

Effect of high pressure and thermal processing on shelf life and quality of strawberry purée and juice

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Abstract

The purpose of the study was to determine the effects of high pressure processing (HPP; 400-600 MPa, 20°C, 1.5 or 3 min) and heat treatment (HT; 85°C for 2 min) of strawberry purée and juice made from the same raw material. Microbiological and enzymatic inactivation, Brix, pH, anthocyanins, vitamin C, colour and sensory properties were analysed after processing and cold storage. The microbiological shelf life of the products was at least 49 days when processed at 500 or 600 MPa. Anthocyanins, vitamin C and colour were well preserved after HPP and HT. During storage, anthocyanins, vitamin C and sensory quality were better conserved in HT than in HPP purées, while there were minor differences between HT and HPP juices. This was probably due to higher enzyme activity in HPP purées, and indicates that raw materials with lower initial enzyme activity, like juices, are more suited for HPP than e.g. purées.

Keywords: high pressure processing; strawberry; juice; puree; storage; polyphenol oxidase; anthocyanins; vitamin C

1 Introduction

Strawberry is a popular fruit grown worldwide with an annual production of more than 9 million tons in 2016 (<http://faostat.fao.org/site/567/>). Strawberries have a delicious appearance and taste. In addition, strawberries are rich in vitamin C, fibres and secondary metabolites, such as polyphenols, and are shown to have several health promoting properties (Giampieri, Forbes-Hernandez, Gasparrini, Alvarez-Suarez, Afrin, Bompadre, et al., 2015). Among the polyphenols, the anthocyanins have received especial attention, both because they are responsible for the bright red colour of the berries and for their promising health effects (Li, Wang, Luo, Zhao, & Chen, 2017). Strawberries are, however, perishable and must be preserved by e.g. freezing or drying, or processed further to products like juice and jam. During processing, cell structures are disrupted and the nutrients and other constituents in the berries become prone to enzymatic and non-enzymatic oxidation (Terefe, Buckow, & Versteeg, 2014). Furthermore, thermal pasteurization, commonly used for inactivation of microorganisms and enzymes, may affect nutritional and sensory quality of the products. The consumers require minimally processed foods with a fresh taste and appearance. Non-thermal processing technologies like high pressure processing (HPP) are shown to keep the original flavour, taste and colour of the food, because molecules with low molecular weights such as sugars, pigments and volatile flavour compounds are unaffected by these preservation techniques (Barrett & Lloyd, 2012; Oey, Lille, Van Loey, & Hendrickx, 2008). Compared with traditional thermal preservation, HPP has shown a better retention of bioactive compounds, like vitamin C, and colour of strawberry products (Patras, Brunton, Da Pieve, & Butler, 2009), though this is not the instance in all cases (Terefe, Kleintschek, Gamage, Fanning, Netzel, Versteeg, et al., 2013). Disruptive enzymes in berries, like peroxidase (POD), polyphenol oxidase (PPO) and pectin methyl esterase (PME) are very resistant against pressure and are only partially inactivated under commercial HP conditions (Terefe, Buckow, & Versteeg, 2014). The incomplete inactivation of these enzymes will affect stability of chemical constituents and thus quality of the berry products during storage. PPO, which is previously shown to be the most pressure resistant and detrimental enzyme in strawberries (Cano, Hernandez, &

DeAncos, 1997; Chisari, Barbagallo, & Spagna, 2007; Terefe, Buckow, & Versteeg, 2014; Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010), will negatively affect polyphenols and colour of strawberry products.

Several types of microorganisms can cause spoilage in fruits and berries. It is generally known that HPP inactivate microorganisms in juices and purées (Guerrero-Beltran, Barbosa-Canovas, & Swanson, 2005). However, the effect is dependent on species and cultivar of the fruits and other factors such as solute concentrations and pH (Koseki & Yamamoto, 2006). Besides some recent studies by a Polish group on strawberry purée after pressurization and cold storage (Krystian Marszalek, Mitek, & Skapska, 2015; K. Marszalek, Wozniak, & Skapska, 2016; K. Marszalek, Wozniak, Skapska, & Mitek, 2017), the shelf life of HPP strawberry products based on survival and regrowth of bacteria, yeast and mould have only been studied to a minor degree. Provided microbiological safety is secured, sensory quality is the most important quality parameter of foods. Thus, it is surprising that very few studies have investigated how HPP affects the sensory quality of strawberry products. The studies conducted to elucidate the effect of HPP on strawberries have mainly focused on enzyme inactivation and chemical composition and quality just after processing (Cao, Bi, Huang, Wu, Hu, & Liao, 2012; Cao, Liu, Wu, Liao, & Hu, 2014; Cao, Zhang, Zhang, Wang, Yi, & Liao, 2011; Krystian Marszalek, Mitek, & Skapska, 2015; Patras, Brunton, Da Pieve, & Butler, 2009; Sulaiman & Silva, 2013; Zabetakis, Leclerc, & Kajda, 2000). This has been done even though it is known that changes in berry products during storage often are more severe than the losses occurring during processing (Aaby, Wrolstad, Ekeberg, & Skrede, 2007; Holzwarth, Korhummel, Kammerer, & Carle, 2012; Howard, Brownmiller, & Prior, 2014; Howard, Prior, Liyanage, & Lay, 2012).

The aims of the present study were (1) to determine the effects of HPP and heat treatment (HT) of strawberry purée and juice just after processing and during storage and (2) to compare the effect of HPP of different products (purée and juice) made from the same raw material, which, to our knowledge,

have not previously been studied. Microbiological and enzymatic inactivation (PPO), colour (L, hue, chroma, ΔE), vitamin C, anthocyanins, Brix, pH and sensory properties of newly processed products and products stored at 6 °C for up to 49 days were determined.

2 Materials and methods

2.1 Chemical used

Catechol, Triton X-100, dehydro-L-(+)-ascorbic acid dimer, tris[2-carboxyethyl]-phosphine (TCEP) and *meta*-phosphoric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-(+)-ascorbic acid, sodium phosphates, potassium chloride, sodium acetate, n-dodecyltrimethylammonium chloride, BactoPeptone, and disodium-EDTA ($\text{Na}_2\text{-EDTA}$) were obtained from Merck KGaA (Darmstadt, Germany). Plate count agar (PCA) was obtained from Oxoid (Hampshire, UK) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) from Difco Laboratories (Sparks, MD, USA). All chemicals and solvents were of analytical or HPLC grade and water was of Milli-Q quality (Millipore Corp., Cork, Ireland).

2.2 Plant material

Strawberries (*Fragaria x ananassa* Duch.) cv. Senga Sengana grown in Western Norway (62.3°N, 7.3° E) in July 2012 were single-frozen and stored at -20 °C for 5-6 months until processing.

2.3 Production and processing of purée and juice

Purée was made by homogenizing partly thawed berries in a cooled food processor (R5, Robot coupe, Vincennes Cedex, France) for 30 sec. Clear juice was produced by pressing thawed strawberries (at about 2 °C) in a juice packing press (50 P1, Voran, Pichl bei Wels, Austria) through a fine-meshed press cloth. The purées and juices were vacuum packed (Webomatic vacuum chamber, Werner Bonk, Bochum, Germany) in heat-sealed 20 µm PA/70 µm PE bags (180 × 140 mm, Lietpak, Vilnius, Lithuania). Each bag contained 50 ± 1 g product.

HPP was carried out in a high hydrostatic pressure machine QFP 2L-700 (Avure Technologies Inc., Columbus, USA). The strawberry products were kept on ice prior to processing and were pressurized at 20 °C for 1.5 or 3 min at 400–600 MPa. Come-up time was 85–115 s. The duration of treatment did not include come-up time. The pressure release was immediate. Heat treatment (HT) was conducted in boiling water for 3 min. Pre-trials had shown that this heat treatment gave a core temperature in the strawberry products of 85 °C for minimum 2 min. All samples were immediately cooled in ice-water after processing. A portion of the samples was not HP or heat treated.

The processing, from thawing the berries to HPP or HT, were performed in triplicates. The HPP treatments were performed in randomised order. HPP and HT samples were stored at 6 °C in the dark and were analysed after 0, 14, 35, and 49 days. The untreated samples (control) were analysed at day 0 only. Microbiological analyses were performed directly after processing and cold storage, while samples for other analyses were frozen at -20 °C prior to analyses.

2.4 Microbiological analysis

The indigenous flora of strawberry purée and juice was determined before and after processing (day 0) and after storage. The samples were diluted 1:10 in peptone water and homogenized for 2 min. The suspensions were plated on PCA for detecting viable cells of total aerobic bacteria (TAB) (colony forming units [cfu] per g). The plates were incubated at 30 °C for 24 h. For detecting viable yeast and mould, the samples were enumerated on DRBC agar. The plates were incubated at 25 °C for 3–5 days. A mechanical spiral plater (Eddy Jet, IUL Instruments, Barcelona, Spain) was mainly used for this purpose, but some manual plating was performed for low dilutions. The detection level on PCA and DRBC was 20 cfu/g. For plates with no colonies detected, the level was set to half of the detection limit.

2.5 Polyphenol oxidase (PPO) activity

The activity of PPO was determined with a method earlier described (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010) with some modifications. PPO was extracted from strawberry products (10 g) with extraction solution (15 mL) consisting of 0.2 M sodium phosphate buffer (pH 6.5) containing 1% (w/v) Triton X-100 and 1 M NaCl. The mixture was homogenized for 1 min at 15 000 rpm (Ultra Turrax, PT3100 Polytron, IL, USA) and then centrifuged at 4 °C for 15 min at 20 000g (Avanti J-26 XP, Beckman Coulter Inc., USA). The supernatant (200 µL) was mixed with 0.07 M catechol in 0.1 M sodium phosphate buffer, pH 5.5 (2 mL). In the blank, the sample was replaced with the extraction solution. Absorbance of the reaction mixture at 20–22 °C was measured at 420 nm every 2nd second for 1 min (Agilent 8453 Spectrophotometer, Agilent Technologies, Waldbronn, Germany). The enzyme activity was determined in the linear range (the first 13 seconds) and expressed as change of absorbance per min per g of fresh weight of the sample (abs/min/g). The assay was performed in triplicate for each sample.

2.6 Colour

Colour measurement (L^* , a^* and b^* values, CIELAB) was conducted on a calibrated Hunter Lab instrument (LabScan XE, Reston, VA, USA). The illuminant was D65, the observer 10° and the port size 0.25 ". L^* defines lightness (0 = black, 100 = white). Hue angle ($\arctan(b^*/a^*)$) designates colour shade where high values indicate a more red-orange colour and low values a more red-bluish colour. Chroma ($(a^{*2} + b^{*2})^{1/2}$) shows transition from grey (low values) to pure colour (high values). The absolute colour difference of the samples after processing and storage was calculated as $\Delta E = ((L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2)^{1/2}$, where L_0^* , a_0^* and b_0^* were the values for the untreated sample.

2.7 pH and brix

pH was determined at room temperature with a pH meter (827 pH lab, Metrohm, Switzerland). Content of soluble solids was determined using a digital refractometer (RE40, Mettler Toledo Inc., Japan) and expressed as °Brix (%). All samples were measured twice.

2.8 Total monomeric anthocyanins (TMA)

TMA was measured directly in the juice and in the supernatant of strawberry purée after centrifugation (39200g for 10 min, Avanti J-26 XP, Beckman Coulter Inc., USA). TMA was determined by the pH-differential method (Giusti & Wrolstad, 2001). The samples were diluted in two buffers; 0.025 M potassium chloride (pH 1) and 0.4 M sodium acetate (pH 4.5). After 30 min at 20–22 °C, absorbance at 520 and 700 nm was measured (Agilent 8453 Spectrophotometer, Agilent Technologies). The concentration of TMA in the samples was calculated as mg cyanidin-3-glucoside equivalents per 100 g of fresh weight (mg/100 g fw).

2.9 Vitamin C

Vitamin C includes both L-ascorbic acid (AA) and the oxidised form dehydroascorbic acid (DHAA). Vitamin C in the samples were determined after reduction of DHAA to AA in accordance with a method previously described (Aaby, Wrolstad, Ekeberg, & Skrede, 2007), with some modifications. Briefly, ascorbic acids in purée or juice (5 g) were extracted with 5% meta-phosphoric acid containing 1 mM disodium-dihydrogen-EDTA (25 mL). DHAA in the extracts (100 μ L) was reduced to AA with 5 mM TCEP, pH 9.0 (50 μ L) and diluted with mobile phase pH 4.2 (350 μ L) prior to analysis. Analysis of AA in the extracts (15 μ L) was performed on an Agilent 1100 Series HPLC system (Agilent Technologies). Separation was performed at 25 °C on a monolithic column; Chromolith® Performance RP-18e (100 mm \times 4.6 mm i.d., Merck KGaA, Darmstadt, Germany) with mobile phase 2.5 mM Na₂H₂PO₄·2H₂O, 2.5 mM *n*-dodecyltrimethylammonium chloride, 1.0 mM Na₂-EDTA, and 2% acetonitrile, adjusted to pH 4.7. AA was detected at 264 nm and quantified by external standard. Concentration of vitamin C was expressed as mg per 100 g of fresh weight.

2.10 Sensory evaluation

Strawberry juices and purées were analysed by sensory profiling using seven trained panellists. The panellists were selected and trained by ISO 6564:1985-E guidelines. The 12 attributes chosen for profiling of purées and juices were colour intensity, colour saturation (pure red to impure brown/grey), viscosity, strawberry odour, strawberry flavour intensity, strawberry flavour typical, sweet taste, sour taste, fresh flavour, ripened flavour, oxidized flavour and off flavour. In addition, size and number of strawberry pieces were evaluated in the purées and fruit pulp was evaluated in the juice. A continuous non-structured scale (from 1.0 to 9.0) was used for evaluation. Each panellist evaluated the samples on a computerized system for recording data (EyeQuestion, Logic8 BV, Utrecht, Netherlands). Coded samples were served in randomised order.

The following 5 purées and 5 juices were analysed; untreated control frozen at day 0 and thawed prior to sensory evaluation and HT- and HPP-samples (400, 500 and 600 MPa) stored for 35 days at 6 °C.

2.11 Statistical analysis

Statistical analysis was performed using Minitab® Statistical Software (version 17.2.1, Minitab Ltd., Coventry, UK). Significant differences ($p < 0.05$) between samples (processing replicates; $n=3$) were determined with one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. General linear modelling ANOVA was performed to determine significant effects of the experimental factors and their interactions.

3 Results

3.1 Strawberry purée; effects of HPP, HT and storage

3.1.1 Microbial inactivation

TAB, yeast and mould were determined in the strawberry purées before and after processing and storage. In the untreated sample, TAB was 3.2 log cfu/g (see Table in Supplementary material). Low levels of yeast and mould were detected (1.5 log cfu/g). Directly after processing, the HT and HPP samples contained 2.2 and 2.2–2.7 log cfu/g of TAB, respectively, and no yeast and mould. There were no significant changes in TAB during cold storage for 49 days. Mould and yeast was only detected in low levels in most samples stored for 49 days. However high levels of yeast and mould was observed at the end of the

storage period for samples treated at 400 MPa. Based on the data from the microbiological analysis, a shelf life of the strawberry purée of at least 49 days at 6 °C when processed at 500 MPa or 600 MPa was found.

3.1.2 *pH, Brix, PPO-activity, TMA, Vitamin C and Colour*

ANOVA showed that Storage time was the experimental factor with the highest influence on pH, Brix, PPO, TMA, vitamin C and colour in HP-treated strawberry purées with explained variances from 22.2 to 95.5% ($p < 0.001$) (**Table 1**). Pressure had significant effect only on PPO-activity and Brix, while Holding time (1.5 or 3 min) only affected PPO.

Chemical composition and colour of non-processed purées (control), HT purées and HPP purées at production and during storage are shown in **Table 2**.

Because of the minor influence of pressure holding time on quality and chemical constituents, and to make the presentation clearer, only results obtained at one pressure holding time (3 min) are presented.

At day 0, the HT purée had significantly higher pH compared with the control and the HPP purées. The pH decreased in all purées during storage, but more in the HPP purées than in the HT purées. The Brix values of HT and HPP purées at day 0 were not significantly different from the control and there were no significant changes in Brix during storage. HT inhibited PPO-activity 25% compared with control, while pressure at 400, 500 and 600 MPa inhibited PPO activity with 11, 12 and 30%, respectively. The PPO-activity of the HT purées was stable in the storage period. The PPO-activity of HPP purées, on the other hand, increased steadily during storage and after 49 days of storage the activity was in average 189% of the activity of freshly prepared purées. TMA decreased during storage in all purées, but more in HPP purées, independent of pressure applied, than in HT purées. For example, after 35 days of storage at 6 °C in average 57 and 67% of anthocyanins were preserved in the HPP and HT purées, respectively. More vitamin C was present in newly made HT purée

compared with the HPP purées. After 14 days of storage 48% of the initial vitamin C was present in the HT purées, while only 40, 18 and 4% remained in the purées processed at 400, 500 and 600 MPa, respectively. After 35 days of storage, almost no vitamin C was left in any of the purées. There were minor changes in instrumentally measured colour after processing and storage. Hue and chroma were not altered by processing, but decreased slightly in HPP purées during storage, and after 35 days of storage chroma was significantly lower than at day 0. The colour difference (ΔE) was highest in the HPP purées after 35 days of storage.

3.1.3 Sensory profile

Sensory profiling of strawberry purées showed that there were differences between HT and HPP purées stored for 35 days and the unprocessed control (**Fig. 1A**). The control sample was fresher, sweeter, purer red (lower colour saturation) and had higher intensities of strawberry flavour and odour than the processed and stored samples. The HT purée was more like the control purée than the HPP purées. The HPP samples showed concurring scores for some attributes, while for others there were differences due to the pressure level applied. Purées treated with higher pressure was more like the fresh control purée. This was especially observed for viscosity, fresh flavour and strawberry flavour and odour.

3.2 Strawberry juice; effects of HPP, HT and storage

Based on the results from the strawberry purée experiment showing that pressure holding time had minor influence on chemical composition and quality of the products, only one holding time (3 min) was chosen in the juice experiment.

3.2.1 Microbial inactivation

The bacterial growth (TAB) at day 0 was below 2.0 log cfu/g (see Table in Supplementary material). Only single colonies were found even in the control sample and there were no significant changes after processing. The control had minor growth of yeast and mould (1.7 log cfu/g). After processing, no yeast and mould were detected. During storage, the bacterial levels in the processed samples did not change. There were only sporadic cases of yeast and mould (< 2.0 log cfu/g), with exception of the 400 MPa juice stored for 49 days that was spoiled by mould and yeast (5.0 log cfu/g). Based on the data from the microbiological analysis, the strawberry juice showed a shelf life at 6 °C of at least 49 days when processed by HPP (≥ 500 MPa) or HT.

3.2.2 pH, Brix, PPO-activity, TMA, Vitamin C and Colour

ANOVA of the HPP juice data showed no significant effects of Pressure or the interaction Pressure x Storage time, while Storage time did significantly affect all the measured quality parameters, except Brix (results not shown).

pH, Brix, TMA, vitamin C and colour parameters of the juices are shown in **Table 3**. No PPO activity was detected in the control strawberry juice. Consequently, PPO activity was not measured in juice after processing or storage. At day 0 all samples had pH of 3.32 ± 0.02 . After 14 days, the pH had declined to 3.26 and 3.21 in HT and HPP samples, respectively, and stayed at this level to the end of the storage period. The average Brix value of the juices was 7.5, with minor differences between the samples. TMA in strawberry juice did not change after HT or HPP, but decreased significantly during storage.

After 35 and 49 days, in average 77 and 65% of originally present TMA, remained in the juice independent of treatment. The vitamin C content was not significantly affected by processing. After 14 days of storage in average only 13 % of the original vitamin C content was present in the juices. Minor differences between the samples in instrumentally measured colour were observed. There was a tendency (not significant) of lower L*-values after processing, that is, the juice became darker. The juice processed at 600 MPa became significant darker during storage. After 35 days of storage the Hue-values of HPP juices had decreased significantly. ΔE increased during storage.

3.2.3 Sensory profiling

Sensory profiling of strawberry juice revealed differences between untreated juice (control) and HPP and HT juices stored for 35 days (**Fig. 1B**). The control juice was fresher and had more strawberry flavour and lower values for colour intensity and colour saturation, i.e. the control juice was lighter and purer red than the processed and stored samples. For the processed samples, concurring scores were seen for most attributes, except for the juice treated at 600 MPa, having higher viscosity than the other juices.

3.3 HPP of purée vs. juice

To determine the effects of Matrix (Purée vs Juice) and possible interactions between Matrix, HP-treatments and Storage time, the results of HPP purées and juices were analysed by ANOVA.

Brix, L*, hue and chroma were highly affected by Matrix with explained variance higher than 67% (**Table 4**). pH, TMA, vitamin C and ΔE were mainly influenced by Storage time (explained variance 65–87%). There were, however, interesting interactions between Storage time and Matrix for pH and TMA, which is illustrated in **Fig. 2**. TMA was the same in juice and purée at day 0, but the anthocyanins were considerably better preserved in the juices than in the purées (**Fig. 2A**) and after 35 days of storage in average 77 and 61% of the anthocyanins remained in the HPP juices and purées, respectively (**Tables 2 and 3**). Just after processing, pH was the same in the juices and in the purées. After 2 weeks of storage, pH in the juices had dropped, but was then stable for the remaining storage period. In the purées, on the other hand, pH continued to decrease the entire storage period (**Fig. 2B**).

4 Discussion

Juice and purée were made from strawberries (cv Senga Sengana) and exposed to HPP (400, 500 or 600 MPa for 1.5 or 3 min) or heat treatment (HT) and stored for up to 49 days at 6 °C. The processing method and subsequent storage affected microorganisms (bacteria, yeast and mould), pH, Brix, PPO, TMA, vitamin C, colour and sensory properties of the products in different ways.

The strawberries in our experiments had low microbial start levels, i.e. 3.2 and 2.0 log cfu/g in purée and juice, respectively. The juices and purées generally showed a long shelf life. All samples had very low bacterial numbers and only single samples contained mould and yeast after storage for 49 days at 6 °C. The exception was samples treated at 400 MPa. There are not many studies on the effects of HPP on microorganisms in strawberries. However, a Polish group have published some studies on HPP of strawberry purées (Krystian Marszalek, Mitek, & Skapska, 2015; K. Marszalek, Wozniak, & Skapska, 2016; K.

Marszalek, Wozniak, Skapska, & Mitek, 2017). They showed that when strawberry purée was exposed to 500 MPa for 1 and 5 min, total microbial counts was reduced from 4.86 to 4.26 log cfu/g, independent of holding time (Krystian Marszalek, Mitek, & Skapska, 2015). Yeast and mould on the strawberries were sensitive towards HPP and below the detection level after 500 MPa for 1 min. In other studies, Marszalek *et al.* (K. Marszalek, Wozniak, & Skapska, 2016; K. Marszalek, Wozniak, Skapska, & Mitek, 2017) showed that strawberry purée can be preserved for more than 28 weeks if HPP (500 MPa, 15 min) was combined with mild temperature (50 °C). These findings are in accordance with other studies on HP-treated fruit juices showing no or low bacterial growth during storage for several weeks (Bull, Zerdin, Howe, Goicoechea, Paramanandhan, Stockman, et al., 2004; Lavinias, Miguel, Lopes, & Mesquita, 2008). Apple juice treated at 400 MPa for 3 min showed no growth of mesophilic bacteria after 8 weeks (Lavinias, Miguel, Lopes, & Mesquita, 2008), and two different types of orange juice (pH 3.8 and 4.2) were both microbiologically stable up to 12 weeks at 4 °C (Bull, et al., 2004). These results suggest that the storage period in the studies, our included, should probably have been longer to be able to give an estimate of the shelf life of the products processed at different HPP conditions.

Brix in the strawberry products were not significantly changed after processing and storage. This is in agreement with a recent study where no changes in Brix were seen in strawberry purée during four weeks of storage (Sulaiman, Farid, & Silva, 2016). Neither pH changed during storage in that study. In our study, on the other hand, pH was not affected by processing, but decreased during storage, and more in HPP than in HT products, and especially in the HPP purées at the end of the storage period. The decrease in pH means that the HPP purées became more acidic during storage. This was also observed by sensory profiling, where the HPP purées were assessed to be more sour than the control and the HT purées. To our knowledge, decrease in pH during storage of strawberry products is not previously reported, but comparable results are found for pressurized avocado paste (600 MPa for 3 min), where 11%

decline in pH was observed during the first 20 days of storage at 4 °C (Jacobo-Velazquez & Hernandez-Brenes, 2010). The authors suggested that the decline in pH could be caused by the gradual migration of organic acids from the intracellular matrix of vegetable cells, being ruptured by HPP. Also in Maoberry juice a slight decrease in pH in juice treated at 400 MPa for 10 min was found after four weeks of cold storage (Chaikham, 2015). The decrease was proposed to be associated with increase in microbes. In the present study, bacterial growth during storage was low and decline in pH was not likely to be caused by bacterial production of lactic acid.

In our study, HT of strawberry purée reduced PPO activity with 24%, while HPP reduced PPO activity with 11–30% with the highest inactivation when the highest pressure (600 MPa) was applied. These results are in agreement with previous studies showing that strawberry PPO is very heat and pressure resistant, and is even activated at medium pressures (300–400 MPa) (Cano, Hernandez, & DeAncos, 1997; Cao, Zhang, Zhang, Wang, Yi, & Liao, 2011; Goessinger, Moritz, Hermes, Wendelin, Scherbichler, Halbwirth, et al., 2009; K. Marszalek, Wozniak, & Skapska, 2016; Terefe, Matthies, Simons, & Versteeg, 2009; Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). For example, PPO activity decreased about 50% after pasteurisation (85 °C for 10 min) (Goessinger, et al., 2009) and PPO in strawberry purée was not inactivated even after heating (100 °C) for 30 min, and only 23% inhibition of PPO was obtained after treatment at 690 MPa and 90 °C (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). Increasing pressure holding times from 5 to 15 or 25 min at 600 MPa increased PPO inhibition in strawberry purée from 30–35% to 52–82 % (Cao, Zhang, Zhang, Wang, Yi, & Liao, 2011; Sulaiman & Silva, 2013). In the present study, increasing holding time, from 1.5 to 3 min, also significantly increased inhibition of PPO activity.

Even it is known that structure modifications of proteins, e.g. enzymes, after pressurization can be reversible (Terefe, Buckow, & Versteeg, 2014), most studies on the effects of HPP on enzyme inactivation measure enzyme activity just after processing and not after storage. In the present study, PPO activity

was measured during storage. The PPO activity in the HPP purées gradually increased and were almost doubled after 49 days of storage, while in the HT purées, the tendency was decline in PPO activity during storage. These results indicate regeneration of structure and activity of PPO after HPP, but not HT. PPO reactivation after HPP has also been found in avocado paste (Jacobo-Velazquez & Hernandez-Brenes, 2010) and increased strawberry PPO activity during frozen and cold storage of untreated strawberries are reported (Chisari, Barbagallo, & Spagna, 2007; Sulaiman & Silva, 2013; Yang, Li, Li, Xin, Zhao, Zheng, et al., 2010). In a study of PPO activity of different strawberry cultivars after thermal treatment, decrease in PPO activity after storage (7 days at 4 °C) were found in most cases, but reactivation of PPO was observed after thermal treatment (90 °C for 5 min) of one cultivar (Holzwarth, Korhummel, Kammerer, & Carle, 2012). The knowledge that activity, as well as stability, of PPO vary between cultivars needs to be considered when choosing cultivars and processing conditions to secure high quality of the products during storage.

PPO activity in strawberry purée in our study was higher than obtained in other studies (Cano, Hernandez, & DeAncos, 1997; Krystian Marszalek, Mitek, & Skapska, 2015; Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). The reason is most likely different procedures used to quantify PPO activity. In our study, PPO activity was determined in the initial, fast linear range of the reaction, while in the other studies absorbance change during 10 min of reaction was recorded.

No PPO activity was detected in the strawberry juice, which indicates that PPO was associated with parts of the strawberries that were discarded during juice processing. Accordingly, 50% decrease in PPO activity after sieving of crushed strawberries were seen (Goessinger, et al., 2009). However, some PPO activity was still present in the final product in that study. PPO has also been detected in strawberry juice (Aguilo-Aguayo, Soliva-Fortuny, & Martin-Belloso,

2010). The diverging findings from our study may be due to different technologies used to produce juice, different strawberry cultivars and/or different methods used for determination of PPO activity.

In agreement with literature (Oey, Lille, Van Loey, & Hendrickx, 2008), minor changes in small molecules like ascorbic acid and anthocyanins, were observed after HPP of the strawberry products. The anthocyanin content (TMA) was neither affected by HT nor HPP. During storage, however, the anthocyanin concentration gradually decreased, with the least retention in HPP purées and the lowest retention in purées treated at 400 MPa. This is in accordance with previous studies, showing that anthocyanins in strawberries were quite stable during HPP, but degraded during storage (Cao, Bi, Huang, Wu, Hu, & Liao, 2012; Cao, Zhang, Zhang, Wang, Yi, & Liao, 2011; Terefe, et al., 2013; Terefe, Matthies, Simons, & Versteeg, 2009). Further, the least retention of anthocyanins was seen when berries were treated at 400 MPa compared to other pressures (200, 600 and 800 MPa) (Suthanthangjai, Kajda, & Zabetakis, 2005; Zabetakis, Leclerc, & Kajda, 2000).

Interestingly, anthocyanins were better preserved during storage in juices than in purées treated with HPP. The higher stability of anthocyanins in the juice compared with the purée is probably due to lower activity of PPO in the juice. Anthocyanins themselves are not good substrates for PPO, but after removal of sugar moieties by β -glucosidase, the resulting anthocyanidins can be oxidized by PPO (Terefe, Buckow, & Versteeg, 2014). Another explanation of the higher stability of anthocyanins in juices compared with purées, could be that less oxygen was trapped in the juice. However, this does not explain that anthocyanins were better preserved in heat treated than in pressure treated purées. Previously, differences in oxygen absorption have been suggested to be the reason for the higher stability of anthocyanins in clear strawberry juice compared with cloudy juice (Cao, Bi, Huang, Wu, Hu, & Liao, 2012), but the explanation could also be differences in enzyme activity, which was not measured in that study.

Vitamin C was, like anthocyanins, not significantly affected by processing and was better preserved in HT than in HPP purées during storage. This indicates that several oxidative enzymes in addition to PPO, e.g. ascorbic acid oxidase, were less inactivated by HPP than by HT. The low stability of vitamin C during storage is in accordance with studies of strawberry purées and of fruit smoothie containing strawberries (Aaby, Wrolstad, Ekeberg, & Skrede, 2007; Keenan, Brunton, Gormley, Butler, Tiwari, & Patras, 2010; K. Marszalek, Wozniak, Skapska, & Mitek, 2017), demonstrating that vitamin C in strawberry products are easily degraded and might be very difficult to preserve.

Colour of the strawberry purées and juices was not affected by the heat or pressure treatments applied. This is in accordance with previous studies demonstrating minor effects of HPP on colour of strawberry products (Cao, Liu, Wu, Liao, & Hu, 2014; Cao, Zhang, Zhang, Wang, Yi, & Liao, 2011; Krystian Marszalek, Mitek, & Skapska, 2015; Patras, Brunton, Da Pieve, & Butler, 2009). ΔE was in the same range as previously found for strawberry purées during processing and storage (K. Marszalek, Wozniak, Skapska, & Mitek, 2017). In the present study, the colour of the HT products was more stable during storage than the colour of the HPP products, where a slight decrease in L^* , hue and chroma was observed. This means that the HPP products became darker, more bluish with lower colour saturation. In accordance with our results, lightness of HP treated strawberry juice and fruit smoothie containing strawberries, decreased during storage (Cao, Bi, Huang, Wu, Hu, & Liao, 2012; Keenan, Brunton, Gormley, Butler, Tiwari, & Patras, 2010). Further, the colour changes were more pronounced in HPP smoothies than in thermally processed smoothies (Keenan, Brunton, Gormley, Butler, Tiwari, & Patras, 2010). Decrease in chroma during storage is also found in HT strawberry products (Holzwarth, Korhummel, Kammerer, & Carle, 2012; Howard, Brownmiller, & Prior, 2014; Mazur, Nes, Wold, Remberg, Martinsen, & Aaby, 2014). However, in opposition to the HPP products in the present study, hue increased, i.e. a more yellow colour developed, in the HT strawberry products. The anthocyanins are the pigments responsible for the bright, red colour of strawberries. The extensive

decrease in TMA during storage was, however, not reflected in a corresponding change in colour, probably because polymeric pigments formed contribute to the colour of the products (Howard, Brownmiller, & Prior, 2014; Howard, Prior, Liyanage, & Lay, 2012).

In accordance with instrumentally measured L^* and chroma, sensory evaluation revealed that newly made, untreated strawberry purée and juice had lower colour intensity and colour saturation, i.e. was lighter and purer red, than stored samples. The HPP purées had lower viscosity than the newly made purée and the HT purée. The loss of viscosity of HPP purées during storage indicates degradation of pectin, which suggests that pectinases, such as PME, were not inactivated by the pressure treatment. The higher viscosity in purées exposed to increasing pressure (400-600 MPa) implied a more extensive inactivation of pectinases with higher pressure applied. The observation is in accordance with previous studies, showing that pressure above 700 MPa usually is needed to inactivate PME at low temperatures and short holding times (Sila, Duvetter, De Roeck, Verlent, Smout, Moates, et al., 2008). All juices had similar texture, except the juice treated at 600 MPa. This juice was thick and had lumps. Gelation of pectin due to insufficient inhibition of PME, as suggested in a study with strawberry purée (Bodelon, Avizcuri, Fernandez-Zurbano, Dizy, & Prestamo, 2013), was probably not the case in our study, as the phenomena did not occur in juices treated at lower pressures. Freshness, strawberry flavour and odour were similar in the stored juices, independent of treatment, while purées exposed to the least severe treatments got lower sensory scores. The finding is in accordance with results of TMA, pH, vitamin C and colour and indicates that several quality-related enzymes in strawberries, in addition to PPO, were similar affected by processing and storage.

Both the HT and HPP applied were too mild to inactivate degrading enzymes in the strawberry purée and more severe treatments could be considered to inactivate enzymes and thereby preserve nutritional and sensory quality during storage. However, with more severe treatments more degradation of vitamin C, polyphenols and other compounds during processing is anticipated. Other approaches to improve stability and quality of strawberry products

during storage are to exclude oxygen during processing and storage and to add inhibitors of PPO and other degrading enzymes (Holzwarth, Wittig, Carle, & Kammerer, 2013; Howard, Brownmiller, & Prior, 2014; Howard, Prior, Liyanage, & Lay, 2012).

5 Conclusion

The microbiological shelf life of the strawberry purée and juice was at least 49 days at 6 °C when heat treated (HT) or processed at 500 or 600 MPa. There were minor differences in PPO activity in HPP and HT purées just after processing, but during storage the PPO activity increased in the HPP purées, but remained stable in the HT purée. This indicates that PPO was reactivated after HPP. Concentrations of vitamin C and anthocyanins declined faster in HPP purées than in HT purées during storage, probably due to insufficient inactivating and regeneration of PPO and other degrading enzymes in the HPP purées. Colour and viscosity also changed more in the HPP purées than in the HT purée during storage. No PPO activity was detected in the strawberry juice. In line with this, there were no differences between HPP- and HT juices in contents of anthocyanins and vitamin C during storage. These results indicate that raw materials with low initial enzyme activity, like juices, are more suited for HPP than e.g. purées. Furthermore, the results clearly demonstrated that major changes in chemical composition and quality occurred during storage, not during processing. Therefore, it is of utmost importance to include storage in studies of HPP and to follow activity of quality-degrading enzymes during storage of the products.

Acknowledgements and conflict of interest

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References

- Aaby, K., Wrolstad, R. E., Ekeberg, D., & Skrede, G. (2007). Polyphenol composition and antioxidant activity in strawberry purees; impact of achene level and storage. *Journal of Agricultural and Food Chemistry*, 55(13), 5156-5166.
- Aguilo-Aguayo, I., Soliva-Fortuny, R., & Martin-Belloso, O. (2010). High-intensity pulsed electric fields processing parameters affecting polyphenoloxidase activity of strawberry juice. *Journal of Food Science*, 75(7), C641-C646.
- Barrett, D. M., & Lloyd, B. (2012). Advanced preservation methods and nutrient retention in fruits and vegetables. *Journal of the Science of Food and Agriculture*, 92(1), 7-22.
- Bodelon, O. G., Avizcuri, J. M., Fernandez-Zurbano, P., Dizy, M., & Prestamo, G. (2013). Pressurization and cold storage of strawberry puree: Colour, anthocyanins, ascorbic acid and pectin methylesterase. *Lwt-Food Science and Technology*, 52(2), 123-130.
- Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E. A., Johnson, R. L., & Stewart, C. M. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science & Emerging Technologies*, 5(2), 135-149.

- Cano, M. P., Hernandez, A., & DeAncos, B. (1997). High pressure and temperature effects on enzyme inactivation in strawberry and orange products. *Journal of Food Science*, 62(1), 85-88.
- Cao, X., Bi, X., Huang, W., Wu, J., Hu, X., & Liao, X. (2012). Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innovative Food Science & Emerging Technologies*, 16, 181-190.
- Cao, X., Liu, F., Wu, J., Liao, X., & Hu, X. (2014). Effects of high hydrostatic pressure combined with blanching on microorganisms and quality attributes of cloudy and clear strawberry juices. *International Journal of Food Properties*, 17(9), 1900-1920.
- Cao, X., Zhang, Y., Zhang, F., Wang, Y., Yi, J., & Liao, X. (2011). Effects of high hydrostatic pressure on enzymes, phenolic compounds, anthocyanins, polymeric color and color of strawberry pulps. *Journal of the Science of Food and Agriculture*, 91(5), 877-885.
- Chaikham, P. (2015). Comparison of high hydrostatic pressure and thermal processing on physicochemical and antioxidant properties of Maoberry (*Antidesma thwaitesianum* Müell. Arg.) juice. *International Food Research Journal*, 22(5), 1993-2001.
- Chisari, M., Barbagallo, R. N., & Spagna, G. (2007). Characterization of polyphenol oxidase and peroxidase and influence on browning of cold stored strawberry fruit. *Journal of Agricultural and Food Chemistry*, 55(9), 3469-3476.
- Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S., Bompadre, S., Quiles, J. L., Mezzetti, B., & Battino, M. (2015). Strawberry as a health promoter: an evidence based review. *Food & Function*, 6(5), 1386-1398.
- Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. In R. E. Wrolstad (Ed.), *Current Protocols in Food Analytical Chemistry*, (pp. Unit F1.2.1-F1.2.13). New York: John Wiley & Sons, Inc.

- Goessinger, M., Moritz, S., Hermes, M., Wendelin, S., Scherbichler, H., Halbwirth, H., Stich, K., & Berghofer, E. (2009). Effects of processing parameters on colour stability of strawberry nectar from puree. *Journal of Food Engineering*, 90(2), 171-178.
- Guerrero-Beltran, J. A., Barbosa-Canovas, G., & Swanson, B. G. (2005). High hydrostatic pressure processing of fruit and vegetable products. *Food Reviews International*, 21(4), 411-425.
- Holzwarth, M., Korhummel, S., Kammerer, D. R., & Carle, R. (2012). Thermal inactivation of strawberry polyphenoloxidase and its impact on anthocyanin and color retention in strawberry (*Fragaria x ananassa* Duch.) purées. *European Food Research and Technology*, 235(6), 1171-1180.
- Holzwarth, M., Wittig, J., Carle, R., & Kammerer, D. R. (2013). Influence of putative polyphenoloxidase (PPO) inhibitors on strawberry (*Fragaria x ananassa* Duch.) PPO, anthocyanin and color stability of stored purees. *Lwt-Food Science and Technology*, 52(2), 116-122.
- Howard, L. R., Brownmiller, C., & Prior, R. L. (2014). Improved color and anthocyanin retention in strawberry puree by oxygen exclusion. *Journal of Berry Research*, 4, 107-116.
- Howard, L. R., Prior, R. L., Liyanage, R., & Lay, J. O. (2012). Processing and storage effect on berry polyphenols: Challenges and Implications for bioactive properties. *Journal of Agricultural and Food Chemistry*, 60(27), 6678-6693.
- Jacobo-Velazquez, D. A., & Hernandez-Brenes, C. (2010). Biochemical changes during the storage of high hydrostatic pressure processed avocado paste. *Journal of Food Science*, 75(6), S264-S270.
- Keenan, D. F., Brunton, N. P., Gormley, T. R., Butler, F., Tiwari, B. K., & Patras, A. (2010). Effect of thermal and high hydrostatic pressure processing on antioxidant activity and colour of fruit smoothies. *Innovative Food Science & Emerging Technologies*, 11(4), 551-556.

- Koseki, S., & Yamamoto, K. (2006). pH and solute concentration of suspension media affect the outcome of high hydrostatic pressure treatment of *Listeria monocytogenes*. *International Journal of Food Microbiology*, *111*(2), 175-179.
- Lavinas, F. C., Miguel, M. A. L., Lopes, M. L. M., & Mesquita, V. L. V. (2008). Effect of high hydrostatic pressure on cashew apple (*Anacardium occidentale* L.) juice preservation. *Journal of Food Science*, *73*(6), M273-M277.
- Li, D. T., Wang, P. P., Luo, Y. H., Zhao, M. Y., & Chen, F. (2017). Health benefits of anthocyanins and molecular mechanisms: Update from recent decade. *Critical Reviews in Food Science and Nutrition*, *57*(8), 1729-1741.
- Marszalek, K., Mitek, M., & Skapska, S. (2015). The effect of thermal pasteurization and high pressure processing at cold and mild temperatures on the chemical composition, microbial and enzyme activity in strawberry puree. *Innovative Food Science & Emerging Technologies*, *27*, 48-56.
- Marszalek, K., Wozniak, L., & Skapska, S. (2016). The application of high pressure-mild temperature processing for prolonging the shelf-life of strawberry puree. *High Pressure Research*, *36*(2), 220-234.
- Marszalek, K., Wozniak, L., Skapska, S., & Mitek, M. (2017). High pressure processing and thermal pasteurization of strawberry purée: quality parameters and shelf life evaluation during cold storage. *Journal of Food Science and Technology*, *54*(3), 832-841.
- Mazur, S. P., Nes, A., Wold, A.-B., Remberg, S. F., Martinsen, B. K., & Aaby, K. (2014). Effects of ripeness and cultivar on chemical composition of strawberry (*Fragaria x ananassa* Duch.) fruits and their suitability for jam production as a stable product at different storage temperatures. *Food Chemistry*, *146*(0), 412-422.
- Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008). Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends in Food Science & Technology*, *19*(6), 320-328.

- Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purees. *Innovative Food Science & Emerging Technologies*, *10*(3), 308-313.
- Sila, D. N., Duvetter, T., De Roeck, A., Verlent, I., Smout, C., Moates, G. K., Hills, B. P., Waldron, K. K., Hendrickx, M., & Van Loey, A. (2008). Texture changes of processed fruits and vegetables: potential use of high-pressure processing. *Trends in Food Science & Technology*, *19*(6), 309-319.
- Sulaiman, A., Farid, M., & Silva, F. V. M. (2016). Strawberry puree processed by thermal, high pressure, or power ultrasound: Process energy requirements and quality modeling during storage. *Food Science and Technology International*.
- Sulaiman, A., & Silva, F. V. M. (2013). High pressure processing, thermal processing and freezing of 'Camarosa' strawberry for the inactivation of polyphenoloxidase and control of browning. *Food Control*, *33*(2), 424-428.
- Suthanthangjai, W., Kajda, P., & Zabetakis, I. (2005). The effect of high hydrostatic pressure on the anthocyanins of raspberry (*Rubus idaeus*). *Food Chemistry*, *90*(1-2), 193-197.
- Terefe, N. S., Buckow, R., & Versteeg, C. (2014). Quality-related enzymes in fruit and vegetable products: Effects of novel food processing technologies, Part 1: High-pressure processing. *Critical Reviews in Food Science and Nutrition*, *54*(1), 24-63.
- Terefe, N. S., Kleintschek, T., Gamage, T., Fanning, K. J., Netzel, G., Versteeg, C., & Netzel, M. (2013). Comparative effects of thermal and high pressure processing on phenolic phytochemicals in different strawberry cultivars. *Innovative Food Science & Emerging Technologies*, *19*, 57-65.
- Terefe, N. S., Matthies, K., Simons, L., & Versteeg, C. (2009). Combined high pressure-mild temperature processing for optimal retention of physical and nutritional quality of strawberries (*Fragaria x ananassa*). *Innovative Food Science & Emerging Technologies*, *10*(3), 297-307.

- 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 & Emerging Technologies*, *11*(1), 52-60.
- Yang, F. M., Li, H. M., Li, F., Xin, Z. H., Zhao, L. Y., Zheng, Y. H., & Hu, Q. H. (2010). Effect of nano-packing on preservation quality of fresh strawberry (*Fragaria ananassa* Duch. cv Fengxiang) during storage at 4 °C. *Journal of Food Science*, *75*(3), C236-C240.
- Zabetakis, I., Leclerc, N., & Kajda, P. (2000). The effect of high hydrostatic pressure on the strawberry anthocyanins. *Journal of Agricultural and Food Chemistry*, *48*(7), 2749-2754.

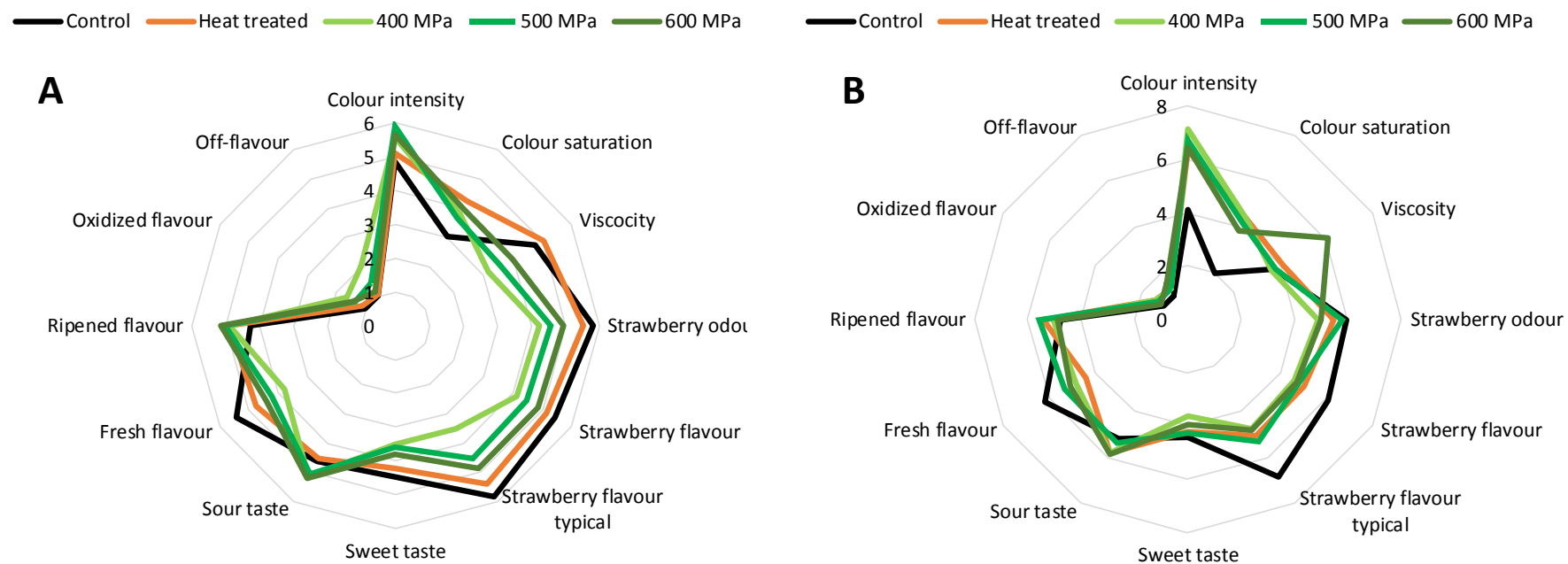


Fig. 1. Sensory profile of strawberry purée (A) and juice (B). The heat- and HP treated products had been stored for 35 days at 6 °C. The control had been frozen at day 0, and was thawed prior to analysis.

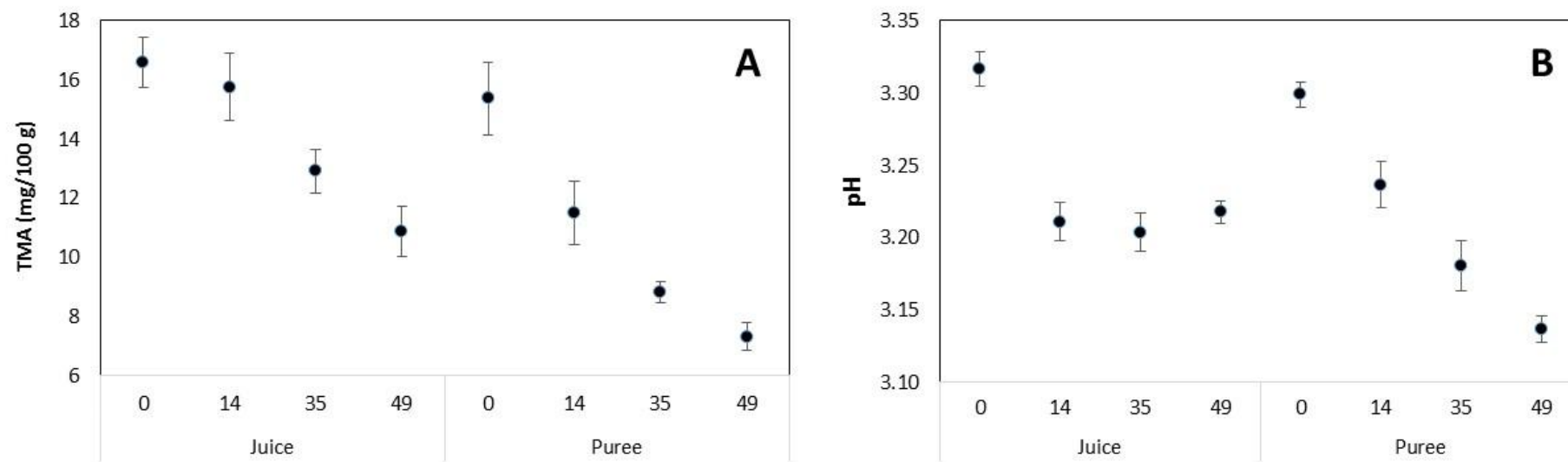


Fig. 3. Matrix \times Storage interaction plots for TMA (A) and pH (B). Data are the means of HPP treatments (400, 500 and 600 MPa), and the vertical bars are standard deviations.

Table 1. ANOVA^a of pH, Brix, polyphenol oxidase (PPO), total monomeric anthocyanines (TMA), vitamin C and colour parameters (L*, Hue, Chroma, ΔE) in strawberry purées after HPP and storage. The numbers are explained variances (%) and the significant levels are represented as stars^b

	DF ^c	pH	Brix	PPO	TMA	Vitamin C	L*	Hue	Chroma	ΔE
Pressure (P)	2	0.0	10.2**	6.0***	0.0	0.6	1.1	4.1	0.9	1.0
Holding time (H-time)	1	0.1	2.7	1.6**	0.0	0.6	0.4	0.0	0.1	0.2
Storage (S)	3(2)	95.5***	22.2***	79.4***	91.5***	76.3***	51.1***	34.2***	83.6***	75.3***
P x H-time	2	0.3	0.6	0.0	0.1	1.4	3.3	4.9*	0.6	0.3
P x S	6(4)	0.9*	11.9	2.5*	0.4	2.0	9.5*	11.0*	0.6	1.9
H-time x S	3(2)	0.0	3.9	1.3*	0.2	1.1	1.9	4.3	0.2	0.1
P x H-time x S	6(4)	0.6	1.9	1.6	1.0	4.3*	7.4*	7.1	0.8	0.3
Residual (Error)	48(36)	2.6	46.5	7.6	6.7	13.5	25.2	34.3	13.3	20.8
R-sq (adj)		0.96	0.31	0.89	0.90	0.80	0.63	0.49	0.80	0.69

^aMain effects and their interactions are included. The factors were Pressure (400, 500 or 600 MPa), Holding time (1.5 or 3 min) and Storage (0, 14, 35 or 49 days). ^bExplained variances are the sum-of-squares as % of total sum-of squares. Significant levels: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. ^cDegrees of freedom. The numbers in brackets are for vitamin C. Vitamin C was not measured after 49 days.

Table 2: pH, Brix, polyphenol oxidase (PPO), total monomeric anthocyanins (TMA), vitamin C and colour parameters (L*, Hue, chroma, ΔE) in strawberry purées after processing and storage^a

Samples	Storage		TMA			Vitamin C				
	(days)	pH	Brix (%)	PPO (abs/min/g)	(mg/100 g)	(mg/100 g)	L*	*Hue	Chroma	ΔE
Control	0	3.30±0.01 b	8.3±0.1 ab	10.0±1.5 de	14.5±0.5 bc	17.0±0.4 ab	20.1±0.5 abcd	32.8±0.2 a	34.4±0.8 a	
HT	0	3.36±0.01 a	8.2±0.2 ab	7.5±0.9 efg	17.8±1.3 a	21.9±4.1 a	21.6±1.1 abc	32.8±0.4 a	35.1±0.8 a	1.8±0.6 de
	14	3.34±0.01 a	8.3±0.2 ab	6.4±1.0 fg	15.0±0.8 b	10.6±0.3 cd	22.0±0.6 ab	31.3±0.4 abc	35.1±0.4 a	2.2±0.3 cde
	35	3.33±0.01 ab	7.9±0.3 b	6.3±0.4 g	12.0±0.8 cd	0.5±0.8 e	20.6±1.9 abcd	31.5±1.5 abc	32.3±2.3 a	4.0±0.8 abcde
	49	3.25±0.01 c	8.3±0.1 ab	6.4±1.3 fg	9.8±1.0 defgh	nm ^b	22.7±0.8 a	30.4±1.1 abcd	32.9±1.9 abc	4.1±0.6 abcde
400 MPa (3 min)	0	3.30±0.01 b	8.3±0.3 ab	8.9±0.6 def	15.7±1.9 ab	14.2±2.8 bc	19.0±0.5 bcde	32.3±0.2 ab	33.1±0.3 ab	1.8±1.2 de
	14	3.24±0.01 c	8.7±0.2 ab	10.8±0.7 cd	11.6±1.5 def	5.7±0.1 de	18.5±0.5 cdef	31.4±0.4 abc	32.1±1.1 abcde	3.1±1.8 bcde
	35	3.18±0.012 f	8.3±0.4 ab	13.2±0.4 bc	8.4±0.1 gh	0.1±0.1 e	19.1±2.1 bcde	28.0±2.5 d	28.9±1.4 de	6.7±1.9 ab
	49	3.15±0.01 fg	8.5±0.9 ab	15.9±1.6 a	7.3±0.5 h	nm	18.9±0.7 bcde	29.2±0.6 cd	29.6±1.0 cde	5.5±1.6 abcd
500 MPa (3 min)	0	3.30±0.01 b	8.1±0.1 ab	8.8±0.5 defg	15.5±1.2 ab	17.5±5.5 ab	19.7±0.5 abcde	31.9±0.4 ab	33.9±0.6 a	1.1±0.3 e
	14	3.23±0.03 cd	8.4±0.2 ab	9.0±0.9 def	11.8±0.2cde	3.1±1.4 e	18.1±0.7 def	31.8±0.2 abc	32.0±0.3 abcde	3.3±1.4 abcde
	35	3.17±0.02 ef	8.6±0.3 ab	11.1±0.6 cd	9.0±0.3 fgh	0.0±0.0 e	16.5±2.3 ef	31.2±0.7 abc	29.9±1.8bcde	6.0±3.1 abc
	49	3.13±0.01 g	8.7±0.1 ab	15.4±0.9 ab	7.5±0.6 h	nm	18.8±0.2 bcdef	29.9±0.2 bcd	29.7±0.9 bcde	5.3±0.5 abcd
600 MPa (3 min)	0	3.30±0.01 b	8.3±0.1 ab	7.0±0.9 fg	14.9±0.6 b	17.4±1.8 ab	19.7±0.7 abc	32.2±0.9 ab	33.7±1.3 a	1.1±0.8 e
	14	3.24±0.01 c	8.7±0.2 ab	8.5±0.2 defg	11.0±1.4 defg	0.7±0.8 e	19.1±0.5 bcde	31.1±0.3 abc	32.5±0.5 abc	3.1±1.1 abcde

35	3.20±0.01 de	8.6±0.1 ab	10.6±0.7 cd	9.0±0.3 efgh	0.0±0.0 e	15.6±0.7 f	32.5±0.7 ab	28.8±0.6 e	7.3±0.9 a
49	3.13±0.01 g	8.9±0.1 a	15.0±0.4 ab	7.2±0.5 h	nm	18.9±0.4 bcde	30.7±0.4 abc	30.0±0.9 bcde	4.8±1.3 abcde

^aControl; untreated. HT; heat treated. 400, 500 and 600 MPa; high pressure treated (holding time 3 min). The results are means and standard deviations of samples from processing parallels (n=3). Values within a column that do not share a letter are significant different ($p < 0.05$) as determined by Tukey's multiple comparison test. ^bNot measured.

Table 3: pH, Brix, total monomeric anthocyanins (TMA), vitamin C and colour parameters (L^* , Hue, chroma, ΔE) in strawberry juices after processing and storage^a

Sample	Storage		Vitamin C						
	(days)	pH	Brix (%)	TMA (mg/100 g)	(mg/100 g)	L^*	*Hue	Chroma	ΔE
Control	0	3.34±0.01 a	7.4±0.0 b	15.9±0.9 a	19.2±0.0 ab	8.6±0.3 a	27.8±0.3 a	26.7±0.1 a	
HT	0	3.34±0.01 a	7.6±0.1 ab	15.5±0.7 ab	16.5±1.1bc	7.0±0.1 abcd	24.3±0.1 bc	25.3±0.1 ab	2.7±0.3 cd
	14	3.26±0.01 c	7.4±0.2 ab	14.8±2.9 abc	2.1±1.0 d	8.0±0.7 ab	25.2±1.4 abc	24.0±0.3 abc	3.1±0.1 bcd
	35	3.26±0.01 c	7.5±0.1 ab	11.5±0.3 de	1.0±0.1 d	6.9±0.7 abcd	23.5±0.9 bc	23.5±0.8 abc	4.1±0.9 abcd
	49	3.26±0.01 c	7.5±0.1 ab	10.1±0.8 e	nm ^b	7.8±0.9 abc	24.7±1.3 abc	22.8±0.6 abc	4.3±0.9 abcd
400 MPa (3 min)	0	3.32±0.01 ab	7.8±0.3 a	16.6±0.9 a	19.8±4.8 ab	7.8±1.0 abcd	26.0±2.0 ab	26.3±0.5 ab	1.5±1.2 d
	14	3.22±0.01 d	6.8±0.8 b	15.5±2.0 ab	2.5±1.9 d	6.8±1.1 abcd	23.8±2.5 bc	23.8±2.3 abc	3.8±2.4 abcd
	35	3.21±0.01 d	7.4±0.1 ab	12.6±0.2 bcde	1.6±0.4 d	6.2±0.7 bcd	22.0±0.6 c	21.5±0.8 abc	6.3±0.9 abc
	49	3.22±0.01 d	7.6±0.2 ab	11.6±0.1 cde	nm	6.4±0.5 bcd	22.5±0.4 c	20.5±0.8 bc	7.0±0.8 a
500 MPa (3 min)	0	3.33±0.01 ab	7.6±0.2 a	16.8±0.8 a	24.3±2.6 a	7.4±0.3 abcd	25.2±0.8 abc	26.3±0.6 ab	1.8±0.8 d
	14	3.20±0.01 d	7.5±0.2 ab	15.9±0.8 a	1.1±1.5 d	6.0±0.6 bcd	22.4±0.7 c	19.0±7.1 c	5.6±1.5 abc
	35	3.20±0.01 d	7.6±0.2 ab	13.8±0.2 abcd	1.0±0.4 d	6.0±0.6 bcd	22.3±0.5 c	20.5±1.3 bc	7.1±1.3 a
	49	3.21±0.01 d	7.5±0.1 ab	11.2±0.4 de	nm	7.4±1.0 abcd	24.1±1.1 bc	21.2±0.8 abc	5.9±1.1 abc
600 MPa (3 min)	0	3.30±0.01 b	7.6±0.3 ab	16.4±1.2 a	11.0±0.1 c	8.0±0.9 ab	26.3±2.0 ab	26.2±0.4 ab	1.3±1.3 d
	14	3.21±0.01 d	7.6±0.1 a	15.8±0.4 a	4.3±1.8 d	6.4±0.5 bcd	22.9±0.6 bc	22.9±1.3 abc	4.9±1.3 acd
	35	3.20±0.02 d	7.4±0.2 ab	12.4±0.5 bcde	1.1±0.2 d	5.7±0.6 d	22.5±0.8 c	21.3±2.3 abc	6.6±2.2 ab

49	3.22±0.01 d	7.4±0.1 ab	9.8±0.4 e	nm	5.8±0.6 d	22.1±0.7 c	20.7±0.8 abc	7.1±0.9 a
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^aControl; untreated. HT; heat treated. 400, 500 and 600 MPa; high pressure treated (holding time 3 min). The results are means and standard deviations of samples from processing parallels (n=3). Values within a column that do not share a letter are significant different ($p < 0.05$) as determined by Tukey's multiple comparison test. ^bNot measured.

Table 4. ANOVA^a of pH, Brix, total monomeric anthocyanines (TMA), vitamin C and colour parameters (L*, Hue, chroma, ΔE) in strawberry purées and juices after HPP and storage. The numbers are explained variances (%) and significant levels are represented as stars^b

	DF ^c	pH	Brix	TMA	Vitamin C	L*	Hue	Chroma	ΔE
Matrix (M)	1	4.6***	67.8***	25.2***	0.3	95.1***	83.2***	72.4***	2.7*
Pressure (P)	2	0.4*	0.9	0.6	1.5**	0.1	0.4	0.2	0.0
Storage (S)	3(2)	79.8***	1.0	64.7***	87.0***	1.5***	6.6***	14.6***	70.0***
M x P	2	0.1	0.7	0.1	0.6	0.0	0.6*	0.5	0.4
M x S	3(2)	11.2***	5.4**	3.5***	0.6	0.1	1.1*	1.2	2.1
P x S	6(4)	0.8*	1.8	0.4	2.9**	0.5*	1.8**	1.0	1.2
M x P x S	6(4)	0.5	4.0	0.6	4.8***	0.4	1.3*	0.7	1.3
Residual (Error)	48(30)	2.6	18.3	4.9	3.7	1.5	4.2	8.3	22.5
R-sq(adj)		0.96	0.73	0.93	0.94	0.98	0.94	0.88	0.66

^aMain effects and their interactions are included. The factors are Matrix (purée or juice), Pressure (400, 500 or 600 MPa for 3 min) and Storage (0, 14, 35 or 49 days). ^bExplained variances are the sum-of-squares as % of total sum-of squares. Significant levels: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. ^cDegrees of freedom. The numbers in brackets are for vitamin C. Vitamin C was not measured after 49 days.

Highlights

- Shelf life of strawberry products was at least 49 days when processed at ≥ 500 MPa
- PPO in HPP strawberry purées was reactivated during storage at 6 °C
- Anthocyanins, vitamin C and colour were better preserved in juices than in purees