in foods. In N. A. Eskin (Ed.), *Biochemistry of foods* (pp. 239–296). (2nd ed.). San Diego, CA: Academic Press, Inc.
- Fang, L., Jiang, B., & Zhang, T. (2008). Effect of combined high pressure and thermal treatment on kiwifruit peroxidase. *Food Chemistry*, *109*, 802–807.
- García-Palazon, A., Suthanthangjai, W., Kajda, P., & Zabetakis, I. (2004). The effects of high hydrostatic pressure on beta-glucosidase, peroxidase and polyphenoloxidase in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria x ananassa*). *Food Chemistry*, *88*, 7–10.
- Gaspar, E. M. S. M., & Lucena, A. F. F. (2009). Improved HPLC methodology for food control – Furfurals and patulin as markers of quality. *Food Chemistry*, *114*, 1576–1582.
- Gimenez, J., Kajda, P., Margomenou, L., Piggott, J. R., & Zabetakis, I. (2001). A study on the colour and sensory attributes of high-hydrostatic-pressure jams as compared with traditional jams. *Journal of the Science of Food and Agriculture*, *81*, 1228–1234.
- Glatt, H., & Sommer, Y. (2006). Health risks of 5-hydroxymethylfurfural (HMF) and related compounds. In K. Skog, & J. Alexander (Eds.), *Acrylamide and other hazardous compounds in heat-treated foods* (pp. 328–357). Cambridge: Woodhead Publishing limited.
- Gökmen, V., Bahçeci, K. S., Serpen, A., & Acar, J. (2005). Study of lipoxygenase and peroxidase as blanching indicator enzymes in peas: Change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. *LWT-Food Science and Technology*, *38*, 903–908.
- Goncalves, E. M., Pinheiro, J., Abreu, M., Brandao, T. R. S., & Silva, C. L. M. (2010). Carrot (*Daucus carota* L.) peroxidase inactivation, phenolic content and physical changes kinetics due to blanching. *Journal of Food Engineering*, *97*, 574–581.
- Greve, L. C., Mcardle, R. N., Gohlke, J. R., & Labavitch, J. M. (1994). Impact of heating on carrot firmness – Changes in cell-wall components. *Journal of Agricultural and Food Chemistry*, *42*, 2900–2906.
- Greve, L. C., Shackel, K. A., Ahmadi, H., Mcardle, R. N., Gohlke, J. R., & Labavitch, J. M. (1994). Impact of heating on carrot firmness – Contribution of cellular turgor. *Journal of Agricultural and Food Chemistry*, *42*, 2896–2899.
- Günes, B., & Bayindirli, A. (1993). Peroxidase and lipoxygenase inactivation during blanching of green beans, green peas and carrots. *LWT-Food Science and Technology*, *26*, 406–410.
- Hart, D. J., & Scott, K. J. (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chemistry*, *54*, 101–111.
- Heinz, V., & Buckow, R. (2010). Food preservation by high pressure. *Journal of Consumer Protection and Food Safety*, *5*, 73–81.
- Heinz, V., & Knorr, D. (2005). High-pressure-assisted heating as a method for sterilizing foods. In G. V. Barbosa-Cánovas, M. S. Tapia, & M. P. Cano (Eds.), *Novel food processing technologies* (pp. 207–232). Boca Raton, FL: CRC Press.
- Holden, J. M., Eldridge, A. L., Beecher, G. R., Buzzard, I. M., Bhagwat, S., Davis, C. S., et al. (1999). Carotenoid content of U.S. foods: An update of the database. *Journal of Food Composition and Analysis*, *12*, 169–196.
- Holdsworth, S. D. (2009). Principles of thermal processing: Sterilization. In R. Simpson (Ed.), *Engineering aspects of thermal food processing* (pp. 3–11). Boca Raton, FL: CRC Press.
- Jolie, R. P., Duvetter, T., Houben, K., Clynen, E., Sila, D. N., Van Loey, A. M., et al. (2009). Carrot pectin methylesterase and its inhibitor from kiwi fruit: Study of activity, stability and inhibition. *Innovative Food Science and Emerging Technologies*, *10*, 601–609.
- Jolie, R. P., Duvetter, T., Van Loey, A. M., & Hendrickx, M. E. (2010). Pectin methylesterase and its proteinaceous inhibitor: A review. *Carbohydrate Research*, *345*, 2583–2595.
- Kim, Y. S., Park, S. J., Cho, Y. H., & Park, J. (2001). Effects of combined treatment of high hydrostatic pressure and mild heat on the quality of carrot juice. *Journal of Food Science*, *66*, 1355–1360.
- Knockaert, G., De Roeck, A., Lemmens, L., Van Buggenhout, S., Hendrickx, M., & Van Loey, A. (2011). Effect of thermal and high pressure processes on structural and health-related properties of carrots (*Daucus carota*). *Food Chemistry*, *125*, 903–912.
- Knorr, D., Froehling, A., Jaeger, H., Reineke, K., Schlueter, O., & Schoessler, K. (2011). Emerging technologies in food processing. *Annual Review of Food Science and Technology*, *2*, 203–235.
- Krebbbers, B., Matser, A. M., Koets, M., & van den Berg, R. W. (2002). Quality and storage-stability of high-pressure preserved green beans. *Journal of Food Engineering*, *54*, 27–33.
- Kreutzmann, S., Christensen, L. P., & Edelenbos, M. (2008). Investigation of bitterness in carrots (*Daucus carota* L.) based on quantitative chemical and sensory analyses. *LWT-Food Science and Technology*, *41*, 193–205.
- Lambert, Y., Demazeau, G., Largeteau, A., & Bouvier, J. M. (1999). Changes in aromatic volatile composition of strawberry after high pressure treatment. *Food Chemistry*, *67*, 7–16.
- Lee, H. S., Rouseff, R. L., & Nagy, S. (1986). HPLC determination of furfural and 5-hydroxymethylfurfural in citrus juices. *Journal of Food Science*, *51*, 1075–1076.
- Lemmens, L., Colle, I., Knockaert, G., Van Buggenhout, S., Van Loey, A., & Hendrickx, M. (in press). Influence of pilot scale in pack pasteurization and sterilization treatments on nutritional and textural characteristics of carrot pieces. *Food Research International*. doi:10.1016/j.foodres.2011.02.030.
- Lemmens, L., Colle, I. J. P., Van Buggenhout, S., Van Loey, A. M., & Hendrickx, M. E. (2011). Quantifying the influence of thermal process parameters on in vitro β-carotene bioaccessibility: A case study on carrots. *Journal of Agricultural and Food Chemistry*, *59*, 3162–3167.
- Lemmens, L., De Vleeschouwer, K., Moelants, K. R. N., Colle, I. J. P., Van Loey, A. M., & Hendrickx, M. E. (2010). β-carotene isomerization kinetics during thermal treatments of carrot puree. *Journal of Agricultural and Food Chemistry*, *58*, 6816–6824.

- Lemmens, L., Van Buggenhout, S., Oey, I., Van Loey, A., & Hendrickx, M. (2009). Towards a better understanding of the relationship between the beta-carotene in vitro bio-accessibility and pectin structural changes: A case study on carrots. *Food Research International*, 42, 1323–1330.
- Lu, R., & Abbott, J. A. (2004). Force/deformation techniques for measuring texture. In D. Kilcast (Ed.), *Texture in food. Solid foods*, 2. (pp. 109–145) Cambridge: Woodhead Publishing.
- Maiani, G., Caston, M. J. P., Catasta, G., Toti, E., Cambrodon, I. G., Bysted, A., et al. (2009). Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Molecular Nutrition & Food Research*, 53, S194–S218.
- Matser, A. M., Krebbers, B., van den Berg, R. W., & Bartels, P. V. (2004). Advantages of high pressure sterilisation on quality of food products. *Trends in Food Science and Technology*, 15, 79–85.
- McInerney, J. K., Seccafien, C. A., Stewart, C. M., & Bird, A. R. (2007). Effects of high pressure processing on antioxidant activity, and total carotenoid content and availability, in vegetables. *Innovative Food Science and Emerging Technologies*, 8, 543–548.
- Nguyen, L. T., Rastogi, N. K., & Balasubramaniam, V. M. (2007). Evaluation of the instrumental quality of pressure-assisted thermally processed carrots. *Journal of Food Science*, 72, E264–E270.
- Nguyen, L. T., Tay, A., Balasubramaniam, V. M., Legan, J. D., Turek, E. J., & Gupta, R. (2010). Evaluating the impact of thermal and pressure treatment in preserving textural quality of selected foods. *LWT- Food Science and Technology*, 43, 525–534.
- Nielsen, M., & Martens, H. J. (1997). Low frequency ultrasonics for texture measurements in cooked carrots (*Daucus carota* L.). *Journal of Food Science*, 62, 1167–1175.
- Nursten, H. (2008). *The Maillard reaction: Chemistry, biochemistry and implications*. Cambridge: The Royal Society of Chemistry.
- Nyman, E. M. G. L., Svanberg, S. J. M., Andersson, R., & Nilsson, T. (2005). Effects of cultivar, root weight, storage and boiling on carbohydrate content in carrots (*Daucus carota* L.). *Journal of the Science of Food and Agriculture*, 85, 441–449.
- O'Donoghue, E. M., Somerfield, S. D., Shaw, M., Bendall, M., Hedderly, D., Eason, J., et al. (2004). Evaluation of carbohydrates in Pukekohe Longkeeper and Grano cultivars of *Allium cepa*. *Journal of Agricultural and Food Chemistry*, 52, 5383–5390.
- O'Neill, M. E., Carroll, Y., Corridan, B., Olmedilla, B., Granado, F., Blanco, I., et al. (2001). A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *British Journal of Nutrition*, 85, 499–507.
- Patras, A., Brunton, N., Da Pieve, S., Butler, F., & Downey, G. (2009). Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purees. *Innovative Food Science and Emerging Technologies*, 10, 16–22.
- Patras, A., Brunton, N. P., & Butler, F. (2010). Effect of water immersion and sous-vide processing on antioxidant activity, phenolic, carotenoid content and color of carrot disks. *Journal of Food Processing and Preservation*, 34, 1009–1023.
- Patras, A., Tiwari, B. K., Brunton, N. P., & Butler, F. (2009). Modelling the effect of different sterilisation treatments on antioxidant activity and colour of carrot slices during storage. *Food Chemistry*, 114, 484–491.
- Patterson, M. F. (2005). Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, 98, 1400–1409.
- Patterson, M. F., Linton, M., & Doona, C. J. (2008). Introduction to high pressure processing of foods. In C. J. Doona, & F. E. Feeherry (Eds.), *High pressure processing of foods*, 1–14. Ames, IA: Blackwell Publishing.
- Pither, R. J. (2003). Quality changes during canning. In B. Caballero, L. Trugo, & P. M. Finglas (Eds.), *Encyclopedia of food sciences and nutrition* (pp. 845–851). (2nd ed.). London: Academic Press.
- Porretta, S., Birzi, A., Ghizzoni, C., & Vicini, E. (1995). Effects of ultra-high hydrostatic-pressure treatments on the quality of tomato juice. *Food Chemistry*, 52, 35–41.
- Ramaswamy, H. S., & Markakis, P. (2006). *Food processing – Principles and applications*. Chapter 3: Thermal processing. Boca Raton, FL: CRC Press.
- Rastogi, N. K., Eshtiaghi, M. N., & Knorr, D. (1999). Effects of combined high pressure and heat treatment on the reduction of peroxidase and polyphenoloxidase activity in red grapes. *Food Biotechnology*, 13, 195–208.
- Reddy, N. R., Solomon, H. M., Fingerhut, G. A., Rhodehamel, E. J., Balasubramaniam, V. M., & Palaniappan, S. (1999). Inactivation of *Clostridium botulinum* type E spores by high pressure processing. *Journal of Food Safety*, 19, 277–288.
- Rodriguez, E. B., & Rodriguez-Amaya, D. B. (2007). Formation of apocarotenals and epoxy-carotenoids from β -carotene by chemical reactions and by autoxidation in model systems and processed foods. *Food Chemistry*, 101, 563–572.
- Rodriguez-Amaya, D. B., & Kimura, M. (2004). *HarvestPlus handbook for carotenoid analysis*. Washington D.C: HarvestPlus.
- Rodriguez-Sevilla, M. D., Villanueva-Suárez, M. J., & Redondo-Cuenca, A. (1999). Effects of processing conditions on soluble sugars content of carrot, beetroot and turnip. *Food Chemistry*, 66, 81–85.
- Sadler, G., Davis, J., & Dezman, D. (1990). Rapid extraction of lycopene and β -carotene from reconstituted tomato paste and pink grapefruit homogenates. *Journal of Food Science*, 55, 1460–1461.
- Sila, D. N., Smout, C., Elliot, F., Van Loey, A., & Hendrickx, M. (2006). Non-enzymatic depolymerization of carrot pectin: Toward a better understanding of carrot texture during thermal processing. *Journal of Food Science*, 71, E1–E9.
- Sila, D. N., Smout, C., Satara, Y., Truong, V., Van Loey, A., & Hendrickx, M. (2007). Combined thermal and high pressure effect on carrot pectinmethylsterase stability and catalytic activity. *Journal of Food Engineering*, 78, 755–764.
- Sila, D. N., Van Buggenhout, S., Duvetter, T., Fraeye, I., De Roeck, A., Van Loey, A., et al. (2009). Pectins in processed fruit and vegetables: Part II – Structure-function relationships. *Comprehensive Reviews in Food Science and Food Safety*, 8, 86–104.
- Simon, P. W., Peterson, C. E., & Lindsay, R. C. (1980). Correlations between sensory and objective parameters of carrot flavor. *Journal of Agricultural and Food Chemistry*, 28, 559–562.
- Skog, K., & Alexander, J. (2006). *Acrylamide and other hazardous compounds in heat-treated foods*. Cambridge: Woodhead Publishing limited.
- Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology*, 9, 152–158.
- Smout, C., Sila, D. N., Vu, T. S., Van Loey, A. M. L., & Hendrickx, M. E. G. (2005). Effect of preheating and calcium pre-treatment on pectin structure and thermal texture degradation: A case study on carrots. *Journal of Food Engineering*, 67, 419–425.
- Soria, A. C., Olano, A., Frias, J., Penas, E., & Villamiel, M. (2009). 2-Furoylmethyl amino acids, hydroxymethylfurfural, carbohydrates and β -carotene as quality markers of dehydrated carrots. *Journal of the Science of Food and Agriculture*, 89, 267–273.
- Soysal, C., Soylemez, Z., & Bozoglu, F. (2004). Effect of high hydrostatic pressure and temperature on carrot peroxidase inactivation. *European Food Research and Technology*, 218, 152–156.
- Terefe, N. S., Yang, Y. H., Knoerzer, K., Buckow, R., & Versteeg, C. (2010). High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. *Innovative Food Science and Emerging Technologies*, 11, 52–60.
- Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L., Van der Plancken, I., et al. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: Impact on overall quality attributes. *Innovative Food Science and Emerging Technologies*, 12, 235–243.
- Trejo Araya, X. I., Smale, N., Zabarás, D., Winley, E., Forde, C., Stewart, C. M., et al. (2009). Sensory perception and quality attributes of high pressure processed carrots in comparison to raw, sous-vide and cooked carrots. *Innovative Food Science and Emerging Technologies*, 10, 420–433.
- Van Buggenhout, S., Sila, D. N., Duvetter, T., Van Loey, A., & Hendrickx, M. (2009). Pectins in processed fruits and vegetables: Part III – Texture engineering. *Comprehensive Reviews in Food Science and Food Safety*, 8, 105–117.
- Verbeyst, L., Bogaerts, R., Van der Plancken, I., Hendrickx, M., & Van Loey, A. (in press). Modelling of vitamin C degradation during thermal and high-pressure treatments of red fruit. *Food and Bioprocess Technology: An international Journal*. doi:10.1007/s11947-012-0784-y.
- Vervoort, L., Van der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M., et al. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part II: Impact on specific chemical and biochemical quality parameters. *Innovative Food Science and Emerging Technologies*, 12, 466–477.
- Vora, H. M., Kyle, W. S. A., & Small, D. M. (1999). Activity, localisation and thermal inactivation of deteriorative enzymes in Australian carrot (*Daucus carota* L.) varieties. *Journal of the Science of Food and Agriculture*, 79, 1129–1135.
- Wilson, D. R., Dabrowski, L. Z., Stringer, S., Moezelaar, R., & Brocklehurst, T. F. (2008). High pressure in combination with elevated temperature as a method for the sterilisation of food. *Trends in Food Science and Technology*, 19, 289–299.
- Wilson, M. J., & Baker, R. High temperature/ultra high pressure sterilization of low acid foods. PCT Patent WO 97/21361.