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Research article

Influence of high pressure processing on qualities of celery-based juices during refrigerated storage

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Abstract

Importance of the work: As a rich source of nutrients, celery has been widely chosen as a food or beverage item. However, a short shelf life is the main concern in celery juice production.

Objectives: To investigate the combination of natural juice acidification and high pressure processing (HPP) to enhance microbial and enzyme inactivation, including shelf life extension of celery-based juice.

Materials & Methods: Lime and apple juices were used to mix with celery juice as natural acidifiers to adjust the juice pH. Three juice formulations—celery, celery-lime and celery-apple—were prepared and treated at 400, 500 or 600 MPa for 5 min. Juice qualities were investigated during 8 wk of storage in refrigerated conditions.

Results: The addition of fruit juice led to differences in the properties of the celery-based juices. Celery-lime juice provided greater inactivation of microorganisms and enzymes than the other two formulations. The highest pressure maintained the levels of important physical properties, total phenolic compounds and antioxidant capacity of juices. Throughout storage, the aerobic count remained under 2 log colony forming units/mL with no detection of yeasts and molds. Residual activity of polyphenoloxidase and peroxidase was reduced to under 36% and 50% in the celery-lime juice and celery-juice, respectively. The antioxidant capacity was maintained at more than 50%, indicating that HPP at 600 MPa was the most suitable condition.

Main finding: HPP showed potential as an alternative preservation method for celery-based juices for up to 8 wk of storage. Furthermore, the combination of HPP and acidifier from low-pH fruits could provide an option for vegetable juice production with greater nutritional value and safety assurance.

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Introduction

The popularity of celery has been clearly shown in recent years with diversified applications in the food industry where each part can be processed in different types of food products for many purposes. For example, juice prepared from the celery stalk has widely been marketed as a nutritious drink rich in vitamins and minerals with considerable health benefits such as anti-inflammatory, anti-oxidant, anti-cancer, and anti-bacterial (Boonruamkaew et al., 2020). However, the short shelf life is one of the important concerns since low-acid vegetable juice are preferably consumed as a fresh product, which facilitates undesirable changes in color, nutrition, flavor and taste derived from browning reactions caused by endogenous enzymes and microbial contamination (Mostafidi et al., 2020). In addition, the combination of celery juice and other fruit juices can produce a mixture having a high sugar content, creating favorable conditions for microbial growth (Gram et al., 2002). High pressure processing (HPP) is a nonthermal food preservation method that has been reported to enhance microbiological safety and extend the shelf life of food products with minimal influence on the quality of foods. In particular, liquid foodstuffs with water activity exceeding 0.98 were suitable for HPP application to ensure the effectiveness on microbial inactivation (Daryaei and Balasubramaniam, 2012). However, every processing method has both advantages and limitations. HPP has to confront with several hindrances, such as the survival of barotolerant bacteria and the condition specificity for different products. Hence, combination with other hurdle methods has been commercially applied, in which acidification has potential. In fact, acidifying agents have been proven for years in the prevention of pathogen and browning enzyme activity. For example, under low pH conditions, a major population of microorganisms is more sensitive to HPP and it is even more challenging for injured cells to recover (Woldemariam and Emire, 2019). Thus, following the market trend of consuming "clean food products" without chemical additives and preservatives, means that low-pH fruit juice as a natural acidifying agent is one of the alternative solutions to not only inhibit microbial growth, but also enhance the nutritional value and health benefit of juice beverages without negatively affecting their consumption.

The objectives of this research were to explore the role of pH adjustment and preservation treatment on celery-based juice processing based on natural adjustment using different ingredients and to investigate the effect of HPP on the qualities of celery-based juices during refrigerated storage.

Materials and Methods

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ), gallic acid, catechol, guaiacol, hydrogen peroxide, polyvinylpyrrolidone (PVP) and polyoxyethylene octyl phenyl ether (Triton X-100) were purchased from Sigma Aldrich (USA). Plate count agar, potato dextrose agar, peptone and Folin-Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). Other chemicals were purchased from Kemaus (New South Wales, Australia). All chemicals and solvents were analytical grade.

Raw materials and sample preparation

Fresh samples of celery (*Apium graveolens*), Granny Smith apple (*Malus domestica*) and lime (*Citrus aurantiifolia*) were purchased at local supermarket (Bangkok, Thailand) and stored in a refrigerator for 1 d before juicing. All ingredients were washed in tap water. Lime juice was extracted using a domestic squeezer; the celery stalks and deseeded apples were each cut into four equal pieces before being pulped using a juicer (MJ-SJ01; Panasonic; Malaysia).

Three formulations of celery-based juice were prepared by mixing the following: celery-lime juice (90% celery juice and 10% lime juice), celery-apple juice (85% celery juice and 15% apple juice) and celery juice (100% celery juice). These proportions were selected after preliminary experiments to achieve celery-based juices with pH levels in the ranges of high-acid juice (pH < 3.5), acidic juice (pH < 4.5) and low-acid juice (pH ~5.0–6.0). After preparation of the three juices, their qualities of untreated fresh juice were analyzed; microbial qualities, pH, total soluble solids content, color parameters, residual enzyme activities, total phenolic content and antioxidant capacity. Juices were individually packed in linear low-density polyethylene pouches (Sripipat Engineering Co., Ltd.; Thailand) using a heat sealer (XP-300 Hanato; Hana Corporation Ltd.; South Korea) to carefully minimize headspace, after which the samples were stored at 4 ± 2°C for 10–12 hr before transporting to the pilot plant where the HPP machine was located.

High pressure processing

HPP treatment was performed using high-pressure equipment (HPP600MPa/5L; Bao Tou KeFA High Pressure Technology Co., Ltd.; China). Pouches containing juice were double bagged and

re-sealed to reduce any risk of leakage. All celery-based juices were treated at 400, 500 or 600 MPa with 5 min holding time. After that, the treated samples were cooled in a box containing ice packs before storage in refrigerated conditions (4 ± 2°C) for further analyses. The quality parameters of the HPP-treated celery-based juices were investigated 1 d after the HPP treatment. Experiments were conducted as a 3×3 factorial in completely randomized design with three juice formulations by the three HPP conditions. In this study, three juice matrices formulations with three different pH levels were treated using HPP with three pressure conditions. Then, the effect of juice pH and HPP treatment on juice qualities were examined.

Microbial analyses

Decimal dilutions of 10 g juice were prepared in sterile 0.1% (weight per weight, w/w) peptone solution. A pour plate technique was applied for total aerobic counts (TAC) using plate count agar (PCA) and for yeasts and molds (YM) using potato dextrose agar (PDA). The numbers of aerobic bacteria were determined after incubation at 35°C for 48 hr, while those of YM were determined at 25°C for 120 hr. The results were reported in units of log colony forming units (CFU) per milliliter of sample.

pH and total soluble solids content

The pH of each sample was measured using a pH meter (C1010PK; Consort bvba; Belgium). The total soluble solids (TSS) contents of juices were measured using a digital refractometer (PAL-α; Atago Co., Ltd.; Japan). All measurements were conducted at room temperature.

Color measurement

Samples were poured into Petri dishes and color parameters (L*, a*, b*) were determined using a spectrophotometer (CM3500D; Konica Minolta, Inc.; Japan). Fresh juice at day 1 after preparation was used as the untreated control. The total color difference (ΔE*) of samples was calculated using Equation 1:

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

where △ indicates the difference between the parameters (L*, a*, b*) of juice at sampling point compared to the untreated control.

Determination of residual enzyme activities

The residual enzyme activities of polyphenoloxidase (PPO) and peroxidase (POD) were determined following the method of Stinco et al. (2019) with slight modification. The extraction mixture was 0.2 M sodium phosphate buffer (pH 6.5) containing 4% (weight per volume) PVP, 1% (volume per volume) Triton X-100. The juice and mixture (1:1, w/w) were thoroughly mixed and stirred at room temperature on a hot plate (C-MAG HS7; IKA®-Werke GmbH & Co. KG; Germany) for 3 min and then centrifuged at 14,000×*g* at 4°C for 30 min (Sorvall D-37520 Osterode; Thermo Fisher Scientific GmbH; Germany). The supernatant was collected as crude enzyme extract. For the PPO activity, the mixture consisted of 1.25 mL of catechol (0.1 M), 0.2 mL crude enzyme extract, with the volume made up to 3 mL with phosphate buffer pH 7 (0.05 M), which was measured at 420 nm using an ultraviolet (UV)-visible spectrophotometer (UV-1900; Shimadzu Corporation; Japan) according to Kaushik et al. (2016). For POD, the substrate was a mixture of 3 mL 30% hydrogen peroxide and 1.9 mL liquid guaiacol, made up to 300 mL with 0.2 M phosphate buffer (pH 6.0). Each mixture sample (0.2 mL) of crude enzyme extract and 3 mL substrate solution was measured at 470 nm (Chen et al., 2010). The residual activity (RA) levels of PPO and POD as percentages were estimated using Equation 2:

$$RA = A / A_c \times 100 \quad (2)$$

where A and A_c are the absorbance of the treated sample and the untreated control sample, respectively.

Determination of total phenolic content

Analyses of the total phenolic content were carried out following the method of Derradji-Benmeziane et al. (2014) using Folin-Ciocalteu assay. Each juice sample (0.5 mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and then added with 2 mL of an aqueous solution of sodium carbonate (7.5%). Samples were mixed thoroughly and kept in the dark for 30 min at room temperature. Absorbance was determined at 765 nm using the UV-visible spectrophotometer and the phenolic compounds were quantified using a prepared gallic acid standard. The results were expressed in milligrams gallic acid equivalent (GAE) per 100 mL of juice.

Antioxidant capacity measurements

Ferric reducing antioxidant power assay

The assay was conducted according to the method of Wong et al. (2006). In brief, the ferric reducing antioxidant power (FRAP) reagent was prepared by mixing sodium acetate buffer

(300 mmol/L, pH 3.6), 10 mmol/L TPTZ solution in 40 mmol/L hydrochloric acid and 20 mmol/L iron (III) chloride solution in proportions of 10:1:1, respectively, by volume. The mixture was warmed in a water bath at 37°C prior to use. The experiment was conducted by adding 0.5 mL of sample to 4.5 mL of the FRAP reagent. The reaction mixture was shaken well and incubated for 30 min at room temperature. Then, the absorbance of the reaction mixture was recorded at 593 nm using the UV-visible spectrophotometer. The standard curve was constructed using iron (II) sulfate solution (100–2,000 mmol/L). The results were expressed as micromoles Fe(II) per milliliter of extract.

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity assay

The scavenging effects of celery-based juice against DPPH radicals were determined according to the modified method described by Shah and Modi (2015). Briefly, 1.5 mL of juice was mixed with 4.5 mL of DPPH solution (1.5 µM). The reaction mixture was shaken well and incubated for 30 min at room temperature. The absorbance of the resulting solution was read at 517 nm using the UV-visible spectrophotometer against a blank. The inhibitory percentage of DPPH was calculated according to Equation 3:

$$\text{Inhibition} = (A_0 - A_s) / A_0 \times 100 \quad (3)$$

where A_0 and A_s are the absorbance of the DPPH radical solution and the extract mixed with DPPH solution, respectively.

Monitoring quality changes of high pressure processing-treated celery-based juices during refrigerated storage

The quality of the HPP-treated celery-based juices was investigated to observe any changes in the juices during storage. The results were recorded at weeks 1, 2, 3, 4, 6 and 8 based on other studies that reported the shelf life of HPP pasteurized juices was approximately 2 mth (Bull et al., 2004; Varela-Santos et al., 2012; Wang et al., 2012).

Statistical analyses

All experiments were conducted with at least two replications. Mean values ± SD were reported. Analysis of variance and Tukey's test were carried out at a significance level of $p < 0.05$. Data analyses were performed using the IBM SPSS Statistics version 28 software (Thaisoftup Co. Ltd.; Thailand).

Results and Discussion

Influence of juice formulations and high pressure processing conditions on juice qualities

Table 1 shows the effect of juice formulation on the quality of the untreated fresh juices while Table 2 presents a summary of the analysis regarding the contribution of juice formulation, HPP treatment and their interaction. Apart for the differences in pH values based on the acidification used initially, significant effects were also found on other qualities ($p < 0.05$). As a high-acid food which can be considered as an important barrier for microbial growth, the initial TAC of the celery-lime juice was more than 3 log CFU/mL (Fig. 1A), while those of celery-apple juice (Fig. 1B) and celery juice (Fig. 1C) were more than 5 log CFU/mL. During storage, the TAC of the celery-apple juice continued to increase since the apple juice was a rich source of sugar that acted as simple nutrient for bacterial growth. The YM numbers of the three fresh juices were greater than 3 log CFU/mL, with it being the greatest in the celery-lime juice. This could have been due to most bacteria growing poorly

Table 1 Quality characteristics of three fresh celery-based juices (untreated control samples)

Characteristic	Celery-lime juice	Celery-apple juice	Celery juice
TAC (log CFU/mL)	3.39±0.09^b	5.74±0.01^a	5.58±0.03^a
YM (log CFU/mL)	3.37±0.07^a	2.95±0.23^c	3.13±0.03^b
pH	3.30±0.08^c	4.50±0.08^b	6.02±0.06^a
TSS (° Brix)	3.87±0.06^b	5.40±0.10^a	3.43±0.06^c
L*	42.28±1.03^a	39.92±0.46^b	33.84±0.03^c
a*	-4.41±0.06^c	-5.73±0.13^b	-7.07±0.04^a
b*	24.96±1.81^a	26.86±0.98^a	25.05±0.35^a
TPC (mg GAE/100 mL)	37.73±0.30^b	46.60±0.50^a	37.01±0.23^b
FRAP (µmol/mL)	73.76±2.10^b	183.35±2.14^a	55.57±2.65^c
DPPH (%)	71.86±0.34^b	86.73±0.18^a	64.87±0.52^c

TAC = total aerobic count; CFU = colony forming units; YM = yeasts and molds; TSS = total soluble solids; L*, a* and b* = color parameters; TPC = total phenolic content; GAE = gallic acid equivalent; FRAP = antioxidant capacity using ferric reducing antioxidant power assay; DPPH = antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity assay.
Mean±SD in same row superscripted with different lowercase letters are significantly different ($p < 0.05$).

or not at all below pH 4, while YM can grow well in low pH conditions, even at a refrigerated temperature, leading to their predominance in acidic foods (Fleet, 2011). Penniston et al. (2008) reported that juices containing apple have a high TSS content, while lime is a rich source of citric acid. Consequently, the celery-apple and celery-lime juices had higher TSS values (5.40 ± 0.10ºBrix and 3.87 ± 0.06ºBrix, respectively) than the formulation containing only celery juice (3.43 ± 0.06ºBrix). Furthermore, the addition of apple juice to the celery juice resulted in a higher TPC in the mixed juice (46.60 mg GAE/100 mL) than in the celery-lime juice (37.73 mg GAE/100 mL) and the celery juice (37.01 mg GAE/100 mL), as shown in Table 1.

Monitoring quality changes of high pressure processing-treated celery-based juices during refrigerated storage

Microbial stability

The initial TAC levels differed among the three juice formulations (Table 2). The celery-lime juice had the lowest level as it has high acidity that acted as an important barrier to microbial growth. However, the untreated celery-based juice showed signs of spoilage after 1 wk of refrigerated storage, as the juice pouches were clearly distended by gas production by the microorganisms along with a fermented odor. HPP assisted in significantly reducing bacterial counts to less than 2 log CFU/mL in all three juice formulations, as well inhibiting the microbial population during storage for 8 wk (Fig. 1). YM are reported to be sensitive to HPP; they can be inactivated with pressures in the range 200–400 MPa (Daryaei and Balasubramaniam, 2012). This would explain its effective contribution as there were no YM detected in any of the HPP-treated juices throughout storage. The differences in pH affected the initial microbial counts and the trend of microbial growth during storage for the three celery-based juices. On the other hand, HPP resulted in microbial inactivation, hence assuring the safety of celery-based juices compared to untreated control for human consumption.

pH and total soluble solids content

The pH of the celery-based juices after HPP remained unchanged throughout storage. In all three formulations, there were no significant differences in pH values among the

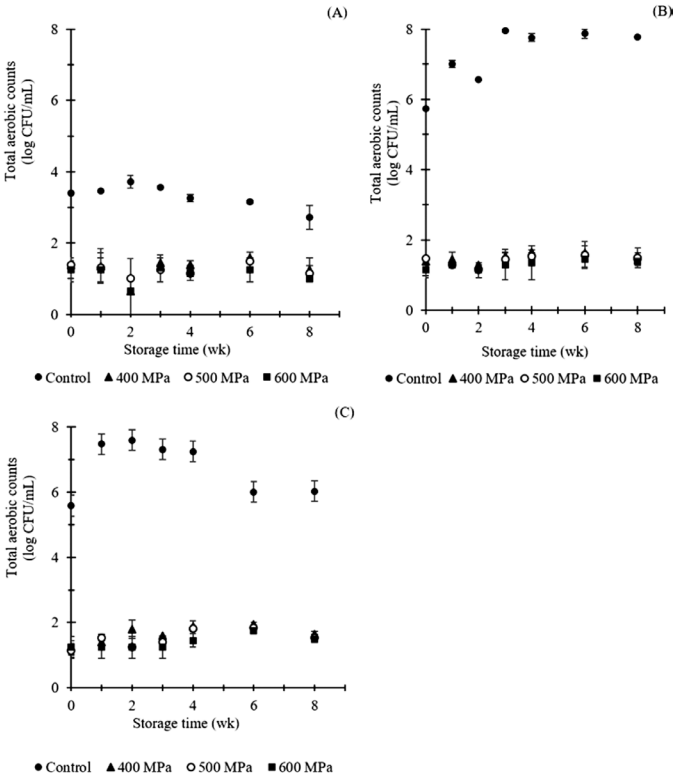

Fig. 1 Total aerobic counts of untreated celery-based juice and juices treated at different pressure levels using high pressure processing followed by 8 wk storage: (A) celery-lime juice; (B) celery-apple juice (C) celery juice, where error bars = ± SD

Table 2 Significance (shown as probability values) of the main effects (juice formulation and high pressure processing [HPP] treatment) and their interaction on quality characteristics of celery-based juices

Characteristic	Juice formulation	HPP treatment	Juice formulation × HPP treatment
TAC	0.000	0.000	0.000
YM	0.000	0.000	0.000
pH	0.000	0.780	0.680
TSS	0.000	0.000	0.000
△E*	0.000	0.000	0.000
PPO	0.000	0.000	0.000
POD	0.000	0.000	0.000
TPC	0.000	0.043	0.874
FRAP	0.000	0.001	0.000
DPPH	0.000	0.000	0.000

TAC = total aerobic count; YM = yeasts and molds; TSS = total soluble solids; △E* = total color difference; PPO = polyphenoloxidase residual activity; POD = peroxidase residual activity; TPC = total phenolic content; FRAP = antioxidant capacity using ferric reducing antioxidant power assay; DPPH = antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity assay.
The tests were considered significant at $p < 0.05$.

three pressure levels ($p > 0.05$). In addition, HPP slightly reduced the TSS levels of the celery-based juices; however; the values varied slightly during storage (3.57–3.80ºBrix for celery-lime juice; 5.20–5.40ºBrix for celery-apple juice; and 3.30–3.43ºBrix for celery juice). Generally, HPP did not significantly affect the levels of pH or TSS in the celery-based juices throughout storage for 8 wk. Similar results were reported in other studies with carrot juice (Zhang et al., 2016) and apple juice (Landl et al., 2010).

Color parameters

The L* values of the juices were slightly reduced after HPP (data not shown), which was consistent with Aaby et al. (2018) and Yu et al. (2013). In the celery-apple and celery juices, there was a higher L* value at a higher pressure. During storage, the change in juice color was mainly related to browning enzyme activity. Based on observation, the color of the celery juice was highly stable, always retaining the signature greenness of fresh celery. However, in the celery-apple juice, the appearance of browning reaction contributed to fading the green color, with a corresponding positive a* value. Along with the presence of the residual activity of PPO and POD due to ineffective enzyme inactivation, the juice color slowly turned yellow and then brown at the end of storage. Unlike the other two formulations, the pressurized celery lime juice had an approximately twice as great decrease in greenness during storage (data not shown), although HPP greatly inhibited browning enzymes. The darker yellow shade of the juice might have been due to the greater influence of pH at a high acidity level. Andrés-Bello et al. (2013) reported that pH had an important effect on pigment, with a green color being retained with higher pH conditions, since chlorophyll degradation to pheophytin and pheophorbide resulted in a change from bright green to olive green or olive yellow. The total color difference ($\triangle E^*$) is a parameter for the quantification of the overall color difference in a given sample (HPP-treated juice) compared to a reference sample (untreated juice at wk 0). Theoretically, the just-noticeable difference (JND) has an estimated ΔE^* value of 2.3 as the threshold of discernability by the human eye (Sharma and Bala, 2017). Fig. 2 shows that after treatment, celery-apple juice and celery juice treated at the highest pressure (600 MPa) had the lowest $\triangle E^*$ compared to the other two pressure conditions for the same holding time, while the values of the treated celery-lime juice were not significantly different among the three pressure levels ($p > 0.05$). Specifically, the $\triangle E^*$ value of the celery-lime juice (3.03 ± 0.45) indicated a medium difference noticed by an untrained eye while that of celery juice (1.50 ± 0.17) was less than the JND threshold. Throughout storage, HPP at 600 MPa had greater ability to retain color except for the celery-apple juice with the highest $\triangle E^*$. The $\triangle E^*$ values of the celery-lime juice and celery juice remained at the same level ($\triangle E^* < 3$), whereas the celery-apple juice was visually changed as $\triangle E^* > 5$ (obvious difference) from the beginning of storage (Mokrzycki and Tatol, 2011). This may have been related to the residual activity of browning enzymes that caused undesirable change in the juice color. Nevertheless, compared to the conventional method, HPP still produced less damage than heat treatment. Jesus et al. (2018) indicated a change from violet to a brown color of açai pulp treated at 85°C, in contrast to HPP-treated samples that showed no difference from the control. In addition, Patras et al. (2009) concluded that HPP preserved the color intensity of tomato and carrot purees better than heat treatment.

Browning enzyme activity

Browning incidence caused by browning enzymes, such as PPO and POD, is a challenging problem, since an oxidation reaction can negatively affect product quality, especially in

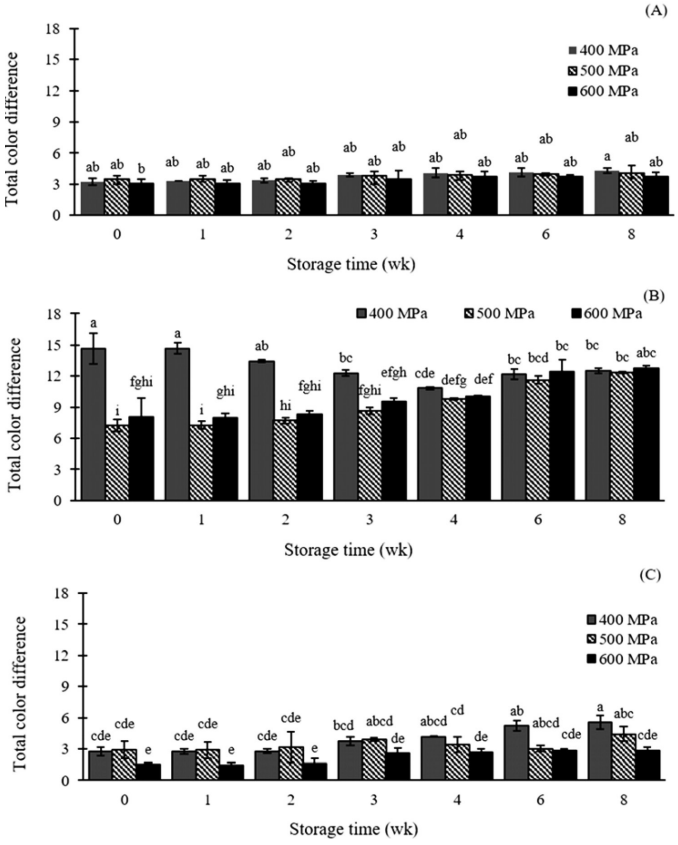

Fig. 2 Total color difference ($\triangle E^*$) in juices treated using high pressure processing followed by 8 wk storage: (A) celery-lime juice; (B) celery-apple juice; (C) celery juice, where the bars and error bars represent mean values and ± SD, respectively; different lowercase letters above bars denote significantly different among treatments ($p < 0.05$)

fresh-cut products, such as fruits and vegetables (Chimvaree et al., 2019). Various methods have been applied to inhibit the negative results of oxidation, depending on product characteristics. In the current study, as shown in Table 2, acidification and the application of HPP treatment significantly affected both enzymes (PPO and POD). The optimal pH for the action of these enzymes was 4.5–5.5, which explained the less effective inhibition in the celery-apple juice (pH 4.5–4.6), despite being treated at the increased pressure levels. Pressure application assisted in the significant reduction of both browning enzyme activity after 1 d of HPP treatment (wk 0), compared to the untreated juice. There was approximately 40–50% of PPO activity in the HPP-treated celery lime juice, while the celery juice was inactivated at wk 0 and then continued decreasing in the following weeks (data not shown). This agreed with another study, where POD was reported to be more pressure-resistant than PPO (Stinco et al., 2019). In the lowest pH sample (celery-lime juice; Fig. 3A), after treatment, the RA of POD was lower than 60% for almost all of the storage period of 8 wk. In particular, 600 MPa pressure for 5 min resulted in a constant inactivation that was lower than for the other two pressure levels, with RA values of 30.37% and 31.15% at weeks 0 and 8, respectively. In the celery-apple juice (Fig. 3B), at 400 and 600 MPa, HPP only assisted in the inhibition to 30–40% of POD activity during weeks 0 and 1, while that at 500 MPa was about 60%. From week 3, the RA of the juices treated at 400 MPa gradually reduced to approximately 50%, while those juices treated at the other two pressures still maintained a level around 80% for the rest of storage time. The RA of POD in celery juice decreased slightly to 70–80% during the first 3 wk and reached around 50–60% for the rest of storage, with no significant differences due to the HPP conditions (Fig. 3C).

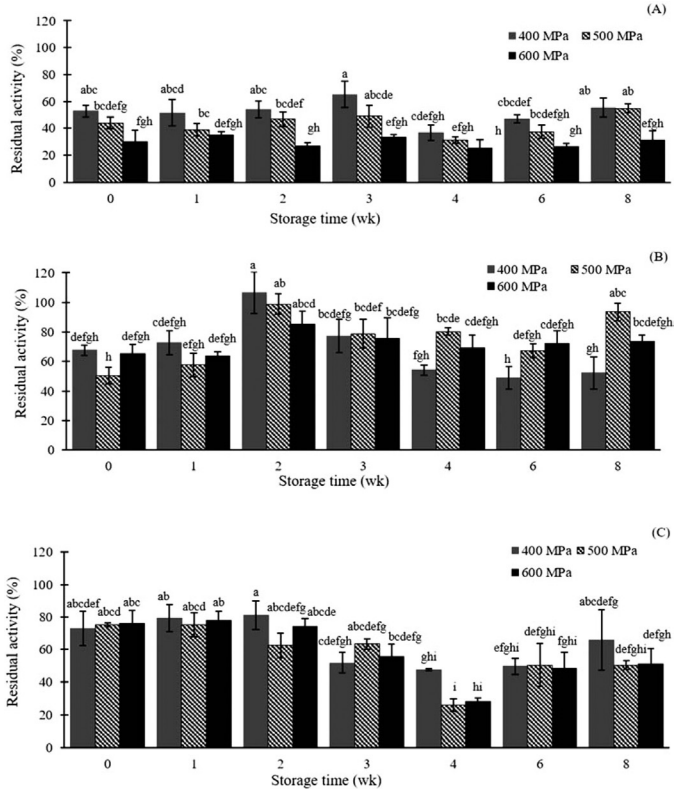

Fig. 3 Residual activities of peroxidase in juices treated using high pressure processing followed by 8 wk storage: (A) celery-lime juice; (B) celery-apple juice; (C) celery juice where the bars and error bars represent mean values and ± SD, respectively; different lowercase letters above bars denote significantly different among treatments ($p < 0.05$)

In summary, an increase or decrease in the RA of enzymes can be affected by several factors, such as pH, temperature and HPP (Seyderhelm et al., 1996). In the current study, both HPP and pH played important roles in the inhibition of browning enzymes. The highest-pressure level resulted in constant inactivation of enzyme activity from the beginning of storage in the celery-lime juice at low pH (pH < 3.5), resulting in greater inhibition. The browning enzymes in the celery-apple juice seemed to be unaffected by the pressure conditions used in this experiment. The lowest RA of enzymes in the celery-lime juice was partly related to the low pH, since lime is a rich source of citric acid. Organic acids such as citric acid and ascorbic acid are considered as acidifying agents. With the presence of citric acid, cooper-chelating action occurs that prevents the oxidation and browning processes. In addition, the application of HPP has been reported to have negative or positive effects on browning enzymes. Under higher pressure condition (> 400 MPa), the native enzyme structure is disrupted due to the breaking of hydrogen bonds, leading to reduced enzyme activity. However, enzyme activation might also occur since HPP induces membrane permeability, which can increase enzyme extractability. In addition, HPP at a lower pressure (< 400 MPa) might cause reversible protein denaturation, which is responsible for the recovery of enzyme activity (Roobab et al., 2021; Yoruk and Marshall, 2003).

Total phenolic content after processing and storage

After HPP treatment, the stability of the TPC levels in the celery-lime juice and celery juice were maintained throughout the 8 wk storage period at 37–38 mg GAE/100 mL, while that of the celery-apple juice had the highest initial value among the three formulations (46–47 mg GAE/100 mL) but this gradually reduced to approximately 38 mg GAE/100 mL in the final wk of storage (Fig. 4). This difference might have been strongly related to the residual activity of browning enzymes when the levels of RA in both PPO and POD in the celery-apple juice were still high, even after HPP, leading to the degradation of polyphenols to quinones during storage, which also resulted

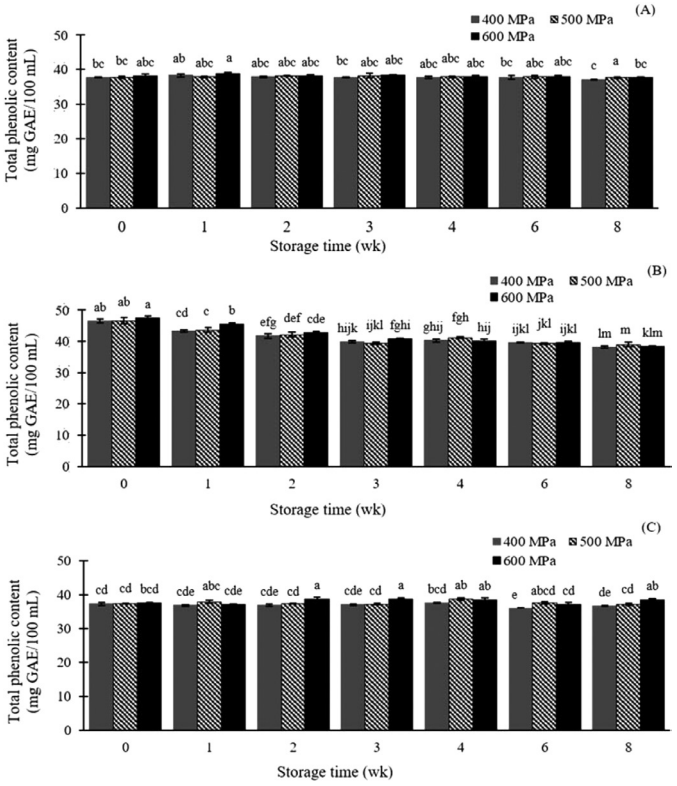

Fig. 4 Total phenolic contents in juices treated using high pressure processing followed by 8 wk storage: (A) celery-lime juice; (B) celery-apple juice; (C) celery juice, where the bars and error bars represent mean values and ± SD, respectively; different lowercase letters above bars denote significantly different among treatments ($p < 0.05$)

in the creation of browning pigments (Marszałek et al., 2018). Pressurizing at 400, 500 and 600 MPa produced similar results for the celery-lime and celery-apple juices ($p > 0.05$), while the celery juice treated at the greatest pressure (600 MPa) had a significantly greater value of TPC (38.45 mg GAE/100 mL at wk 8) than at the other two pressure levels from the beginning to the end of storage. Nevertheless, has not been possible to reach a definitive conclusion regarding the influence of HPP on the TPC in fruit and vegetable juices due to the impacts of other factors, such as treatment conditions (pressure level and time), raw ingredients and processing ambience, with various results reporting that HPP may heighten (Barba et al., 2013), maintain (Patras et al., 2009) or reduce phenolic compounds (Barba et al., 2012).

Antioxidant capacity after processing and storage

FRAP is a method based on the ability of juice to reduce the ferric (Fe^{3+}) ion to the ferrous (Fe^{+2}) ion, while DPPH assay is based on the reduction of 2,2-diphenyl-1-picryhydrazyl (violet color) caused by antioxidants. In line with the above qualities, the dissimilar pH levels in the raw ingredients in each formulation produced significant difference in the antioxidant capacity of the three celery-based juices. As supported by available published studies of juices, the effect of HPP on the antioxidant capacity of beverages after treatment is uncertain. In particular, the results of the current study were consistent with the reports by Hurtado et al. (2015) for a fruit smoothie and by Indrawati et al. (2004) for orange and carrot juices. In contrast, de Ancos et al. (2000) reported an increase in antioxidant capacity immediately after HPP treatment, while Wang et al. (2012) reported no significant difference. During storage, the three juice formulations had similar trends in their FRAP values which continued to decline. However, their levels of decrease differed until the final week, with the reductions in antioxidant capacity of approximately 14%, 46% and 60% for celery-lime juice, celery juice and celery-apple juice, respectively, which could have related to the oxidase activity of the browning enzymes mentioned above (Fig. 5). In addition, the DPPH inhibition percentage of the juices reduced throughout storage, with the greatest loss for the celery-apple juice (from 80% to 50%), as shown in Fig. 6. Beverages in general and juices in particular have a tendency to reduce in quality parameters at different levels despite

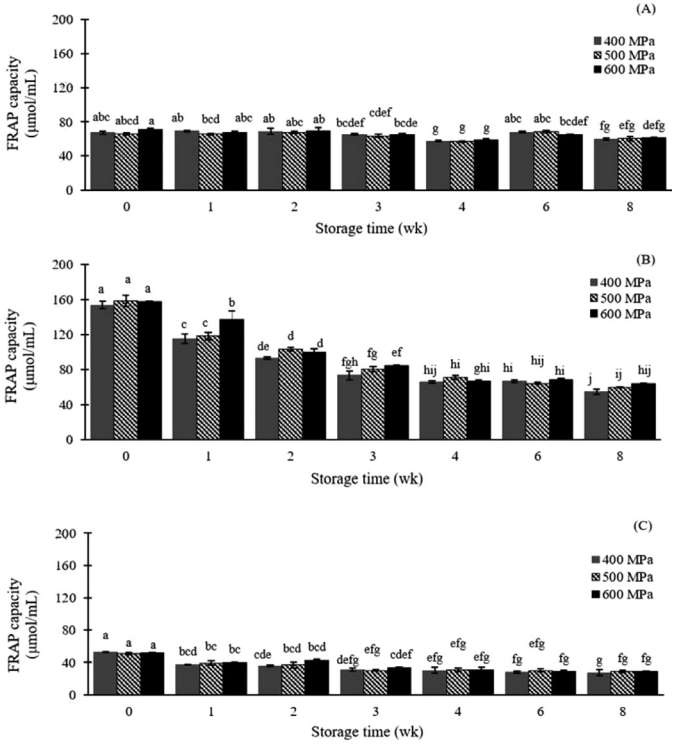

Fig. 5 Antioxidant capacity based on ferric reducing antioxidant power (FRAP) assay in juices treated using high pressure processing followed by 8 wk storage: (A) celery-lime juice; (B) celery-apple juice; (C) celery juice, where the bars and error bars represent mean values and ± SD, respectively; different lowercase letters above bars denote significantly different among treatments ($p < 0.05$)

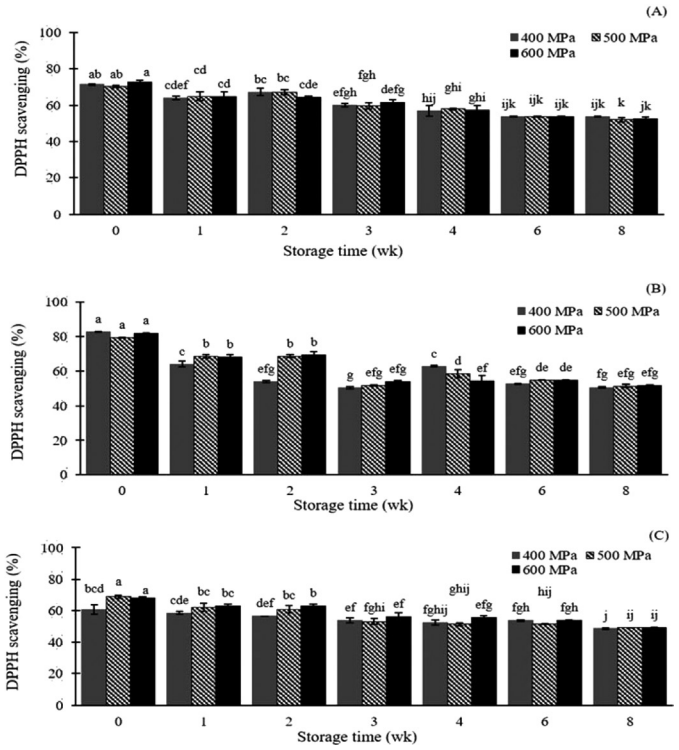

Fig. 6 Antioxidant capacity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay in juices treated using high-pressure processing followed by 8 wk storage: (A) celery-lime juice; (B) celery-apple juice; (C) celery juice, where the bars and error bars represent mean values and ± SD, respectively; different lowercase letters above bars denote significantly different among treatments ($p < 0.05$)

the same conditions and type of processing or preservation being applied. For example, Puttongsiri and Haruenkit (2010) noted the reduction of ascorbic acid in tangerine during 5 wk at 4, 12 and 20°C. A storage study for 30 d by Touati et al. (2016) reported a considerable degradation in antioxidant compounds in oranges and grapes and in pear nectar after 3 d storage at 4°C and after 1 d at 37°C.

In conclusion, natural acidification from fruit juice produced different results on storage quality for the three juice formulations. HPP showed considerable potential to preserve celery-based juices, offering a shelf life of up to 8 wk, while untreated juices were spoiled after 1 wk. The pH, and the total soluble solids and total phenolic contents of the three tested juices remained almost unchanged compared to their values at wk 0. Of the tested HPP conditions, pressure at 600 MPa for 5 min resulted in significant inactivation of microorganisms and the inhibition of browning enzyme activity (PPO and POD), while the physical properties, total phenolic content and antioxidant capacity were maintained after treatment. According to the excellent quality level maintained during the complete storage period of 8 wk, a commercially available pressure level of 600 MPa could be recommended for the treatment of the three formulations of celery-based juices. However, longer holding times may be necessary for more prominent inactivation of enzyme activity.

Conflict of Interests

The authors declare that there are no conflicts of interest.

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References

Aaby, K., Grimsbo, I.H., Hovda, M.B., Rode, T.M. 2018. Effect of high pressure and thermal processing on shelf life and quality of strawberry purée and juice. Food Chem. 260: 115–123. doi.org/10.1016/j.foodchem.2018.03.100

Andrés-Bello, A., Barreto-Palacios, V., García-Segovia, P., Mir-Bel, J., Martínez-Monzó, J. 2013. Effect of pH on color and texture of food products. Food Eng. Rev. 5: 158–170. doi.org/10.1007/s12393-013-9067-2

Barba, F.J., Cortés, C., Esteve, M.J., Frígola, A. 2012. Study of antioxidant capacity and quality parameters in an orange juice–milk beverage after high-pressure processing treatment. Food Bioprocess Technol. 5: 2222–2232. doi.org/10.1007/s11947-011-0570-2

Barba, F.J., Esteve, M.J., Frigola, A. 2013. Physicochemical and nutritional characteristics of blueberry juice after high pressure processing. Food Res. Int. 50: 545–549. doi.org/10.1016/j.foodres.2011.02.038

Boonruamkaew, P., Sukketsiri, W., Chonpathompikunlert, P. 2020. Celery. In: Jaiswal, A.K. (Ed.). Nutritional Composition and Antioxidant Properties of Fruits and Vegetables. Academic Press. San Diego, CA, USA, pp. 107–120.

Bull, M.K., Zerdin, K., Howe, E., et al. 2004. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. Innov. Food Sci. Emerg. Technol. 5: 135–149. doi.org/10.1016/j.ifset.2003.11.005

Chen, J.L., Zhang, J., Song, L., Jiang, Y., Wu, J., Hu, X.S. 2010. Changes in microorganism, enzyme, aroma of hami melon (*Cucumis melo* L.) juice treated with dense phase carbon dioxide and stored at 4°C. Innov. Food Sci. Emerg. Technol. 11: 623–629. doi.org/10.1016/j.ifset.2010.05.008

Chimvaree, C., Wongs-Aree, C., Supapvanich, S., Charoenrat, T., Tepsorn, R., Boonyaritthongchai, P. 2019. Effect of sericin coating on reducing browning of fresh-cut mango cv. 'Nam Dok Mai No. 4'. Agr. Nat. Resour. 53: 521–526. doi.org/10.34044/j.anres.2019.53.5.11

Daryaei, H., Balasubramaniam, V.M. 2012. Microbial decontamination of food by high pressure processing. In: Demirci, A., Ngadi, M.O. (Eds.). Microbial Decontamination in the Food Industry: Novel Methods and Applications. Woodhead Publishing Limited. Philadelphia, PA, USA, pp. 370–406.

de Ancos, B., Gonzalez, E., Cano, M.P. 2000. Effect of high-pressure treatment on the carotenoid composition and the radical scavenging activity of persimmon fruit purees. J. Agric. Food Chem. 48: 3542–3548. doi.org/10.1021/jf990911w

Derradji-Benmeziane, F., Djamai, R., Cadot, Y. 2014. Antioxidant capacity, total phenolic, carotenoid, and vitamin C contents of five table grape varieties from Algeria and their correlations. J. Int. Sci. Vigne Vin. 48: 153–162. doi.org/10.20870/oeno-one.2014.48.2.1564

Fleet, G. H. 2011. Yeast spoilage of foods and beverages. In: Kurtzman, C.P., Fell, J.W. Boekhout, T. (Eds.). The Yeasts. Elsevier. San Diego, CA, USA, pp. 53–63.

Gram, L., Ravn, L., Rasch, M., Brhun, J.B., Christensen, A.B., Givskov, M. 2002. Food spoilage—Interactions between food spoilage bacteria. Int. J. Food Microbiol. 78: 79–97. doi.org/10.1016/S0168-1605(02)00233-7

Hurtado, A., Picouet, P., Jofré, A., Guàrdia, M.D., Ros, J.M., Bañón, S. 2015. Application of high pressure processing for obtaining "fresh-like" fruit smoothies. Food Bioprocess Technol. 8: 2470–2482. doi.org/10.1007/s11947-015-1598-5

Indrawati, V.L.A., Hendrickx, M. 2004. Pressure and temperature stability of water-soluble antioxidants in orange and carrot juice: A kinetic study. Eur. Food Res. Technol. 219: 161–166. doi.org/10.1007/s00217-004-0929-y

Jesus, A.L.T., Leite, T.S., Cristianini, M. 2018. High isostatic pressure and thermal processing of açaí fruit (*Euterpe oleracea* Martius): Effect on pulp color and inactivation of peroxidase and polyphenol oxidase. Food Res. Int. 105: 853–862. doi.org/10.1016/j.foodres.2017.12.013

Kaushik, N., Kaur, B.P., Rao, P.S. 2016. Inactivation of polyphenol oxidase and peroxidase enzymes during pulsed, static and cyclic pressurization of litchi (*Litchi chinensis*) juice. Food Bioprod. Process. 100: 412–423. doi.org/10.1016/j.fbp.2016.07.015

Landl, A., Abadias, M., Sárraga, C., Viñas, I., Picouet, P.A. 2010. Effect of high pressure processing on the quality of acidified Granny Smith apple purée product. Innov. Food Sci. Emerg. Technol. 11: 557–564. doi.org/10.1016/j.ifset.2010.09.001

Marszałek, K., Woźniak, Ł., Barba, F.J., Skąpska, S., Lorenzo, J.M., Zambon, A., Spilimbergo, S. 2018. Enzymatic, physicochemical, nutritional and phytochemical profile changes of apple (*Golden Delicious* L.) juice under supercritical carbon dioxide and long-term cold storage. Food Chem. 268: 279–286. doi.org/10.1016/j.foodchem.2018.06.109

Mokrzycki, W.S., Tatol, M. 2011. Colour difference ΔE - A survey. Mach. Graph. Vis. 20: 383–411.

Mostafidi, M., Sanjabi, M.R., Shirkhan, F., Zahedi, M.T. 2020. A review of recent trends in the development of the microbial safety of fruits and vegetables. Trends Food Sci. Technol. 103: 321–332. doi.org/10.1016/j.tifs.2020.07.009

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 purées. Innov. Food Sci. Emerg. Technol. 10: 16–22. doi.org/10.1016/j.ifset.2008.09.008

Penniston, K.L., Nakada, S.Y., Holmes, R.P., Assimos, D.G. 2008. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. J. Endourol. 22: 567–570. doi.org/10.1089/end.2007.0304

Puttongsiri, T., Haruenkit, R. 2010. Changes in ascorbic acid, total polyphenol, phenolic acids and antioxidant activity in juice extracted from coated kiew wan tangerine during storage at 4, 12 and 20°C. Kasetsart J. (Nat. Sci.). 44: 280–289.

Roobab, U., Abida, A., Afzal, R., Madni, G.M., Zeng, X.A., Rahaman, A., Aadil, R.M. 2021. Impact of high-pressure treatments on enzyme activity of fruit-based beverages: An overview. Int. J. Food Sci. Technol. 57: 801–815. doi.org/10.1111/ijfs.15492

Seyderhelm, I., Boguslawski, S., Michaelis, G., Knorr, D. 1996. Pressure induced inactivation of selected food enzymes. J. Food Sci. 61: 308–310. doi.org/10.1111/j.1365-2621.1996.tb14182.x

Shah, P., Modi, H.A. 2015. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. Int. J. Res. Appl. Sci. Eng. Technol. 3: 636–641.

Sharma, G., Bala, R. 2017. Digital Color Imaging Handbook. CRC Press. Boca Raton, FL, USA.

Stinco, C.M., Szczepańska, J., Marszałek, K., et al. 2019. Effect of high-pressure processing on carotenoids profile, colour, microbial and enzymatic stability of cloudy carrot juice. Food Chem. 299: 125112. doi.org/10.1016/j.foodchem.2019.125112

Touati, N., Barba, F.J., Louaileche, H., Frigola, A., Esteve, M.J. 2016. Effect of storage time and temperature on the quality of fruit nectars: Determination of nutritional loss indicators. J. Food Qual. 39: 209–217. doi.org/10.1111/jfq.12189

Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J.E., Pérez-Won, M., Briones-Labarca, V., Morales-Castro, J. 2012. Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. Innov. Food Sci. Emerg. Technol. 13: 13–22. doi.org/10.1016/j.ifset.2011.10.009

Wang, Y., Liu, F., Cao, X., Chen, F., Hu, X., Liao, X. 2012. Comparison of high hydrostatic pressure and high temperature short time processing on quality of purple sweet potato nectar. Innov. Food Sci. Emerg. Technol. 16: 326–334. doi.org/10.1016/j.ifset.2012.07.006

Woldemariam, H.W., Emire, S.A. 2019. High pressure processing of foods for microbial and mycotoxins control: Current trends and future prospects. Cogent Food Agric. 5: 1622184. doi.org/10.1080/23311932.2019.1622184

Wong, C.C., Li, H.B., Cheng, K.W., Chen, F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. 97: 705–711. doi.org/10.1016/j.foodchem.2005.05.049

Yoruk, R., Marshall, M.R. 2003. Physicochemical properties and function of plant polyphenol oxidase: A review. J. Food Biochem. 27: 361–422. doi.org/10.1111/j.1745-4514.2003.tb00289.x

Yu, Y., Lin, Y., Zhan, Y., He, J., Zhu, S. 2013. Effect of high pressure processing on the stability of anthocyanin, ascorbic acid and color of Chinese bayberry juice during storage. J. Food Eng. 119: 701–706. doi.org/10.1016/j.jfoodeng.2013.06.036

Zhang, Y., Liu, X., Wang, Y., Zhao, F., Sun, Z., Liao, X. 2016. Quality comparison of carrot juices processed by high-pressure processing and high-temperature short-time processing. Innov. Food Sci. Emerg. Technol. 33: 135–144. doi.org/10.1016/j.ifset.2015.10.012