

High hydrostatic pressure treatment and storage of carrot and tomato juices: Antioxidant activity and microbial safety

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Abstract: The application of high hydrostatic pressure (HHP) (250 MPa, 35 °C for 15 min) and thermal treatment (80 °C for 1 min) reduced the microbial load of carrot and tomato juices to undetectable levels. Different combinations of HHP did not cause a significant change in the ascorbic acid content of either juice ($P > 0.05$). Both heat treatments (60 °C for 5–15 min and 80 °C for 1 min) resulted in a significant loss ($P < 0.05$) in the free-radical scavenging activity as compared to untreated samples. HHP-treated juices showed a small loss of antioxidants (below 10%) during storage. The ascorbic acid content of pressurized tomato and carrot juices remained over 70 and 45% after 30 days of storage, respectively. However, heat treatment caused a rapid decrease to 16–20%. Colour changes were minor ($\Delta E = 10$) for pressurized juices but for heat-pasteurised samples it was more intense and higher as a result of insufficient antioxidant activity. HHP treatment (250 MPa, 35 °C for 15 min) led to a better product with regard to anti-radical scavenging capacity, ascorbic acid content and sensory properties (colour, pH) of the tomato and carrot juices compared to conventional pasteurisation. Therefore, HHP can be recommended not only for industrial production but also for safe storage of fresh juices, such as tomato and carrot, even at elevated storage temperatures (25 °C).

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Keywords: high hydrostatic pressure; heat treatment; tomato juice; carrot juice

INTRODUCTION

Consumer demand has increasingly required minimally processed foods to have more natural flavour and colour with high nutritional quality and storage that is sufficient for distribution and consumption. This can be achieved by novel minimal processing methods such as pulsed electric fields, high hydrostatic pressure (HHP), high intensity light and ultrasound applications which inactivate micro-organisms and enzymes with little loss of pigments, flavour compounds and vitamins.

Microbial spoilage of juice products may lead to off flavours, odours, turbidity and gas production.¹ A limited range of yeasts, moulds and aciduric bacteria are able to grow at the low pH of orange juice, typically pH 3.3–4.0.² To extend storage, mild heat treatments (60–65 °C) are required to destroy yeasts and most fungal spores, while higher temperatures (89–95 °C) are required for inactivation of lactic acid bacteria. However, *Alicyclobacillus* spp. and the ascospores of some heat resistant moulds may still not be inactivated at these higher temperatures.³ Historically, acid foods such as fruit juices have been considered safe but recent outbreaks of food-borne diseases which have been attributed to unpasteurised juices contaminated with pathogens such as *Salmonella* spp. and *Escherichia coli* O157:H7 have demonstrated that unpasteurised juices can be a vehicle for food-borne illnesses.^{4–6}

HHP can be used to avoid the detrimental effects, including vitamin losses, instead of traditional thermal pasteurisation of many foods.⁷ Traditional thermal processing of orange juice causes vitamin losses.⁸ Ascorbic acid and carotenoids are very reactive compounds and have been reported to be minimally affected by HHP treatment.^{9–12} Pressure treatments of orange juice had minimal effect on other quality parameters, including pH and soluble solids content, with an extended refrigerated storage.¹³ On the other hand, Bignon¹⁴ observed that the vitamin A, C, B₁, B₂ and E content of fruit and vegetable products is not significantly affected by pressure treatment in contrast to thermal treatment. Besides, in the case of strawberries and guava puree, the decrease in ascorbic acid content during storage after pressure treatment (400–600 MPa, 15–30 min, 20 °C) was found to be much lower compared to the fresh products.¹² The kinetic study of pressure–temperature stability of ascorbic acid in buffer, orange and tomato juices was performed by Van Den Broeck *et al.*¹⁵ They found only significant degradation of ascorbic acid when pressures of about 850 MPa was combined with temperatures between 60 and 80 °C, and more in tomato and orange juice than in buffer. For many fruit products such as fruit jam, strawberries, tomato juice, guava, avocado and banana puree, HHP treatment was noted largely to preserve fresh

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colour.^{16–20} Park *et al.*²¹ reported that the cloud and colour of carrot juice was not significantly affected after combined treatment of high-pressure carbon dioxide (4.90 MPa) and HHP treatment of up to 600 MPa. The brightness (*L* value) and redness/greenness (*a* value) of pressure-treated products were found to be superior compared with their thermally treated counterparts. However, during storage of guava and banana puree, the colour change was observed because of browning as a result of residual polyphenoloxidase activity.^{20,22} The longest storage time was achieved by using high pressure, low pH and refrigerated storage. Recent studies showed that HHP treated juices have better antioxidant retention compared to thermally processed ones.^{23,24} Ascorbic acid constituted the most important antioxidant compound reacting instantaneously with free radicals, while the reaction of other antioxidants of orange juice (flavonoids and other polyphenolic compounds) was time dependent. A treatment of 600 MPa at 40 °C for 4 min led to a better retention of antioxidant activity during post processing storage of orange juice at 0–30 °C compared to conventional thermal pasteurisation (80 °C, 60 s), mainly due to lower degradation rates of ascorbic acid.²⁴ Fernández-García *et al.*²⁵ and Indrawati *et al.*²⁶ also reported that antioxidant activity of orange–lemon–carrot and orange and lemon juices was not significantly affected by HHP treatment, respectively. It was evident that the thermal treatments induced a decrease in the free-radical scavenging activity and were contemporarily responsible for the degradation of ascorbic acid in blood-orange juice.²³ Sánchez-Moreno *et al.*²⁷ and De Ancos *et al.*²⁸ also reported that HHP treatments of 50–250 MPa, 30–60 °C, 15–30 min did not significantly affect the anti-radical scavenging of orange juices.

The objective of this research was to study the effects of HHP on total aerobic bacteria, antioxidant activity, ascorbic acid content, colour and pH of tomato and carrot juices in comparison to conventional heat pasteurisation. The changes of these parameters were also determined during storage at 4 and 25 °C for 30 days.

MATERIALS AND METHODS

Fresh tomatoes (*Lycopersicon esculentum* cv. 144) and carrots (*Daucus carota* L.) were purchased from a local market. They were all harvested in mid-season in Finike (Antalya, Turkey). Juice was extracted from tomatoes and carrots by using a domestic fruit processor (Moulinex, Spain) and the samples were filtered by using cheese cloth.

High hydrostatic pressure treatment

HHP treatments were performed in a designed and constructed laboratory-scale unit (capacity, 30 cm³; maximum pressure, 500 MPa). The rate of pressure increase and pressure release was approximately

5–10 s for the designed system. A mixture of deionised water and glycol was used as the pressure transmitting medium. The equipment consists of a pressure chamber of cylindrical design, two end closures, a means for restraining the end closures, a pressure pump, and a hydraulic unit to generate high pressure for system compression and also a temperature control device. The pressure vessel was made of hot galvanised carbon steel and the piston was hard chrome plated and polished to mirror finish (steel-type heat treated special K) which was processed into the required sizes at the Electrical and Electronic Engineering Department of Middle East Technical University, Ankara, Turkey. The liquid was heated prior to pressurisation to the desired temperature (25 and 35 °C) by an electrical heating system surrounding the unit. For pressurisation two independently prepared cryovials (Simport Plastic, Quebec, Canada) were filled with freshly squeezed tomato and carrot juices and closed with great care, avoiding as many air bubbles as possible. They were placed inside the cylindrical vessel of the HHP equipment and the chamber was closed. As HHP is a process that involves three variables – pressure, time and temperature – it is necessary to study the interaction of these factors in order to determine the effective use of HHP in food preservation. In addition, it is also necessary to determine the minimum conditions for obtaining desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality. Therefore samples were treated at 150, 200 and 250 MPa for 5, 10 and 15 min at 25 and 35 °C; based on the effect on total microbial count presented in this study and also according to previous studies by the group.^{29,30} The samples were held in the insulated pressurisation chamber for 1–2 min for temperature equilibration before pressurisation; this temperature and time relation for equilibration had been determined earlier.²⁹ Immediately after pressurisation, the vials were removed and cooled in an ice bath. Pressurisation time reported in this study did not include the pressure increase and release times. Reported temperature is the actual process temperature during hold time at reported pressure levels. The pressure level, time and temperature of pressurisation were all recorded during the pressurisation cycle. Controls were not pressurised.

For heat pasteurisation, samples were heat treated in a water bath at 60 °C for 5, 10 and 15 min (representative of the mildest pasteurisation treatment used by the industry) and at 80 °C for 1 min (industrial pasteurisation application).²³ Two tubes containing the same amount (2 mL) of sample were placed in the water bath that was set to the desired temperature. Tubes were continuously stirred during pasteurisation to improve heat transfer. When the samples reach the desired temperature as measured by a digital temperature probe, one of the tubes was taken out from the water bath and immediately cooled in an

ice bath. Another tube with the sample was kept at the desired temperature for the reported process time. In this way the increased effect is determined and subtracted from the total effect. All experiments and measurements were replicated twice on separate days.

Storage

Duplicate samples pressurised at 250 MPa at 35 °C for 15 min or heat treated at 80 °C for 1 min were used for storage analysis. These treatment conditions were selected due to total microbial inactivation as given in the results and discussion section. All the measurements were replicated twice except colour measurements (ΔE), which were replicated three times. The samples were stored at 4 and 25 °C in the dark for up to 1 month and analysed at 2-day intervals. New cryovials were opened each time. Untreated samples were used as controls.

Microbiological analysis

Serial dilutions of HHP and heat treated juices were performed in 0.1% peptone (Merck, Darmstadt, Germany) water. Total aerobic count was determined by the spread plate technique on tryptic soy agar (TSA) (Merck). Duplicate agar plates were used for each sample and incubated at 37 °C \pm 1 °C for 48 h. Plates containing 25–250 cfu mL⁻¹ were selected for counting. Two separate cryovials filled with same juice were pressurised and two sterile Petri dishes were prepared by the spread plate method for each cryovial. All experiments and measurements were replicated twice on separate days. Average results were presented.

Antioxidant scavenging activity

Antioxidant activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma, Steinheim, Germany). Scavenging activity of juices was measured by DPPH radical quenching.³¹ One millilitre of juice sample was added to 4 mL of methanol (Riedel, Inc., Steinheim, Germany) and homogenised in a refrigerated centrifuge (Sorvall RC5C, Du Pont, Wilmington, USA) at 41692 \times g for 5 min. An aliquot of 0.1 mL of this product is added to 2 mL of methanolic solution containing DPPH (0.025 g DPPH L⁻¹ methanol) in a spectrophotometer cuvette. The reaction mixture was shaken and left to stand for 15 min at room temperature in the dark. The absorbance values were measured in a spectrophotometer (Novaspec II; Pharmacia LKB, Cambridge, UK) at 517 nm against a blank of methanol without DPPH. The DPPH concentration in the reaction medium was calculated from the calibration curve, determined by linear regression: $A_{517} = 30.59D - 0.0386$, where A_{517} is the absorbance read at 517 nm and D is the amount of the free radical as grams of DPPH per litre. Data were evaluated with the initial change in DPPH concentration. Results are given as $c = D_{t=0} - D_{t=15}$, where c is the antioxidant activity, $D_{t=0}$ is the amount of DPPH at the beginning of the reaction (i.e. 0 min)

and $D_{t=15}$ is the amount of DPPH 15 min after the reaction starts.

For the storage analysis, the retention of antioxidant activity was estimated by using the equation $c_R = \frac{c_t}{c_{t=0}} \times 100$, where c_R is the remaining antioxidant activity (%), c_t is the antioxidant activity during the storage period and $c_{t=0}$ is the antioxidant activity of the unprocessed sample.

Measurement of ascorbic acid and pH

Ascorbic acid content of the samples was analysed by using the 2,6-dichlorophenolindophenol titrimetric method.³² The pH values of the samples were determined by using a pH meter (WTW 537 pH meter; WTW, Weilheim, Germany) at 20 °C.

Measurement of colour

The colour of the samples was determined by using an Avantes spectrophotometer (Avaspec-2048; Avantes, Eerbeek, The Netherlands) with a light source set on D65. L , a and b values were measured and the colour change (ΔE) is calculated using the formula below,³³ where L_0 , a_0 and b_0 values are the values for a standard white solution (0.05 g TiO₂ 100 mL⁻¹ water).

$$\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{\frac{1}{2}}$$

Statistical analysis of the data

The results of HHP and heat treatment were submitted to one-way analysis of variance (ANOVA). For the shelf-life analysis a three-way ANOVA was used with treatments, replication of the experiments, storage time and temperature as factors. Significant differences between means were tested using Duncan's multiple range test with a probability level fixed at $P < 0.05$. Differences at $P < 0.05$ were considered to be significant. Statistical treatments were carried out with SPSS (Chicago, IL, USA) 10.0 for Windows.

RESULTS AND DISCUSSION

Values for log₁₀ reductions calculated from the total aerobic counts are presented on Fig. 1 after HHP and thermal treatments. The initial microbial loads of tomato (pH = 4.5) and carrot (pH = 6.0) juices were 4.5 and 5.5 log₁₀ cfu mL⁻¹, respectively. Pressure treatment of 250 MPa, 35 °C, 15 min and heat treatment at 80 °C, 1 min were sufficient to reduce the population levels below the detection limit (<1 cfu mL⁻¹). The destructive effect of pressure, in a range much higher than that was used in our study (615 MPa, 15 °C, 2 min), on *E. coli* O157:H7 and *Salmonella* in unpasteurised grapefruit, orange, apple and carrot juices was reported.³⁴ Also, the effect of fruit juice pH in the range 3.4–4.5 on the inactivation of pressure resistant *E. coli* O157:H7 in orange juice at 550 MPa, 20 °C, 5 min and on the inactivation of acid resistant *Alicyclobacillus acidoterrestris* at 350 MPa, 50 °C, 20 min

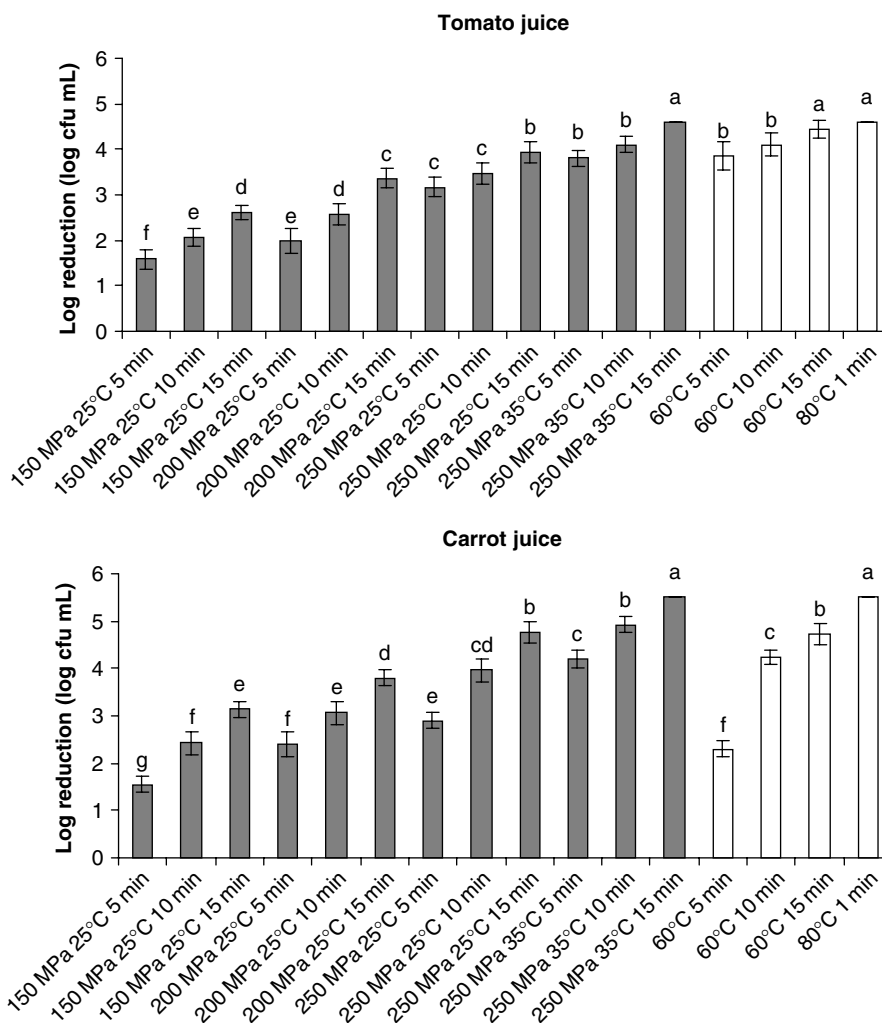


Figure 1. The reduction of total aerobic bacteria in juices treated by high pressure and heat. The initial microbial loads of tomato (pH = 4.50) and carrot (pH = 6.0) juices were 4.5 and 5.5 log₁₀ cfu mL⁻¹ respectively. Different letters imply significant changes (P < 0.05).

is reported.^{35,36} A pressure of 600 MPa at 20 °C for 20 s was required for a reduction of 4.5 log₁₀ cfu mL⁻¹ of viable aerobic bacteria in Valencia and Navel orange juices.³⁷ These studies reveal that, depending on pressure and/or acid resistance of the micro-organism, higher pressures combined with shorter processing times or higher pressurisation temperatures (50 °C) is needed for complete inactivation, as compared to results (250 MPa, 35 °C, 15 min) presented in this study.

Ascorbic acid is an indicator of nutritional quality in fruit juices. HHP treatments provided more ascorbic acid retention than thermal treatments as compared to the untreated control sample (Fig. 2). The change in ascorbic acid content of both juices by different HHP combinations was not statistically significant (P > 0.05) Polydera *et al.*³⁸ reported that high pressure treatment of 500 MPa, 35 °C, 5 min led to a better retention of ascorbic acid in orange juice when stored at 0–15 °C compared to thermal pasteurisation (80 °C, 30 s). However, in tomato puree, stronger HHP treatment even at lower temperatures (400 MPa, 25 °C, 15 min) and higher pasteurisation temperatures even at shorter

times (70 °C, 30 s or 90 °C, 1 min) significantly decrease the ascorbic acid and vitamin C content as compared to the untreated samples.³⁹ Therefore when selecting the HHP parameters consideration of the product is of great importance. For instance, for tomato juice 250 MPa, 25 °C, 15 min and 60 °C 10 min treatments are equivalent in intensity, and are insignificant statistically (P > 0.05) in terms of microbial inactivation (around 3.5 log reduction) although the former results in a lower loss of ascorbic acid than does the latter (P < 0.05). Sancho *et al.*¹² reported that minor variations were found among the vitamins (B₁, B₆ and C) after pressurisation (200, 400, 600 MPa for 30 min at 20 °C), where ascorbic acid was not affected by the intensity of HHP applied.

Figure 3 shows the antioxidant activity of high pressure and thermal treated juices. The antioxidant capacity of orange juice is reported to decrease more quickly than that of carrot juice as pressure is increased from 100 to 800 MPa at a temperature range of 75 to 120 °C.²⁶ However, for thermal treatments the activity decreases as the treatment becomes harsher (longer processing time, higher processing temperature). Lo Scalzo *et al.*²³ reported that the

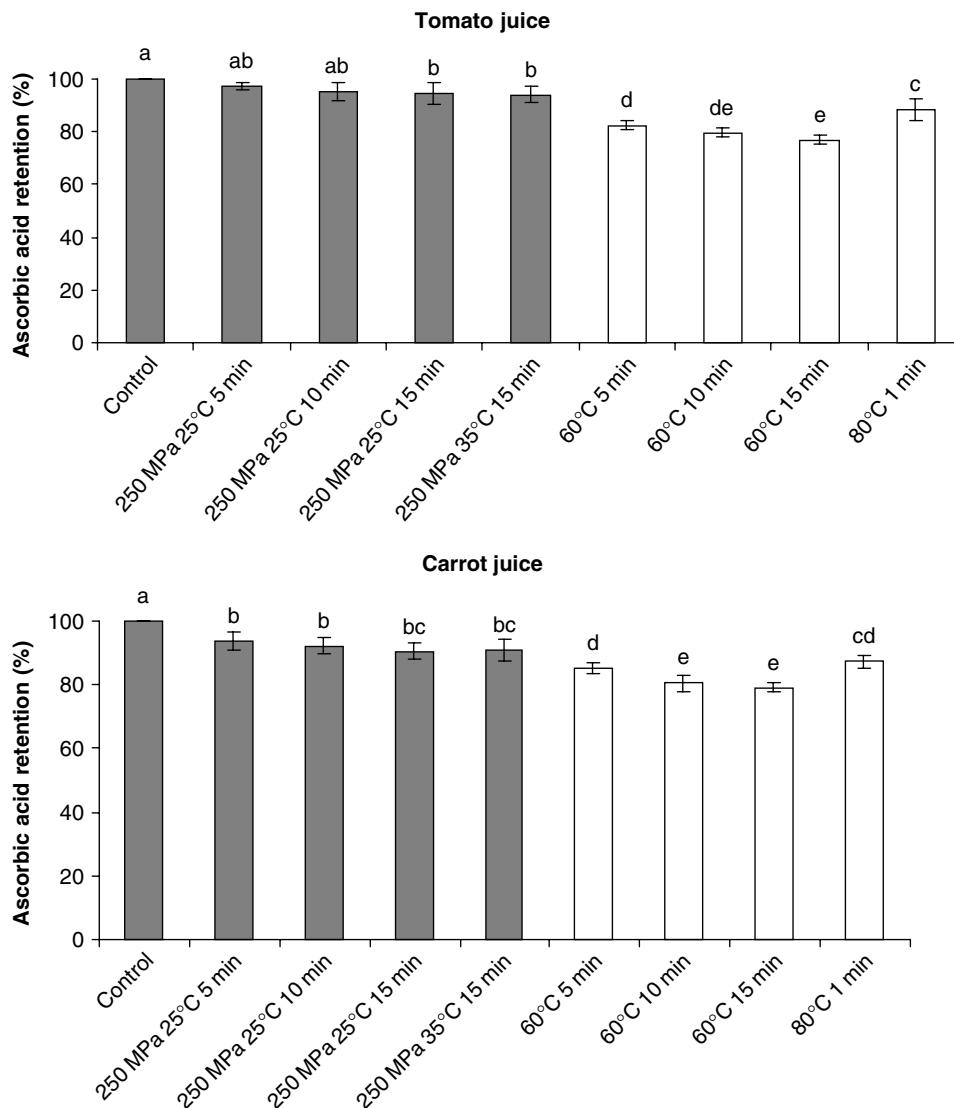


Figure 2. The retention of ascorbic acid in juices treated by high pressure and heat. The error bars denote the standard deviation. Different letters imply significant changes ($P < 0.05$).

thermal treatments induced a decrease in the free radical scavenging activity of blood orange juice as the samples were blanched (80 °C for 6 min), pasteurised (80 °C for 1 min) and blanch-pasteurised (subjected to blanching and then pasteurisation) and compared to the untreated juice samples for radical scavenging activity. For carrot and tomato juices, there was no significant difference between 250 MPa, 35 °C, 15 min pressure treatment and 80 °C, 1 min heat treatment ($P < 0.05$). Likewise, a high temperature, short time, heat treatment did not affect those compounds responsible for anti-radical scavenging. HHP treatment of carrot juice below 40 °C is recommended as the increase in the antioxidant capacity of carrot juice was reduced by a pressure increase at temperatures above 40 °C.²⁶ Generally, antioxidant activity is related to the antioxidant vitamins, carotenoids and polyphenol contents of fruits and vegetable products. In this study, a positive correlation was found between ascorbic acid and antioxidant activity ($r = 0.955$, $p = 6.28 \times 10^{-5}$ for

carrot juice and $r = 0.934$, $p = 2.27 \times 10^{-4}$ for tomato juice).

Total microbial inactivation was achieved by either HHP (250 MPa, 35 °C, 15 min) or thermal treatments. Overall, HHP applications gave better antioxidant and ascorbic acid values than heat treatments studied. Both juices were microbiologically stable with no microbial growth observed throughout storage period of 30 days at 4 and 25 °C (data not shown).

The ascorbic acid content of pressurised tomato and carrot juices, when stored at both 4 and 25 °C, remained over 70% and 45% after 30 days of storage, respectively (Fig. 4). However, tomato and carrot juices treated at 80 °C for 1 min displayed a rapid decrease of ascorbic acid when stored at both 4 and 25 °C. Carrot juice had no ascorbic acid after 16 and 18 days for 4 and 25 °C storage, respectively; whereas tomato juice had only around 15% at both storage temperatures. Throughout the storage period, HHP-treated juices were judged to be of superior quality than the conventional, thermally processed ones in

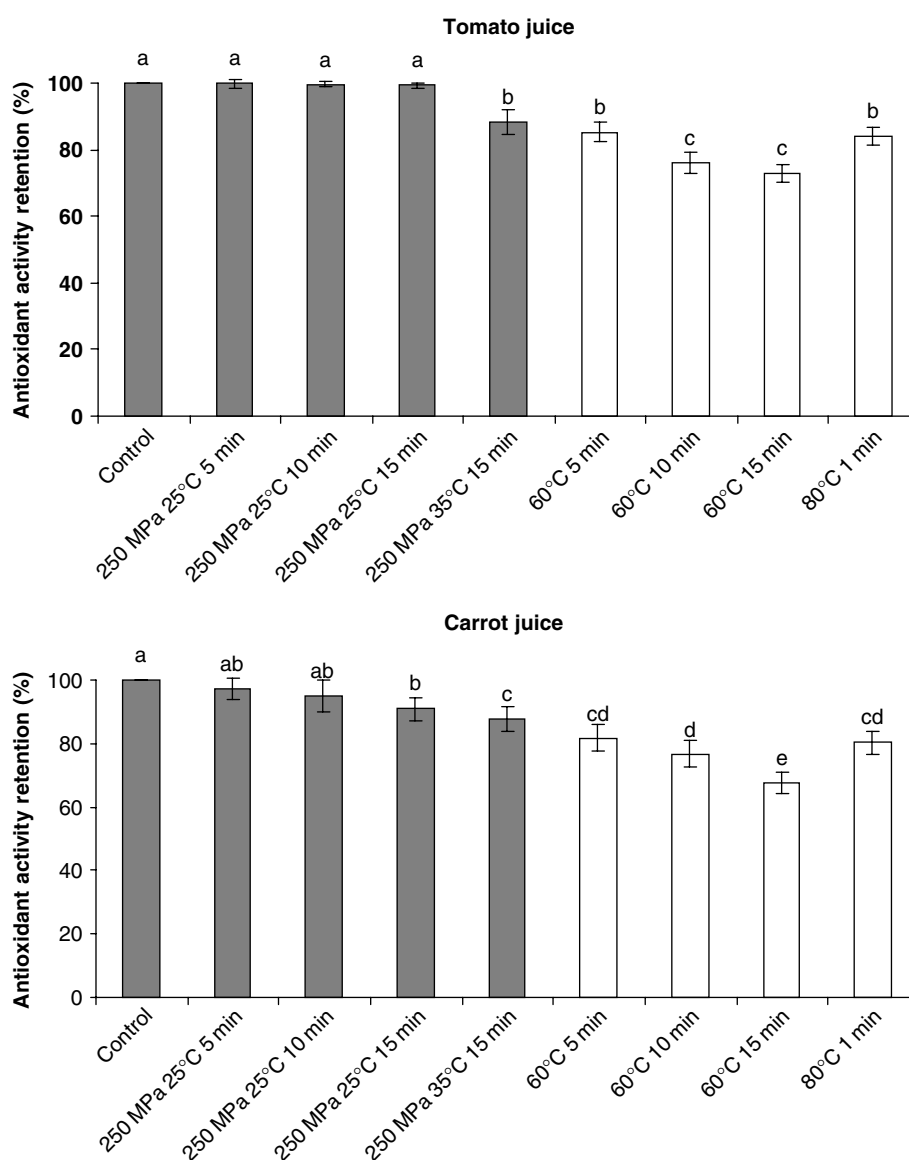


Figure 3. Antioxidant activity of juices treated by high pressure and heat. The error bars denote the standard deviation. Different letters imply significant changes ($P < 0.05$).

terms of microbiological stability (data not shown), ascorbic acid retention and antioxidant activity.

Among the compounds exhibiting antioxidant activity in juices, ascorbic acid is the most important and accounts for 65–90% of the total antioxidant activity of orange juice.^{40,41} The reaction between L-ascorbic acid and the DPPH radical occurs instantly. In contrast, the reaction between most flavonoids and the radical is time dependent, resulting in continuously decreasing absorbance with time.²⁴ Therefore, a better retention of the antioxidant activity of the juices due to ascorbic acid was observed for high pressurised juices in our study. In summary, the decrease of total antioxidant activity during storage of the juices studied can be mainly attributed to the loss of ascorbic acid. Furthermore, the higher overall antioxidant activity of high pressure treated juices compared to thermally treated ones during storage (4 and 25 °C) was the result of better retention of ascorbic acid, which was observed for high pressurised juices.

HHP-treated juices showed a small (20%) loss of antioxidants at both storage temperatures (4 and 25 °C) whereas the loss is higher in heat-treated tomato juice (70%) through storage (Fig. 5). De Ancos *et al.*⁴² reported that after 30 days of storage at 4 °C the untreated orange juice showed a significant decrease of 18% in free-radical-scavenging capacity and orange juices treated at 350 MPa, 30 °C showed approximately 20% inhibition at different time combinations. Compared to conventional pasteurisation, HHP treatment led to higher total antioxidant activity immediately after processing as well as during storage at both 4 and 25 °C. Similar results were also reported by Polydera *et al.*²⁴ in a kinetic study of orange juice, where the antioxidant activity was described mathematically as a function of storage time and temperature conditions. Sánchez-Moreno *et al.*²⁷ also reported that high pressure treatments (100 MPa, 60 °C, 5 min; 350 MPa, 30 °C, 2.5 min and 400 MPa, 40 °C, 1 min) did not affect the bioactive compounds responsible

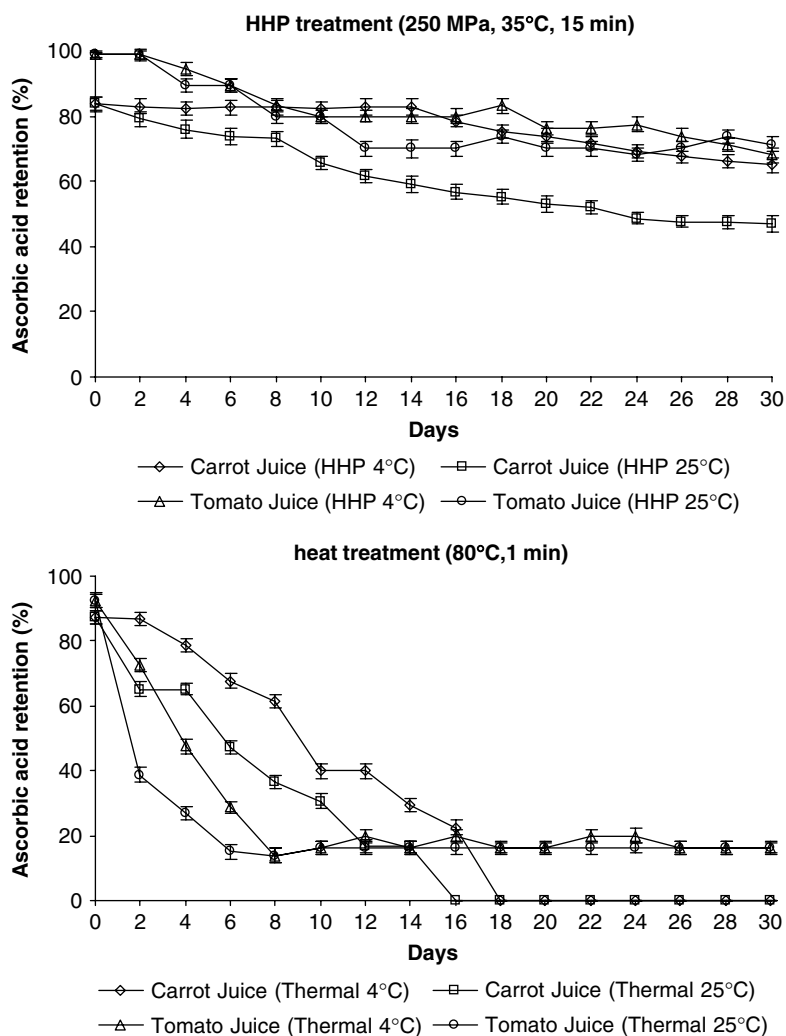


Figure 4. The retention of ascorbic acid during the storage of treated juices at 4 and 25°C (ascorbic acid retention of unprocessed juice: 100%). The error bars denote the standard deviation.

for the radical scavenging capacity of freshly squeezed orange juices, either immediately after treatment or during the chilled storage period. It is clear that radical-scavenging capacity is steady throughout the storage period for both juices at the selected storage temperatures.

The colour difference of juices is another way of correcting the change in antioxidant activity. As ascorbic acid and other antioxidant compounds oxidise, the colour is affected. The application of HHP had a smaller effect on colour change than did thermal treatment throughout the storage period (Fig. 6). This small change was predicted and can be attributed to the effect of HHP on the release of carotenoids from protein complexes or the disruption of chloroplasts, such as in raspberries.²⁸ For pressurised samples, the colour changes (ΔE) were about or lower than 15 for tomato and carrot juices, but for heat-treated samples, the colour changes were more intense and higher at the end of storage period of 30 days as compared with ΔE of untreated samples mainly due to insufficient antioxidant activity. Although the additional increase in ΔE value with storage time in tomato juice is higher in HHP-treated samples than heat-treated ones, the

final ΔE values of HHP-treated samples are still lower than heat-treated ones at the end of the storage period. This high level of change in colour by heat may also be explained by the positive effect on phenolic substances (anthocyanins and hydroxycinnamates).²³

Initial pH values of tomato (pH = 4.50) and carrot (pH = 6.00) juices were not affected by treatment, storage temperature or storage time. The pH values were also stable with no significant change (variation of ± 0.5 unit) during the storage period (data not shown).

The effects of variables (treatment, storage temperature and time) on the parameters studied (antioxidant activity, ascorbic acid, colour and pH) are reported in Table 1. Apart from pH, all parameters were significantly affected by treatment, storage time and temperature. For tomato juices, the interaction of storage time and temperature, and treatment, storage time and temperature did not affect antioxidant activity and pH. Ascorbic acid was not affected by the interaction of treatment and storage temperature. Analysis of carrot juice showed that interactions of storage time and temperature, and treatment, storage time and temperature did not affect antioxidant activity.

Table 1. Effects of the three variables^{a,b} – treatment, storage temperature and storage time – on the properties of tomato and carrot juices

Property	Treatment (e)	Storage temperature (f)	Storage time (g)	e × f	e × g	f × g	e × f × g
Tomato juice							
Antioxidant activity	✓	✓	✓	✓	✓		
Ascorbic acid	✓	✓	✓	✓	✓	✓	✓
Colour	✓	✓	✓	✓	✓	✓	✓
PH	✓		✓	✓	✓		
Carrot juice							
Antioxidant activity	✓	✓	✓	✓		✓	
Ascorbic acid	✓	✓	✓	✓	✓	✓	✓
Colour	✓	✓	✓	✓	✓	✓	✓
PH	✓		✓	✓	✓	✓	✓

^a Significance at $P < 0.05$.

^b ✓ means the parameter is affected by the variable.

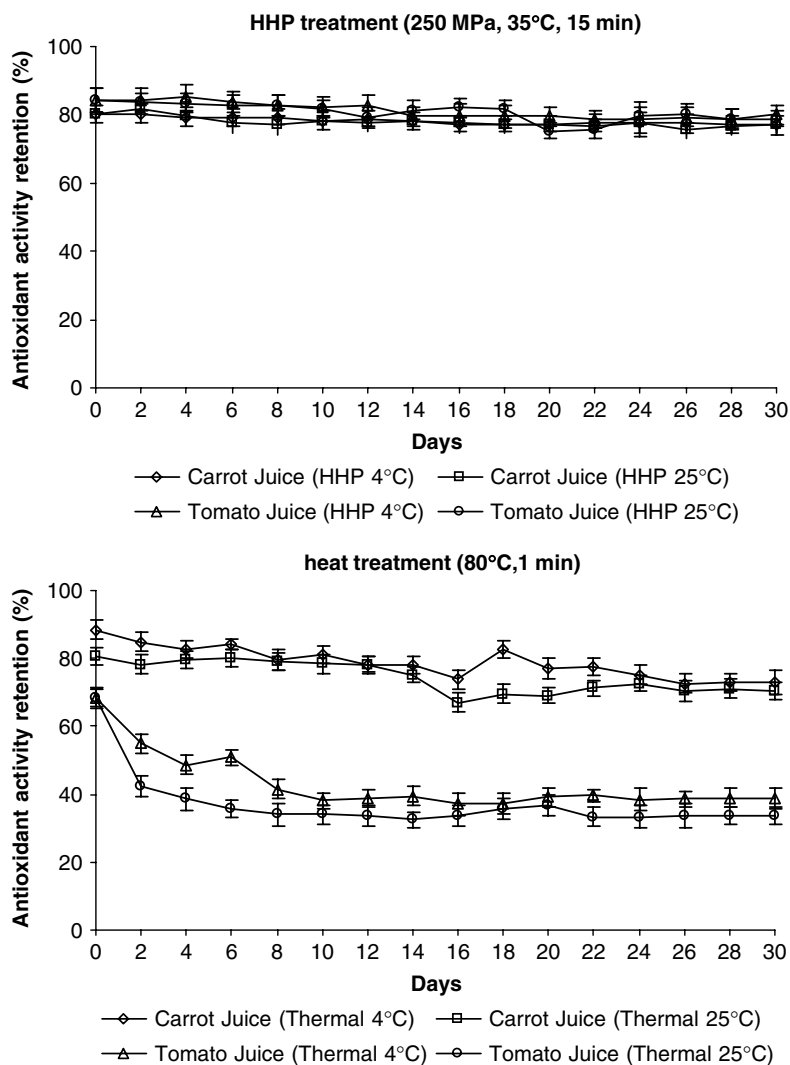


Figure 5. The retention of antioxidant activity during the storage of treated juices at 4 and 25 °C (antioxidant activity retention of unprocessed juice: 100%). The error bars denote the standard deviation.

CONCLUSION

Both HHP and heat treatment (within the experimental conditions studied) were able to produce microbiologically stable products although fresher products are achieved by HHP treatment at 250 MPa in terms of ascorbic acid content, anti-radical scavenging activity, pH and colour. Throughout the storage period,

HHP-treated juices were judged to be of superior quality than the conventional, thermally processed ones in terms of microbiological stability, ascorbic acid retention and antioxidant activity. HHP processing (250 MPa, 35 °C for 15 min) can be an alternative method to conventional thermal pasteurisation for preserving fruit and vegetable juices as freshly squeezed

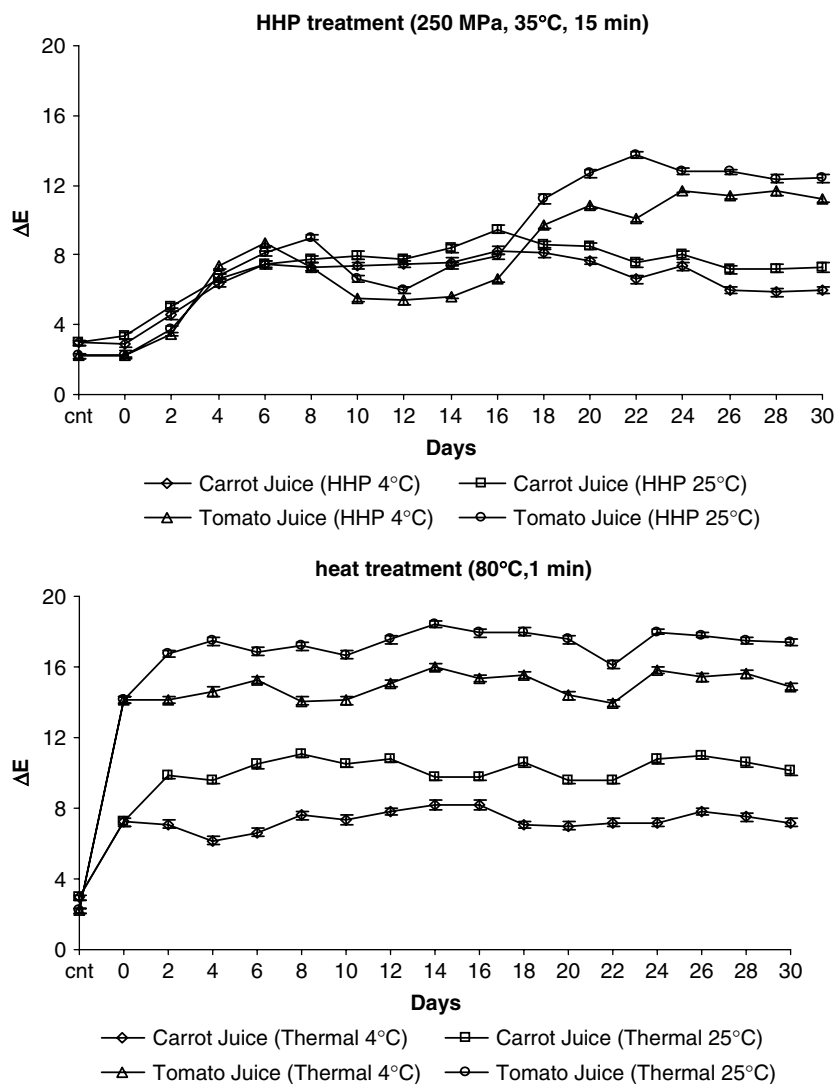


Figure 6. Colour change during the storage of treated juices at 4 and 25°C. cnt; ΔE of untreated juices. The error bars denote the standard deviation.

for up to 30 days. In order to select the most suitable processing conditions, not only microbiological stability but also sensory characteristics must be taken into account.

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