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Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées ☆

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ABSTRACT

The present study was undertaken to assess the effect of high pressure treatments and conventional thermal processing on antioxidant activity, levels of key antioxidant groups (polyphenols, ascorbic acid and anthocyanins) and the colour of strawberry and blackberry purées. Bioactive compounds (cyanidin-3-glycoside, pelargonidin-3-glucoside, ascorbic acid) and antioxidant activity were measured in strawberry and blackberry purées subjected to high pressure treatment (400, 500, 600 MPa/15 min/10–30 °C) and thermal treatments (70 °C/2 min). Samples were assessed immediately after processing. Different pressure treatments did not cause any significant change in ascorbic acid ($p>0.05$). In contrast, following thermal processing ($P_{70}\geq 2$ min) ascorbic acid degradation was 21% ($p<0.05$) as compared to unprocessed purée. However, no significant changes in anthocyanins were observed between pressure treated and unprocessed purées ($p>0.05$), whereas conventional thermal treatments significantly reduced the levels ($p<0.05$). In general, antioxidant activities of pressure treated strawberry and blackberry purées were significantly higher ($p<0.05$) than in thermally processed samples. Colour changes were minor (ΔE) for pressurised purées but the differences were slightly higher for thermally treated samples. Redness of purées was well retained in high pressure treated samples. Therefore processing strawberry and blackberry by high pressure processing could be an efficient method to preserve these products quality. Hence high pressure processing (HPP) at moderate temperatures may be appropriate to produce nutritious and fresh like purées.

Industrial relevance: This research paper provides scientific evidence of the potential benefits of high pressure processing in comparison to thermal treatments in retaining important bioactive compounds. Antioxidant activity (ARP), ascorbic acid, and anthocyanins after exposure to high pressure treatments (400–600 MPa) were well retained. Our results also show that redness and colour intensity of strawberry and blackberry purées were better preserved by high pressure processing than conventional thermal treatment. From a nutritional perspective, high pressure processing is an attractive food preservation technology and offers opportunities for horticultural and food processing industries to meet the growing demand from consumers for healthier food products. Therefore high pressure processed foods could be sold at a premium than their thermally processed counterparts as they will have retained their fresh-like properties.

1. Introduction

Consumers are demanding high quality and convenient products with natural flavour and taste, and greatly appreciate the fresh appearance of minimally processed food (Oey, Van der Plancken, Van Loey, & Hendrickx, 2008; Patras, Brunton, Butler, & Gerard, 2008b). In order to increase microbiological stability and extended shelf life they are often thermally processed either alone or in-situ in the product. Thermal processing has been shown in many studies to affect the antioxidant capacities of foods (Dewanto, Adom, & Liu, 2002; Puupponen-Pimia et al., 2003; Sikora, Cieślik, Leszczyńska, Filipiak-Florkiewicz, & Paweł, 2008; Zhang & Hamauzu, 2004). While in most cases thermal processing results in a decrease in antioxidant capacity, some authors have also reported an increase in antioxidant capacity (Patras, Brunton, Tiwari, & Butler, 2008a; Patras, Brunton, Butler, & Gerard, 2008b).

Fruit purées are used in a variety of products including jams, conserves and smoothies and contain many health promoting antioxidants. The role of antioxidant compounds in reducing the risk of many chronic diseases such as cancer, coronary heart disease, immune system decline has been well documented (Kaur & Kapoor, 2001). Several studies have demonstrated a relationship between consumption of fruits and a lower incidence of degenerative diseases such as heart disease, arthritis and aging (Blumberg, 2003). Therefore there is a need for alternate methods of processing which can increase microbiological stability and will aid in preserving nutritional characteristics. Non-thermal processing methods such as high

☆ Work dedicated to my parents (Iris and Parvez).

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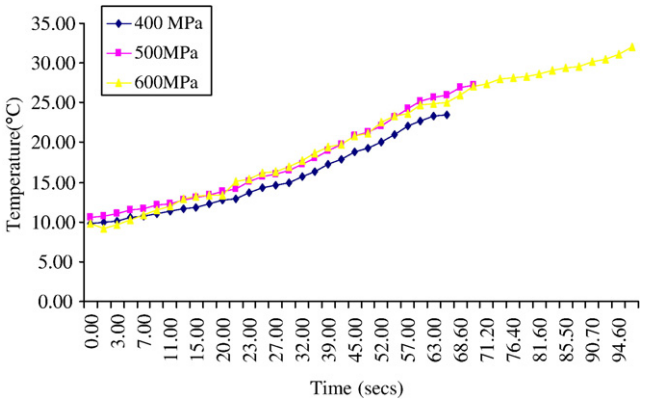

Fig. 1. A typical time and temperature profile of strawberry purées processed at different pressure treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydrostatic pressure processing (HPP) could potentially fill this role. HPP uses water as a medium to transmit pressures from 0 to 800 MPa. One of the main advantages of this process is the almost instantaneous isostatic pressure transmission to the product, independent of size, shape and food composition yielding highly homogeneous products (Patterson, Quinn, Simpson, & Gilmour, 1996). Food treated in this way has been shown to keep its original freshness, flavour, taste and colour changes are minimal (Dede, Alpas & Bayindirli, 2007). While the structure of high-molecular-weight molecules such as proteins and carbohydrates can be altered by high pressure processing, smaller molecules such as volatile compounds, pigments, vitamins, and other compounds connected with the sensory, nutritional, and health promoting are unaffected (Cheftel, 1992; Oey et al., 2008). High-pressure treatment in comparison with those of traditional thermal processing results in better retention of levels of bio-active compound groups (Patras et al., 2008b), increasing microbiological stability (Meyer, Cooper, Knorr, & Lelieveld, 2000) and decreasing enzyme activity (Weemaes, Ludikhuyze, Van den Broeck, & Hendrickx, 1999).

Fruit colour is a major determinant of quality in red berry fruits and their products and is due to the presence of anthocyanins, a group of water-soluble pigments with antioxidant properties. The main anthocyanins present in strawberry and blackberry are pelargonidin-3-glucoside and cyanindin-3-glucoside (Zabetakis, Delphine, & Kajda, 2000) respectively and recent work has suggested involvement of anthocyanins in various health benefits and cancer prevention (Zhang, Kou, Fugal, & McLaughlin, 2004). Despite the fact that high pressure processing has been used in Japan commercially and other countries for some years, the effect of high pressure processing on antioxidant activity, and different antioxidant groups (anthocyanins, phenols, ascorbic acid) has not been extensively studied in blackberry and strawberry purées. The objective of the present work was to study the impact of high pressure treatments in comparison with those of traditional thermal processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées with the aim of preserving the nutritional quality initially present in fresh fruits.

2. Materials and methods

2.1. Chemicals and preparation of fruit purées

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox), pyrogallol, Folin-Ciocalteu reagent (2 N), sodium carbonate, gallic acid and L-ascorbic acid were obtained from Sigma Aldrich (Dublin, Ireland). Hexane, acetone, metaphosporic acid and methanol (HPLC grade) were purchased from BDH England. (Poole, BH15, ITD). Cyanidin and pelargonidin standards were obtained from Extrasynthèse (Lyon, France).

Strawberries (*Fragaria*×*ananassa* cv, *EI Santa*) and blackberries (*Rubus fruticosus* cv, *Loughness*) were obtained locally (Keeling & St Margarets, Dublin, Ireland). After washing and dicing, samples were blended in a mechanical blender (Robot Coupe, Blixer 4, mono, France). To minimise oxidation of the purée during processing, it was vacuum mixed (Stephen mixer, Stephen U Söhne GmbH & Co., Hameln, Germany) at 500 rpm at 1 °C. Samples were vacuum packed using Vac-star S220 vacuum sealer (Vicquip Ltd., Dublin, Ireland) and stored at −21 °C until required for thermal and non-thermal processing. The procedures of freezing, thawing and freeze drying were applied to both unprocessed and processed purées.

2.2. High pressure and thermal processing treatments

After thawing overnight at 4 °C the vacuum packed samples (250 g) were placed in a high pressure vessel (100 mm internal diameter×254 mm internal height, Pressure Engineered System, Belgium) filled with a mixture of water and rust inhibitor (Dowcal N, 60% v/v in distilled water) and subjected to pressures of 400, 500 or 600 MPa for 15 min at ambient temperature (≈20 °C). Time taken to reach the target pressure was approximately (60–100 s) and depressurisation took 10 s. Temperature in the sample was monitored and illustrated in Fig. 1.

Vacuum packed unprocessed purées (250 g) were boiled in water until; they had achieved a core temperature of 70 °C (Fig. 2). They were held at this temperature until they had reached a time–temperature (P_{70}≥2 min) equivalent to a six log reduction in numbers of vegetative cells of the target pathogen (*L. monocytogenes*), (FSAI, 2006) as monitored using E-lab time–temperature recorder (Ellab, Ltd., Norfolk, UK). Sample core temperature profile was recorded during the process, using an Ellab E-Val TM TM9608 data module (Ellab [UK] Ltd., Norfolk, England) connected to a laptop (Fig. 2). Standard Ellab SSA-12080-G700-TS temperature probe was inserted through an Ellab GKM-13009-C020 packing gland (20 mm) into a vacuum bag. After processing, samples were removed; freeze dried (Frozen in Time Ltd., York, UK) at a temperature and pressure of −50 °C and 0.03 mbar respectively for more than two days and tested for antioxidant indices and instrumental colour. Prior to any experiment, all Ellab unit probes were calibrated against JOFRA (ATC-155B) calibration unit at temperatures of 70 and 80 °C and all results associated with the calibration did not exceed ±0.1 °C.

2.3. Chemical and physical analysis

2.3.1. Total antioxidant capacity and phenolic content

Methanolic extracts of freeze-dried fruit purées were prepared by adding 25 mL of HPLC grade methanol added to 1.25 g of milled

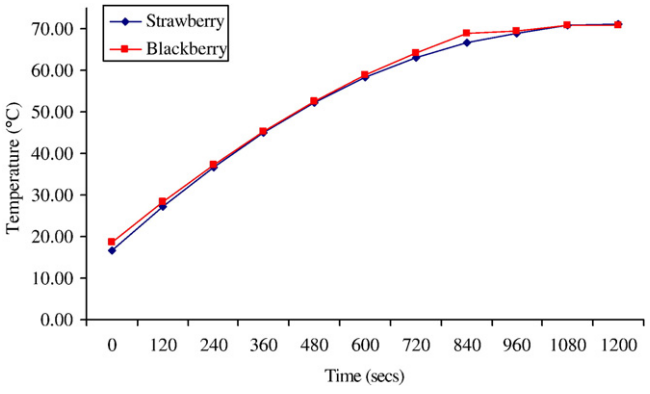

Fig. 2. Example of a time–temperature profile of thermally processed purée.

(Tecator ab, S314., Swedan) freeze dried powder and homogenising for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogeniser (Janke & Kunkel, IKA®-Labortechnik, Saufen, Germany). The samples were mixed with a V400 Multitude Vortexer (Alpha laboratories, North York, Canada) for 20 min at 1050 rpm and centrifuged for 15 min at 2000 g (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK). 10 mL of the sample was filtered through PVDF Acrodisc syringe filters (pore size 0.45 μm, Sigma, Ireland) and stored at −20 °C for subsequent analysis. Total antioxidant capacity was measured using the DPPH assay as described by Goupy, Hugues, Boivin, and Amiol (1999) (Patras et al., 2008a). Briefly, 500 μL of diluted sample and 500 μL of the DPPH (0.238 mg/mL^{-1}) working solution were added to a micro-centrifuge tube. After vortexing, the tubes were left in the dark for 30 min at room temperature after which the absorbance was measured against methanol at 515 nm using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Milton Keynes). Antioxidant activities were expressed as the IC_{50} i.e., the concentration of antioxidant required to cause 50% reduction in the original concentration of DPPH. For ease of interpretation antiradical powers (ARP) were also calculated and defined as the inverse of the IC_{50} value. Finally the antioxidant capacity of the extracts was compared to that of a synthetic antioxidant (Trolox) and expressed as Trolox equivalent antioxidant capacity values (TEAC).

Total phenolic content (TP) in the purée was determined using the Folin-Ciocalteu reagent according to the method of Singleton, Orthofer, and Lamuela-Raventos (1999). Briefly 100 μL of methanolic extract, 100 μL of MeOH, 100 μL Folin-Ciocalteau reagent (FC) and 700 μL of Na_2CO_3 were added to 1.5 mL microcentrifuge tubes and the samples were vortexed. The tubes were then left in the dark for 20 min at room temperature. Following this, the samples were centrifuged (Eppendorf, Centrifuge 5417R., Germany) at 13,000 rpm for 3 min. The absorbance of the sample was read at 735 nm using aqueous Gallic acid (10–400 mg/L) as a standard. Results were expressed as mg of Gallic acid equivalent per 100 g of dry weight of sample.

2.3.2. Ascorbic acid analysis

Extraction of ascorbic acid was carried out using 6% metaphosphoric acid and 1.25 g of freeze dried powder as described for antioxidant assay extractions. Ascorbic acid determination was carried out by high performance liquid chromatography (HPLC) according to the method of Lee and Coates (1999). The chromatographic system (Shimadzu-Model no SPD-M10A VP, Mason Technology, Dublin 8, Ireland) consisted of a pump, a vacuum degasser, a Diode-Array Detector and it was controlled through EZ Start 7.3 software (Shimadzu) at 40 °C. A hypersil ODS column (15 cm × 4.6 cm, 5 μm, Supelco., US) fitted with hypersil ODS guard column (Gemini C18 [4 mm L × 3.0 mm ID], Phenomenex., UK) was utilised with a mobile phase (isocratic) of 25 mM monobasic potassium phosphate adjusted to pH 3 at a flow rate of 1 mL/min. The detector was set at 245 nm. For quantification external calibration curves for ascorbic acid in metaphosphoric acid (6%) were prepared at concentrations from 25 μg/mL to 500 μg/mL. The total run time was 4.0 min.

2.3.3. Measurement of anthocyanin content of samples

Anthocyanins were analysed by the method described by Zabetakis et al. (2000) with a slight modification. Briefly 1.25 g of freeze-dried samples was extracted with the mixture of methanol, acetic acid, and water in ratios 25:1:24 and the rest of the isolation was identical to that described for antioxidant extractions assay. The mobile phase for HPLC analyses consisted of acetonitrile (83 mL); methanol (33 mL) and acetic acid (170 mL) which was mixed with trichloroacetic acid (0.65 g). HPLC analysis was carried out using the same chromatographic system as used for ascorbic acid with different chromatographic conditions. Separations were conducted on a Zorbax SB C_{18}, 5 μm, 150 × 4.6 mm column (Agilent Technologies, Dublin, Ireland). The injection volume was 20 μL with an isocratic flow rate of 1 mL/min and the total run time was less than 4 min. Detection was carried out at 520 nm. For quantification external calibration curves for cyanidin-3-glucoside (Cy3gl) and pelargonidin-3-glucoside (Pg3gl) were prepared at concentrations from 25 μg/mL to 100 μg/mL.

2.3.4. Measurement of instrumental colour

The colour of the samples was measured using a Hunter-Lab colour meter (Hunter Lab DP-9000 colour difference meter, Hunter Associates Laboratory, Virginia, USA) fitted with a 2.5 cm diameter aperture. The instrument was calibrated using the black and white tiles provided. Colour was expressed in Hunter Lab units L^*(Lightness/darkness), a^*(redness/greenness) and b^*(yellowness/blueness). Samples of purée were filled into a plastic petri dishes (i.d. 50 mm) taking care to exclude air bubbles and placed under the aperture of the colour meter. Three measurements were performed and results were averaged. In addition, hue angle and total colour difference (ΔE) were calculated using the following equations, where L_0, a_0, b_0 are the control values for unprocessed purées.

$$\text{Hue angle} = \tan^{-1}(b/a) \quad (1)$$

$$\Delta E = \left[(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2\right]^{1/2}. \quad (2)$$

2.3.5. Statistics

Analysis of variance with Tukey's test (5% significance level) was used to assess the effect of different treatments on bioactive compounds and colour parameters using Minitab version (MINITAB 15.1.1.0, 2007). Data are presented as means ± standard deviation of 3 observations. Three observations of different samples for one treatment were carried out.

3. Results and discussion

3.1. Chemical indices of antioxidant activity

3.1.1. Free radical scavenging activity

Antioxidant activities are expressed as anti-radical powers (ARP), the inverse of the IC_{50} index which gives a more convenient

Table 1
Antioxidant indices of thermally and high pressure processed (HPP) strawberry and blackberry purées.

Treatment	Anti-radical power, $(g/L)^{-1}$		Total phenols, mg GAE/100 g DW[e]		Anthocyanin, mg/100 g DW		Ascorbic acid, mg/100 g DW	
	Strawberry	Blackberry	Strawberry	Blackberry	Strawberry[f]	Blackberry[g]	Strawberry	Blackberry
Unprocessed	1.55 ± 0.07^{a}	2.86 ± 0.23^{a}	855.02 ± 6.52^{a}	1694.19 ± 3.0^{a}	202.27 ± 0.50^{a}	1004.90 ± 8.60^{a}	633.10 ± 9.31^{a}	nd
Thermally processed	1.16 ± 0.01^{b}	2.78 ± 0.26^{a}	817.01 ± 5.26^{b}	1633.62 ± 8.4^{a}	145.82 ± 6.40^{b}	975.28 ± 7.90^{b}	496.11 ± 0.04^{b}	nd
HPP400MPa	1.25 ± 0.05^{b}	3.87 ± 1.11^{a}	859.03 ± 6.56^{a}	1546.26 ± 8.0^{a}	173.34 ± 6.51^{ab}	1039.21 ± 4.51^{a}	574.30 ± 3.93^{c}	nd
HPP500MPa	1.30 ± 0.02^{ab}	3.70 ± 0.57^{a}	926.00 ± 5.93^{a}	1724.65 ± 0.7^{b}	202.53 ± 5.40^{a}	1014.21 ± 0.10^{a}	577.10 ± 6.52^{c}	nd
HPP600MPa	1.33 ± 0.02^{a}	4.80 ± 1.79^{b}	939.01 ± 0.99^{c}	1778.44 ± 6.0^{b}	204.30 ± 1.60^{a}	1014.47 ± 1.00^{a}	599.11 ± 0.60^{c}	nd

Values are mean ± standard deviation, $n = 3$, mean values in a column with different letters are significantly different at $p < 0.05$.
[e]Dry weight.
[f]Expressed as mg/100 g DW pelargonidin-3-glucoside.
[g]Expressed as mg/100 g DW cyanidin-3-glucoside.

illustration of radical scavenging activity as it is directly related to it. Results are presented in Table 1. ARP values for strawberry and blackberry purées ranged from 1.16–1.55 $(g/L)^{-1}$ and 2.78–4.80 $(g/L)^{-1}$ respectively amongst all the samples analysed. Higher ARP values for blackberry purées were presumably related to higher phenolics and anthocyanin levels (Table 1). Unprocessed strawberry and thermally treated purées had anti-radical powers of 1.55 and 1.16 $(g/L)^{-1}$ respectively representing an overall significant decrease of 25% ($p<0.05$) during processing. Lo Scalzo, Iannoccari, Summa, Morelli, and Rapisarda (2004) also reported that thermal treatments induced a decrease in free radical scavenging activity of blood orange juice as compared to untreated samples as juices were blanched and pasteurised (80 °C for 6 min). No significant decrease in anti-radical power of blackberry purées was detected following conventional thermal processing ($p>0.05$). For strawberry purées high pressure treated samples at 400 MPa had significantly lower antioxidant capacities when compared to unprocessed samples ($p£0.05$). However, for purées treated at 500 and 600 MPa, mean ARP values were higher than thermally processed samples ($p<0.05$). In contrast, for blackberry purées no significant differences in ARP values were detected between unprocessed, thermally processed, HPP400 or HPP500 whereas pressure treatments at 600 MPa produced significant increase of 67% as compared to unprocessed counterpart. The possible reason for the increase in ARP could be due to better extractability of antioxidant components. Other authors have also reported that high pressure processing either increase or does not affect antioxidant activity of liquid foods. For example, the content of the flavanones naringenin and hesperetin in orange juice increased due to pressure treatment of 400 MPa/40 °C/1 min, by 20.16% and 39.88% respectively (Sánchez-Moreno, Plaza, DeAncos, Martin, & Cano, 2005). Fernández Garcia, Butz, and Tauscher (2000) reported that high pressure treatments of 600 MPa/60 °C/30 min only slightly affected antioxidant capacity (determined as TEAC value) of apple juice. Sánchez-Moreno, Plaza, De Ancos, and Cano (2006) indicated that total scavenging activity (DPPH) in aqueous and organic fractions of tomato purée was unaffected by a HP treatment of 400 MPa/25 °C/15 min.

3.1.2. Phenolic content

Phenolic contents of strawberry and blackberry purées before and after thermal and high pressure treatment are presented in Table 1. Levels of phenols for unprocessed strawberry and blackberry purées were in the range of those reported by other authors (Aaby, Wrolstad, Ekeberg, & Screeds, 2007). Phenols appeared to be relatively resistant to the effect of processing. Levels of phenols in high pressure treated strawberry purées treated at 600 MPa (939.0 mg/100 g DW) increased significantly (9.8%, $p<0.05$) as compared to unprocessed purée (855.0 mg/100 g DW). Similar trend was observed for blackberry purées. This increase in total phenolic content may be related to an increased extractability of some of the antioxidant components following high pressure processing. For example, Corramles, Toepfl, Butz, Knorr, and Tausche (2008) reported increase in total phenolic content of grape by-products following high pressure processing, ultrasonics and pulsed electric field. No significant differences were detected on phenolic concentration after thermal treatments. Pressure treatment at 400 and 500 MPa produced a slight but non-significant increase on total phenolic content. For blackberry purées neither high pressure (400 MPa) nor thermal processing had a significant effect on levels of phenols. Similar to the case for strawberry purées, levels of phenols were significantly higher at 600 MPa or 500 MPa than all other treatments ($p<0.05$).

3.1.3. Anthocyanin content

The main anthocyanin present in strawberry purée is Pelargonidin-3-glucoside (Pg3gl) (Zheng, Wang, Wang, & Zheng, 2007). In the present study, levels of this anthocyanin in strawberry purées ranged from 145–204 mg/100 g DW (Table 1), these values are in the range of those reported elsewhere by other authors (Zheng et al., 2007). HPLC chromatograms of unprocessed and processed strawberry purée are illustrated in Fig. 3. Pg3gl contents of unprocessed and thermally processed purées were 202.27 and 145.82 mg/100 g DW with an overall significant reduction of (27.9%, $p<0.05$). Degradation of anthocyanin pigments in strawberry purée may have been catalyzed by the presence of oxidase enzymes during or after processing, as has been demonstrated in several fruit systems (Jackman, Yada, Stung, & Speers, 1987). No significant difference between levels of Pg3gl in high pressure treated samples was detected for strawberry purées regardless of pressure level ($p>0.05$). The main anthocyanin present in blackberry purée is cyanindin-3-glycoside (Cy3gl) (Wang & Xu, 2007). Cy3gl levels ranged from 975.28 to 1039.21 mg/100 g DW, averaging 1009.61 mg/100 g DW. Values reported here are in the range of those reported by other authors (Wang & Xu, 2007). Similar to the results reported for Pg3gl levels in strawberry purées subjected to conventional thermal treatment, levels of Cy3gl in thermally processed blackberry purée were significantly lower than for unprocessed sample ($p<0.05$). This decrease could be due to simple thermal degradation of the samples although the thermal treatment was relatively short (70 °C, 2 min). However, anthocyanin degradation in processed berry products has been reported to arise as a result of indirect oxidation by phenolic quinones generated by polyphenol oxidase and peroxidase (Kader, Rovel, Girardin, & Metche, 1997; Wesche-Ebeling & Montgomery, 1990; Skrede, Wrolstad, & Durst, 2000). Mean anthocyanin levels for high pressure treated samples at 400, 500 or 600 MPa were higher than for fresh samples, but the effect was not significant ($p>0.05$). In general, other authors have also reported that anthocyanins are stable to HP treatment at moderate temperature. For example Garcia-Palazon, Suthanthangjai, Kajda, and Zabetakis (2004) reported that pelargonidin-3-glucoside and pelargonidin-3-rutinoside in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria*×*ananassa*) were stable to HP treatment at 800 MPa (18–22 °C/15 min). In contrast, some authors have also reported increased extractability of coloured pigments in food components at extreme pressures (Sánchez-Moreno et al., 2005; De Ancos, Sgroppo, Plaza, & Cano, 2002, Patras et al., 2008b). In general, anthocyanins (Pg3gl and Cy3gl) were well retained at all pressure treatments and this was reflected in better retention of antioxidant activity of both purées as shown in Table 1. Moreover, a positive significant correlation was also found between anthocyanin content of strawberry (Pg3gl) and anti-radical power ($r=0.64$, $p<0.05$).

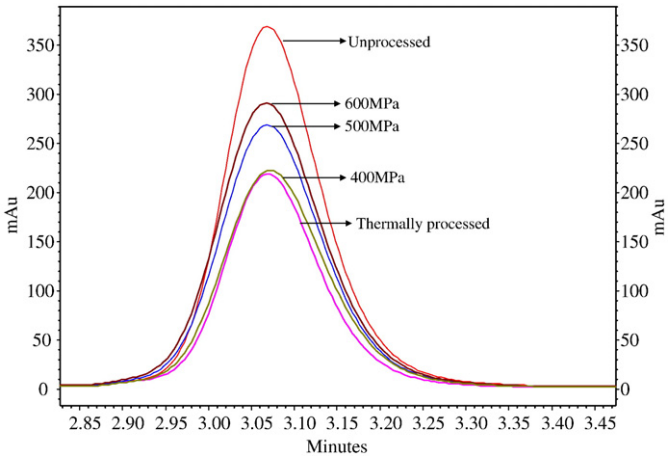

Fig. 3. HPLC chromatogram of extract from freeze dried strawberry purée of unprocessed, thermally (70 °C, 2 min) and high pressure processed (400–600 MPa, 20 °C) samples. Major peak is pelgargonidin-3-glucoside as identified by comparison to authenticated standards.

Table 2
Instrumental colour parameters of thermally and high pressure processed (HPP) strawberry and blackberry purées.

Treatment	Total colour change (ΔE)		Hunter a^*		Hue angle	
	Strawberry	Blackberry	Strawberry	Blackberry	Strawberry	Blackberry
Unprocessed	0^{a}	0^{a}	32.34 ± 0.01^{a}	20.67 ± 0.20^{a}	20.59 ± 0.05^{a}	15.93 ± 0.15^{a}
Thermally processed	5.67 ± 0.07^{b}	3.17 ± 0.09^{b}	29.00 ± 0.02^{b}	17.40 ± 0.08^{b}	24.60 ± 0.01^{b}	14.82 ± 0.01^{b}
HPP400MPa	2.05 ± 0.07^{c}	3.67 ± 0.01^{c}	30.74 ± 0.01^{c}	16.64 ± 0.01^{c}	21.41 ± 0.01^{c}	15.29 ± 0.01^{ab}
HPP500MPa	2.44 ± 0.04^{c}	2.71 ± 0.06^{d}	30.65 ± 0.02^{c}	17.58 ± 0.05^{bc}	20.91 ± 0.01^{c}	15.19 ± 0.05^{ab}
HPP600MPa	2.12 ± 0.07^{c}	2.15 ± 0.03^{d}	31.17 ± 0.03^{c}	18.14 ± 0.07^{d}	20.51 ± 0.05^{a}	16.01 ± 0.01^{c}

Values are mean ± standard deviation, $n = 3$, mean values in a column with different letters are significantly different at $p < 0.05$.

3.1.4. Ascorbic acid content

Ascorbic acid content in unprocessed and processed strawberry purée ranged from 496–633.1 mg/100 g DW. These values are in the range of those previously reported by other authors (Klopotek, Otto, & Bohm, 2005). Purées treated by the conventional thermal process had an ascorbic acid content of 496.1 mg/100 g DW representing an overall significant decrease of 22.6% (Table 1, $p < 0.05$). For example, Taoukis et al. (1998) also reported ascorbic acid losses of 20–25% in pineapple juice after heating at 45 °C this figures rises to 60–70% at temperatures in excess of 75 °C. Levels of ascorbic acid in samples treated at 400 and 500 MPa were significantly lower than in fresh samples ($p < 0.05$), this was also true for samples pressurised at 600 MPa. Results indicates that pressurisation at 600 MPa preserved the majority of the ascorbic acid in the samples (94%). In fact ascorbic acid levels were significantly higher for all high pressure treated samples as compared to conventional thermal processed samples ($p < 0.05$). Again this is in line with reports from other authors. For example, Sánchez-Moreno et al. (2005) reported only 9% loss of ascorbic acid in orange juice after HPP at 400 MPa/40 °C/1 min. This compares well with a figure of 9.2% in the present study given the longer pressurisation time (15 min). Yen and Lin (1996) reported that 88.68% of the initial content of ascorbic acid in strawberry coulis and in strawberry nectar was retained after treatment at 400 MPa/20 °C/30 min. Polydera, Stoforos, and Taoukis (2003) reported that a high pressure treatment of 500 MPa, at 35 °C for 5 min led to better retention of ascorbic acid during processing as compared to conventional thermal pasteurisation (80 °C, 30 s) for orange juice. Ascorbic acid levels in blackberry samples were not detectable. Ascorbic acid and anti-radical power of strawberry purée were positively correlated ($r = 0.81$, $p < 0.01$), indicating that ascorbic acid plays an important role in scavenging activity of strawberry purée. In general, the decrease of anti-radical power during TP and HPP of purées can be attributed to the loss of ascorbic acid but other antioxidant groups cannot be discarded.

3.2. Instrumental colour parameters

Instrumental colour parameters of strawberry and blackberry purées as affected by thermal and high pressure processing are illustrated in Table 2. Thermal treatment caused a significant ($p < 0.05$) decrease of Hunter a^* value (redness) of strawberry and blackberry purées as compared to unprocessed sample. Red colour of both fruits is mainly due to the presence of anthocyanins and it is interesting to note that levels of anthocyanins were also significantly lower in thermally processed strawberry and blackberry purées (Table 1, $p < 0.01$). In fact Py3gl and Cygl levels of strawberry and blackberry purées were positively correlated ($r = 0.64$, $p = 0.009$, $r = 0.72$, $p = 0.001$) with Hunter a^* values indicating anthocyanins in strawberry and blackberry purées significantly influenced redness of purée. In addition to simple thermal degradation of anthocyanins, loss of colour at higher temperatures has also been attributed to increased rates of enzyme mediated losses via enzymes such as peroxidase, polyphenol oxidase and glucosidase (Cano, Hernandez, & De Ancos, 1997). High pressure processed samples also had significantly higher a^* values as compared to thermally treated samples (Table 2, $p < 0.05$). However a^* values for both purées were significantly lower than for fresh samples (Table 2, $p < 0.05$). Amongst all the pressure treatments, 600 MPa had the highest retention (96%, $p < 0.05$, Table 2] of redness in strawberry purée. Porretta, Birzi, Ghizzoni, and Vicini (1995) reported higher a^* values in of HPP treated tomato juice compared to conventional cooked sample. The authors attributed this to compacting and homogenising effects of the HPP treatment. In a study carried out by Gimenez, Kajda, Margomenou, Piggott, and Zabetakis (2001), the authors reported better colour retention in jams and grape juices when treated by HPP due to better extraction of anthocyanins from the fruit matrix. It is well known that high pressure treatments combined with low temperatures can minimise colour and quality losses. An inconsistent affect of processing was observed on both purées in regard to hue angle. Hence this index should not be used as a quality parameter for strawberry and blackberry purées. Total colour change (ΔE) in processed purées was significantly different to unprocessed samples. The ΔE^* values for strawberry and blackberry decreased at high pressure treatments as compared to thermal processing with the exception of blackberry purée treated at 400 MPa. It is quite apparent that application of HPP had a smaller effect on colour changes than thermal processing. For pressurised strawberry purées, ΔE were about or less than 2.50, but for heat treated the colour change was more intense (5.67) as indicated in Table 2. A similar trend was observed for blackberry purée. Dede et al. (2007) reported similar results for carrot and tomato juice. They observed that high pressure treatment of 250 MPa/35 °C/15 min produced a lower colour difference compared to fresh sample than thermally processed juices.

4. Conclusions

High pressure treatment at pressures from 400–600 MPa significantly retained more phenols, anthocyanins and ascorbic acid in strawberry purées than thermal treatment. In blackberry purées greater retention of anthocyanins was noted as compared to thermally treated purées and total anti-radical powers of high pressure treated samples were significantly higher than in fresh and thermally processed samples. Colour retention as assessed using the Hunter a^* values (redness) was higher in pressure treated samples than in thermally processed for both strawberry and blackberry purées. Processing strawberry and blackberry by high pressure processing could be an efficient method to preserve these products. High pressure processing at moderate temperatures can maintain nutritional quality of purées and could be used in commercial production of high quality products with superior characteristics than thermally processed counterparts.

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