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Effects of Thermal and High Hydrostatic Pressure Processing and Storage on the Content of Polyphenols and Some Quality Attributes of Fruit Smoothies

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The aim of the present study was the evaluation of high hydrostatic pressure (HHP) processing on the levels of polyphenolic compounds and selected quality attributes of fruit smoothies compared to fresh and mild conventional pasteurization processing. Fruit smoothie samples were thermally (P_{70} > 10 min) or HHP processed (450 MPa/1, 3, or 5 min/20 °C) (HHP1, HHP3, and HHP5, respectively). The polyphenolic content, color difference (ΔE), sensory acceptability, and rheological (G'; G''; G^*) properties of the smoothies were assessed over a storage period of 30 days at 4 °C. Processing had a significant effect ($p < 0.001$) on the levels of polyphenolic compounds in smoothies. However, this effect was not consistent for all compound types. HHP processed samples (HHP1 and HHP3) had higher ($p < 0.001$) levels of phenolic compounds, for example, procyanidin B1 and hesperidin, than HHP5 samples. Levels of flavanones and hydroxycinnamic acid compounds decreased ($p < 0.001$) after 30 days of storage at 2−4 °C). Decreases were particularly notable between days 10 and 20 (hesperidin) and days 20 and 30 (chlorogenic acid) ($p < 0.001$). There was a wide variation in ΔE values recorded over the 30 day storage period ($p < 0.001$), with fresh and thermally processed smoothies exhibiting lower color change than their HHP counterparts ($p < 0.001$). No effect was observed for the type of process on complex modulus (G^*) data, but all smoothies became less rigid during the storage period ($p < 0.001$). Despite minor product deterioration during storage ($p < 0.001$), sensory acceptability scores showed no preference for either fresh or processed (thermal/HHP) smoothies, which were deemed acceptable (>3) by panelists.

KEYWORDS: Polyphenols; fruit smoothies; high hydrostatic pressure; sensory; rheology; color

INTRODUCTION

Smoothies are drinks prepared by blending fruit, fruit juice, ice, and yogurt or milk. The market share for this product grew 214% between 2002 and 2006 in the Republic of Ireland and was worth an estimated €4 million (*1*). They are typically purchased freshly prepared from juice bars or mildly pasteurized under chilled storage in local retail outlets. Despite worsening global economic conditions, smoothies remain a popular and convenient way of consuming fruits. Smoothies contain a high quantity of fruit rich in important phytochemicals (*2*), in particular, polyphenolic compounds. Similar to other antioxidants, polyphenols function to protect the fruit from oxidative deterioration (*3*). Therefore, they are an important determinant of fruit quality. Furthermore, the antioxidant and anti-inflammatory characteristics of polyphenols may confer potential health benefits on consumers (*4*). Most smoothies have apples as a core ingredient, and the positive physiological effects of apple phytochemicals on human health are reported in a comprehensive review paper which highlighted both in vitro and animal studies that have demonstrated decreased lipid oxidation, a lowering of cholesterol, and inhibitory effects on cancer cell proliferation with apple consumption (*5*). However, polyphenolic compounds also play a key role in enzymatic browning in fruits, as they are preferred substrates of oxidative enzymes (*6*).

In line with their desire for healthy products, such as smoothies, consumers are demanding convenient foods with fresh-like flavor, taste, and appearance. Therefore, many researchers have investigated alternatives to thermal processing of fruit drinks to counteract the possible negative impact that thermal processing may have, resulting in minimally processed fresh-like products without compromising storage life (*7*). The application of high hydrostatic pressure (HHP) (up to 700 MPa; however, the upper limit for industrial equipment is typically 600 MPa) can result in enzyme inactivation and a mild pasteurization of foods at ambient temperatures (*8*, *9*). In this regard, it may result in foods with more fresh-like characteristics by overcoming deleterious reactions more commonly associated with traditional thermal pasteurization, such as undesirable changes in organoleptic, textural, and nutritional properties (*10*). Adoption of the

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technique has been limited within the food industry, primarily because of the substantial capital investment required (U.S. $1.5−2.5 million) (*11*). Despite this, development of the technique has continued and has resulted in successful commercial products, such as guacamole, sliced meats, orange juice, smoothies, and shellfish, introduced to the marketplace (*12*). Research suggests that HHP could be useful in retaining nutritional qualities of antioxidant-rich fruits as it affects only the structure of high molecular weight molecules such as proteins and carbohydrates in foods but does not affect smaller molecules associated with sensory, nutritional, and health-promoting properties such as volatile compounds, pigments, and vitamins (*13*). Fruit has a low pH (≤4.5), which is an intrinsic preservation factor as most spoilage microorganisms are controlled and spore formers cannot proliferate. Vegetative cells are relatively pressure sensitive, making fruit products an ideal matrix for HHP processing (*2*). Although extensive investigation into the affects of HHP on single-fruit purees have been undertaken (*2*, *6*, *12*−*16*), only a small number have assessed HHP effects on mixtures. In addition, the small number of studies that have been undertaken have reported observations contrary to those expected. For example, a recent study reported that the application of HHP can affect the bioactive content of fruits, with vitamin C levels of apple purees (cv. Granny Smith) decreasing by 21.5% after pressurization (600 MPa/5 min/20 °C) (*12*). Therefore, a more comprehensive study of the effects of HHP on the individual polyphenolic makeup of processed fruit products would be of benefit.

The objective of the present study was to compare the effectiveness of HHP processing (450 MPa/1, 3, or 5 min/20 °C) on fruit smoothies with a desired storage life of 30 days at 4 °C. Storage duration was chosen on the basis of comparisons with equivalent products currently available at retail and are in agreement with the literature (*17*). The effect of HHP was evaluated on physicochemical (color, sensory, and rheological parameters) and nutritional quality parameters (individual polyphenols by HPLC) and compared to fresh controls and a mild conventional pasteurization treatment (P_{70} ≥ 10 min) as a function of treatment and storage time (30 days/4 °C).

MATERIALS AND METHODS

Sample Preparation. Smoothie formulation was based on a commercially available smoothie (GLSA, Portugal) (*18*). Strawberries (cv. Sabrosa; Spain), apples (cv. Braeburn; France), concentrated apple juice, bananas (cv. Nino; Cameroon), and oranges (cv. Navel-late; Spain) were obtained from a local retailer. Smoothie composition by weight consisted of whole apple (29.5%), apple juice from concentrate (29.5%), strawberry (21%), banana (12%), and orange (8%). Fruit was blended (Robot Coupé Blixer 4 mono, Bourgogne, France) for 3 min. All smoothie samples were sealed into 250 mL HHP grade polyethylene terephthalate bottles (The Packaging Centre, product code 18PBC250J, Dublin, Ireland). Controls (fresh) were chilled (2−4 °C) immediately, whereas other samples were subjected to subsequent processing. Average results for a range of fundamental physicochemical properties of fresh smoothies are presented in **Table 1**.

HHP and Thermal Processing Treatments. HHP processing was carried out 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). Two smoothie samples (250 mL bottles) were sealed inside a high-pressure vessel and subjected to a pressure of 450 MPa for 1, 3, or 5 min (HHP1, HHP3, or HHP5, respectively) at ambient temperature (≈20 °C) (*20*). The time taken to reach the target pressure was 60−100 s, and depressurization took 10 s. HHP vessel temperature was monitored by two type J thermocouples (1210/1260-S) and increased from 15 to 37.5 °C during high-pressure processing. During this time, sample temperature increased by an average of 2 °C (for the 5 min dwell time). Thermal processing was carried out in a pilot-scale retort (Barriquand Steriflow, Roanne, France) using an Ellab E-Val TM TM9608 data module [Ellab (U.K.) Ltd., Norfolk, U.K.) connected to a laptop to record core, temperature, and processing profiles and initial pasteurization value (P_0). Smoothie samples were probed (Ellab SSA-12080-G700-TS) and inserted through a 20 mm packing gland (Ellab GKM-13009-C020) to record cook cycle. Temperature was monitored every 10 s. The samples were heated to a time temperature equivalent of P_{70} ≥ 10 min to achieve a process equivalent to a six log reduction of vegetative cells (*18*). Samples were stored for 1, 10, 20, and 30 days at 4 °C. After processing and appropriate storage time, fresh, thermally processed, and HHP processed samples were tested for sensory acceptability, rheological analysis, instrumental color, and polyphenolic content.

Table 1. Physicochemical Attributes of Fresh Smoothies

parameter[a]	mean value (n = 6)	parameter	mean value (n = 6)
TP	431.4 ± 39.4	moisture	86.31 ± 0.1
TAC	222.4 ± 11.2	*L*	37.1 ± 0.4
TTA	0.56 ± 0.0	*a*	12.6 ± 0.2
pH	3.76 ± 0.0	*b*	10.6 ± 0.2
TSS	12.09 ± 0.3		

[a] TP, total phenolics (mg GAE 100 g^{-1}) by Folin−Ciocalteu assay; TAC, total antioxidant capacity (mg TE 100 g^{-1}) by 2,2-diphenyl-1-picrylhydrazyl (DPPH); TTA, total titratable acidity (mg malic acid 100 g^{-1}); TSS, total soluble solids (°Brix).

Chemical and Physical Analysis. *Extraction of Polyphenols.* All smoothie samples were freeze-dried for a minimum of 5 days (A6/14 freeze-dryer, Frozen in Time Ltd., York, U.K.) for polyphenol analysis. Extracts were prepared by adding 25 mL of HPLC grade methanol HPLC grade (BDH England, Poole, U.K.) to 1.25 g of the freeze-dried powder (Kenwood Mutli-Pro food processor, FP698 series, Kenwood Ltd., Havant, U.K.) and homogenizing for 30 s at 10000 rpm and 1 min at 20000 rpm using an Ultra-Turrax T-25 tissue homogenizer (Janke & Kunkle, IKA-Labortechnik, Saufen, Germany). Samples were vortexed with a V400 Multitude Vortexer (Alpha Laboratories, North York, Canada) for 20 min at 1050 rpm and centrifuged at 2218*g* (3000 rpm) for 15 min (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, U.K.). The resultant supernatant was filtered from a 10 mL disposable pipet (BD Plastipak, Becton Dickson S.A.S., Augistin del Gaudalix, Madrid, Spain) through PVDF Acrodisc syringe filters (pore size = 0.2 μm, Sigma-Aldrich, Ireland) and stored at −20 °C for subsequent analysis.

HPLC-DAD Analysis of Polyphenolic Composition. HPLC analysis of polyphenolic content of smoothies was carried out as previously described (*6*). Data were analyzed by EZ Start software (version 7.3) controlling an SPD-M10A vp Shimadzu chromatographic system (Shimadzu, U.K. Ltd., Milton Keynes, U.K.) equipped with a pump, degasser, and diode ray detector (DAD). A Zorbax SB C_{18}, 5 μm, 150 × 4.6 mm column (Agilent Technologies, Dublin, Ireland) was used for separations The mobile phase was prepared with 6% acetic acid in 2 mM sodium acetate (pH 2.55, v/v, line A) and 100% acetonitrile (line B) as previously described (*19*). The solvent gradient program was 95% A, 5% B (initial conditions); 0−45 min, 0−15% B; 45−60 min, 15−30% B; 60−65 min, 30−50% B; 65−70 min, 50−100% B. Column temperature was 37 °C with a flow rate of 1 mL min^{-1}. Samples were injected in 10 μL aliquots. Monitored wavelengths were 280 nm for hydroxybenzoic acids, dihydrochalcones, flavanones, and flavanols; 320 nm for hydroxycinnamic acid derivatives; 360 nm for flavonols; and 520 nm for anthocyanins. Sample peaks were identified using a spectral library comprising retention times and light absorbing spectra (200−900 nm) of authenticated standards [procyanidin B1 and hesperidin; Extrasynthèse (Lyon, France); chlorogenic acid and *p*-coumaric acid (Sigma-Aldrich, Dublin, Ireland)] under the above chromatographic conditions. Standard curves of the aforementioned standards were used for quantification. A compound with the same spectrum as a library standard but a different retention time was classified as derivative of the standard and quantified using the appropriate standard curve. Results were expressed as milligrams per 100 grams of fresh weight (FW). The detection limits for the four polyphenolic compounds identified were as follows: procyanidin B1, 274−300 nm; hesperidin, 252−350 nm; chlorogenic acid, 310−340 nm; and *p*-coumaric acid derivatives, 310−330 nm. Representative chromatograms for fresh and spiked samples are presented in **Figure 1**.

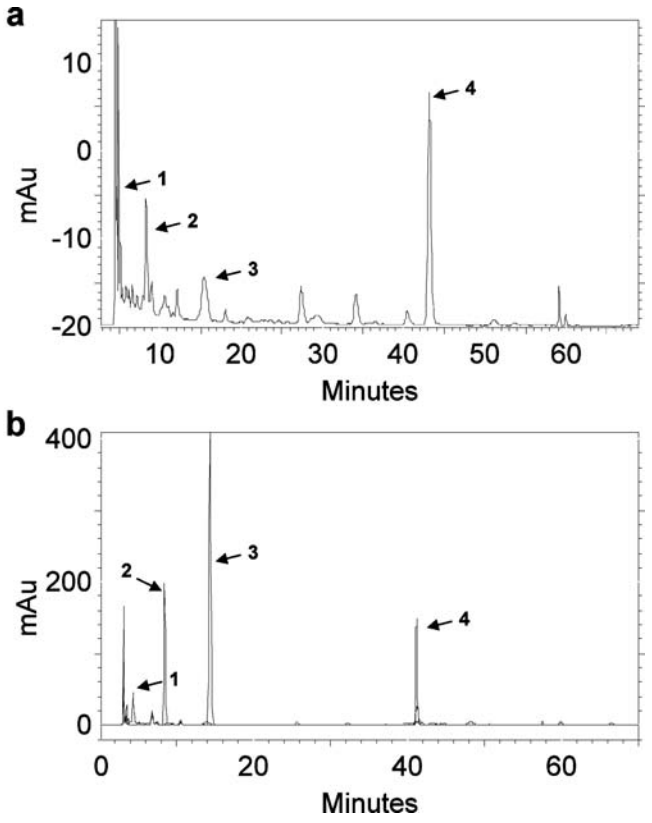

Figure 1. HPLC chromatograms at 280 nm of (**a**) fresh smoothie sample and (**b**) smoothie spiked with authenticated standards. In both instances, the peaks identified were (1) procyanidin B1, (2) chlorogenic acid, (3) *p*-coumaric acid, and (4) hesperidin.

Instrumental Color. Instrumental color (HunterLab) of fruit smoothies was measured in triplicate using a HunterLab D25A DP-9000 (HunterLab, Reston, VA) calibrated against a white and black tile (illuminant D65 and 10° observer angle). Sampling procedure involved filling smoothies into a low reflectance Agtron Color/quality control sample cup (Magnuson Engineers Inc., San Jose, CA) (i.d. = 25 mm) and placing the cup over the aperture of the color meter. Color was expressed as ΔE values (color change) using fresh smoothie on day 1 as initial value and given by the equation

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

where L (lightness/darkness), a (redness/greenness), and b (yellowness/blueness) units.

Sensory Evaluation. Sensory acceptability of fruit smoothies was assessed by an untrained 15 member taste panel (age range of 23−67 years) (*20*). Tasting was performed in a sensorial testing room, with individual booths and controlled lighting. Panelists were presented with five smoothies (20 mL in plastic thimbles), that is, HHP1, HHP3, and HHP5, thermally processed, and a fresh control. Responses were recorded on days 1, 10, 20, and 30 of the storage period using a 6 cm scale with endpoints of 0 (unacceptable) and 6 (very acceptable). The midpoint of the scale (>3) was chosen as an arbitrary threshold of assessing the acceptability; that is, >3 was deemed acceptable and <3 as unacceptable.

Dynamic Oscillatory Measurements. Rheological measurements were performed on a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) fitted with parallel plate (50 mm; smooth) geometry. Samples were placed onto the base plate with a test gap of 1 mm, and the testing geometry was covered by the temperature hood. Samples were rested (5 min) to achieve a constant test temperature (25 °C) and relaxation of residual stresses. A preliminary amplitude sweep was performed to identify the linear viscoelastic region of the samples and the strain (0.1%) that should be used for the resultant frequency sweep. A frequency sweep from 0.1 to 10 Hz was performed, and the results for storage modulus (G'), loss modulus (G''), and complex modulus (G^*) were recorded.

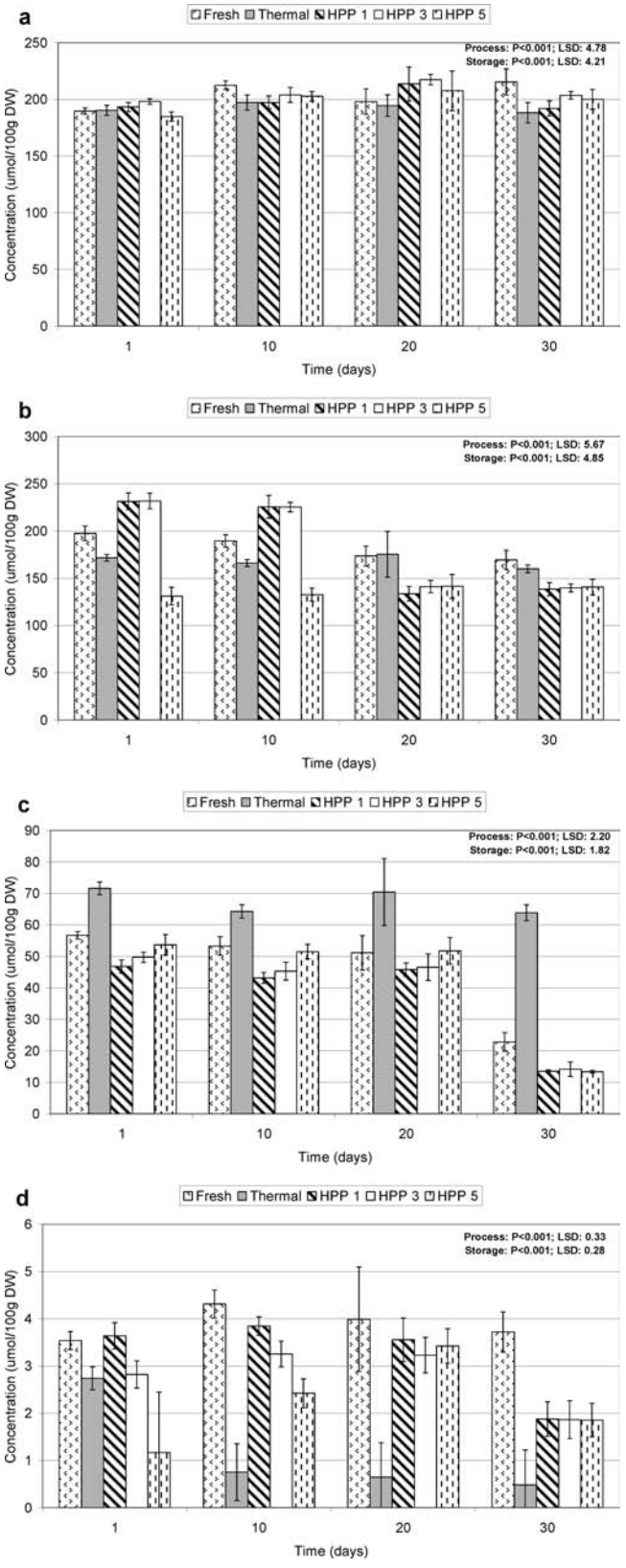

Figure 2. Effect of process and storage on the (**a**) procyanidin B1, (**b**) hesperidin, (**c**) chlorogenic acid, and (**d**) *p*-coumaric acid contents of fruit smoothies.

Statistical Design. The following statistical design was used: five processes (fresh, thermal, HHP1, HHP3, HHP5) × four storage days (1, 10, 20, and 30) × three replicates. Analysis of variance (ANOVA) was used

to determine the significant differences over the storage period (Genstat 5, version 3.2, Lawes Agricultural Trust, Rothamsted, Harpenden, U.K.).

RESULTS AND DISCUSSION

Polyphenols by HPLC-DAD. The major polyphenolic compounds present in fresh and processed (thermal and HHP) smoothies were quantified by HPLC analysis of methanolic extracts over four storage days [1, 10, 20, and 30 (**Figure 2**)]. The compounds detected were procyanidin B1 (**Figure 2a**), hesperidin (**Figure 2b**), chlorogenic acid (**Figure 2c**), and *p*-coumaric acid derivatives (**Figure 2d**). The majority of procyanidin B1, chlorogenic acid, and *p*-coumaric acid derivatives are most likely derived from the relatively high content (59% juice and flesh) of apple (*21*−*23*), whereas the hesperidin content will almost exclusively be from the orange (8%) present in the smoothie. The levels of three of the compounds detected in the present study are lower than those reported elsewhere for whole fruits, that is, hesperidin in oranges (58.50 mg 100 mL^{-1} FW cv. Valencia) (*24*) and chlorogenic acid and *p*-coumaric acid derivative compounds in apples (4.00−31.98 and 0.06 mg 100 g^{-1} FW, respectively) (*25*,*26*). However, procyanidin B1 (0.52−17.28 mg 100 g^{-1} FW) was directly within the range for apples (*22*). Lower values were within the same order of magnitude and could be explained by their percentage composition within the smoothie and processing effects; for example, the short exposures of pressure treatment (450 MPa for 1, 3, and 5 min) may have been inadequate for complete inactivation of oxidative enzymes. A number of matrix effects may have had an impact also. The homogenization of fruits when the smoothie was blended would place oxygen, intercellular oxidative enzymes [polyphenol oxidase (PPO)], and antioxidants in contact, leading to enzymatic and oxidative degradation of these compounds. Banana (12% of total smoothie) contains significant quantities (48 μg g^{-1} FW) of PPO (*27*), the major enzyme responsible for the degradation of polyphenols in fruits; that is, it readily degrades certain flavan-3-ol and hydroxycinnamic acid compounds, for example, procyanidin B1 (*28*) and chlorogenic acid (*14*). This enzymatic degradation could explain the low levels of both chlorogenic acid and other hydroxycinnamic acids. However, it would be unwise to extrapolate from studies on individual fruits to mixtures, as this does not take into account the possible matrix effects resulting from mixing intracellular contents from different sources.

Processing affected ($p < 0.001$) all of the polyphenolic compounds present in the fruit smoothies. However, the effect was not consistent for all compound types. Procyanidin B1 and hesperidin (**Figure 2a**,**b**) were present in the highest concentrations and *p*-coumaric acid derivatives (**Figure 2d**) in the lowest concentrations throughout the storage period. Processing effects followed similar trends for these compounds. HHP processing had contrasting effects, resulting in both the highest (HHP3) and also the lowest (HHP5) levels of procyanidin B1 (16.05 and 14.97 mg 100 g^{-1}) and hesperidin (19.82 and 11.21 mg 100 g^{-1} FW) present in the smoothies ($p < 0.001$). However, the severity of the difference between HHP3 and HHP5 varied considerably between the two compounds, that is, 7% lower in procyanidin B1 compared to 76% lower in hesperidin. Similarly, HHP5 samples had the lowest levels (0.025 mg 100 g^{-1} FW) of *p*-coumaric acid derivatives and differed significantly ($p < 0.001$) from all other processing treatments. However, no differences in the levels of *p*-coumaric acid derivatives were observed between fresh and thermally and high pressure treated samples (HPP1 and HHP3). These data indicate that the effects of processing on procyanidin B1 levels in fruit smoothies were less severe than hesperidin levels, as no differences were observed between fresh, thermal, and HHP5 samples. Other studies have reported contrasting effects of processing on levels of procyanidin compounds; for example, the processing of blueberries into various forms (juices and purees) has been shown to trigger significant losses of total procyanidins (*29*), whereas thermally processing peaches has resulted in decreases of procyanidin monomers, dimers, trimers, and oligomers (*30*). It has been hypothesized that depolymerization of the higher oligomeric and polymeric procyandins, which constitute a much larger proportion of the flavan-3-ols, into dimers and trimers, would result in an increase in procyandins B1, B2, B3, and B4 (*31*). This could explain the high levels of procyanidin B1 relative to other flavan-3-ol compounds recovered in the present study. On the other hand, hesperidin levels (**Figure 2c**) in fresh samples were significantly different ($p < 0.001$) from all other treatments, as were HHP5 and thermally processed samples. Although the level of hesperidin in thermally processed samples was the second lowest of the processing types (14.66 mg 100 g^{-1} FW), a study reported no significant decreases in hesperetin (aglycone derivative) content of orange juice following thermal treatments (70 °C for 30 s or 90 °C for 1 min) but an increase in hesperetin (39.88%) content in high pressure treated orange juice (400 MPa/1 min/40 °C) compared to fresh samples (*32*). These are similar to the trends for some of the high-pressure treatments (450 MPa/1 and 3 min/20 °C) in the present study. However, the reason for decreased hesperidin content in HPP5 samples remains unclear.

The effects of processing on chlorogenic acid levels in smoothies differed substantially from those on the other polyphenolic compounds. Thermally processed smoothies had the highest levels of chlorogenic acid (3.55 mg 100 g^{-1} FW), which were significantly different ($p < 0.001$) from all of the processing treatments. HHP treated samples (450 MPa/5 min/25 °C) had levels of chlorogenic acid similar to those of fresh samples (2.66 and 2.81 mg 100 g^{-1} FW, respectively). Furthermore, high-pressure treatments with dwell times of 1 and 3 min had the lowest overall concentrations of chlorogenic acid. High levels of chlorogenic acid in thermally processed samples could be attributed to increased extractability of bioactive compounds. However, chlorogenic acid is generally regarded to be sensitive to heat. For example, chlorogenic acid content in potatoes was reduced by 46, 60, and 100% by heating after microwave cooking, boiling, and oven baking, respectively (*33*). Although the effects of HHP on the total phenolic content of fruits have been investigated (*13*, *18*), little or no literature data could be obtained on its effects on individual phenolic compounds by the authors of the present study.

Storage had a significant impact on the levels all of the polyphenolic compounds of the smoothies ($p < 0.001$), although the individual compounds were affected differently. Procyanidin B1 levels in smoothies were relatively stable throughout storage as was the case with processing effects. No effect on procyanidin B1 levels was observed between days 10, 20, and 30. However, procyanidin B1 levels were lower (overall mean = 15.50 μmol 100 g^{-1} DW) on day 1 than all of the proceeding days of the storage period ($p < 0.001$), with the biggest difference occurring between days 1 and 20, that is, 7.83%. However, the differences were small in practical terms. Other authors have reported that the storage stability of procyanidin appears to be time and temperature dependent, with complete degradation of procyanidin compounds being observed in some instances, that is, apple juice stored at 25 °C over 9 months (*34*). In the present study, smoothies were stored at chilled temperatures (4 °C) for a shorter period. Hawthorn drinks were shown to have a relatively stable procyanidin B2 content at chilled temperatures (4 °C) in line with data reported in this study, but significantly lower contents at

Table 2. Instrumental Color (ΔE), Sensory Acceptability, and Rheological Properties (G^*, Complex Modulus at a Frequency of 1 Hz) of Fruit Smoothies following Thermal ($P_{70} \geq 10$ min) and High Hydrostatic Pressure (HHP) Processing (450 MPa) over Storage at 4 °C

process	storage (days)	ΔE	sensory acceptability (0−6)	G^*, 1 Hz (kPa)
fresh	1	0.00 ± 0.00	4.21 ± 0.95	4.47 ± 0.59
	10	2.35 ± 1.02	4.11 ± 0.18	5.54 ± 0.98
	20	3.30 ± 0.65	3.47 ± 1.62	4.38 ± 0.68
	30	4.24 ± 0.26	1.84 ± 1.63	4.44 ± 1.22
thermal	1	1.74 ± 1.00	3.45 ± 1.49	4.66 ± 1.69
	10	2.42 ± 0.32	3.76 ± 1.42	3.57 ± 2.18
	20	3.26 ± 0.84	2.98 ± 1.68	2.95 ± 0.68
	30	3.84 ± 0.09	3.61 ± 1.26	3.21 ± 0.79
HHP (1 min)	1	4.22 ± 2.03	4.20 ± 0.90	5.79 ± 0.31
	10	4.67 ± 0.31	3.91 ± 1.61	5.02 ± 1.85
	20	7.87 ± 1.11	3.77 ± 1.41	4.88 ± 0.45
	30	6.69 ± 0.33	3.92 ± 1.12	3.05 ± 0.76
HHP (3 min)	1	4.14 ± 0.19	4.18 ± 1.29	5.49 ± 2.24
	10	4.20 ± 0.13	4.14 ± 1.29	5.99 ± 1.35
	20	7.15 ± 0.27	3.76 ± 1.33	3.23 ± 0.45
	30	6.50 ± 0.48	3.54 ± 1.32	2.50 ± 1.86
HHP (5 min)	1	3.24 ± 1.04	3.90 ± 1.43	5.80 ± 0.86
	10	8.41 ± 0.52	3.79 ± 1.24	4.40 ± 0.36
	20	8.51 ± 1.11	3.25 ± 1.43	3.78 ± 0.52
	30	6.84 ± 0.30	3.87 ± 1.07	2.32 ± 0.59
F[a] test process, p		<0.001	ns[c]	ns
LSD[b]		0.56	0.49	1.20
F test storage, p		<0.001	<0.01	<0.01
LSD		0.44	0.40	1.24

[a] Fischer test. [b] Least significant difference. [c] Not significant.

ambient (23 °C) and elevated (40 °C) storage temperatures over a 6 month period (*35*). During chilled storage, the most notable decreases in hesperidin content occurred in fresh and HHP treated (HHP1 and HHP3) samples. The rate of decline was initially low between days 1 and 10 (4.08, 2.47, and 2.82%, respectively), but this increased substantially ($p < 0.001$) from days 10 to 20 (8.41, 40.83, and 37.36%). In the case of HHP5 samples, hesperidin levels were low on day 1 but remained relatively stable throughout the storage period. A similar degradation ($p < 0.001$) pattern was observed in chlorogenic acid levels for fresh and some HHP treated samples between days 20 and 30 of the storage, with a >70% decrease in some HHP samples (HHP1). Similar trends were observed in a related study where an increased level of degradation of total phenolic and antioxidant contents between days 20 and 30 for high pressure treated fruit smoothies (*18*) could be attributed to residual enzyme activity. These "slow" and "fast" phase degradation trends are characteristic of biphasic degradation kinetics that are common in enzymatic degradation (*36*). In contrast, hesperidin and chlorogenic acid contents of thermal samples were relatively stable throughout the storage period with small fluctuations over 30 days. This is in line with previous studies that have reported the hesperidin content was relatively stable in orange juices stored for up to 6 months at ambient (18 °C) or higher temperatures (28 and 38 °C) (*17*). Conversely, *p*-coumaric acid derivatives in thermally processed smoothies decreased by 72.63% between days 1 and 10 (0−0.75 μmol 100 g^{-1} DW) and remained low over the remaining test days. Fresh samples were lowest on day 1 (3.52) and highest on day 10 (4.31 μmol 100 g^{-1} DW). For HHP treated samples, the levels fluctuated between days 1 and 20 with no pattern emerging until day 30, when significant ($p < 0.01$) decreases were observed in HHP1, HHP3, and HHP5 samples (47.19, 42.41, and 45.91%). These fluctuations could be attributed to the inherent variability within the smoothie mixture. However, *p*-coumaric acid derivatives have been shown to be susceptible to degradation over storage (0−5 days) in fresh-cut, skin-on Braeburn apple wedges (*6*).

Color Change (ΔE). Instrumental color of fresh and processed smoothies is presented in **Table 2**. In the present study these values are presented as ΔE, which represents the difference in a fresh smoothie that had not been stored. Significant differences ($p < 0.001$) were observed in the color change of the smoothies for both the type of process and the storage period (**Table 2**). Thermally processed smoothies had a lower ($p < 0.001$) color change (1.74) than HHP1, HHP3, or HHP5 samples (4.22, 4.14, and 3.24, respectively) compared to fresh controls. In general, this study showed that HHP processing led to higher rates of color change compared with fresh or thermal processing. In contrast, other studies have shown HHP treatments to have a positive impact on the color of many foods (*13*,*37*). It is generally accepted that increased color degradation is more associated with thermal processing because it increases the formation of degradation products, which result in color loss (*10*). The color loss observed for HHP treatments in this study could be due to the releasing bound cell constituents, such as PPO from the cell vacuole (*18*). Although similar disruption would be present in thermally processed samples, the applied heat treatment would inactivate any enzymes potentially released into the sample. As mentioned elsewhere, the applied HHP treatments (450 MPa/25 °C/1, 3, or 5 min) may have been inadequate to completely inactivate enzymes present. This is supported by correlation data ($r^2 = -0.50$) between chlorogenic acid content [a good substrate for PPO (*14*) and responsible for the formation of brown color melanins] and the degree of color change in this study, which

shows color change increased as chlorogenic acid content decreased. Color change for all treatments increased steadily over the storage period for both fresh and thermal treatments. This could also be attributed to oxidation (*38*), residual enzyme activity (*9*), or inadequate reduction of endogenous microorganisms (*8*). Although microbial spoilage can be responsible for color change, it seems unlikely in the context of this study as all smoothies remained within safe limits throughout the storage (data not shown). Incomplete enzyme inactivation would be the most obvious factor affecting color change. However, high-pressure treatments were less consistent. For example, data from **Table 2** show HHP1 samples (least severe of the HHP treatments) had the greatest color change over days 1−20, whereas thermal treatments (most invasive) experienced lower, more gradual changes. However, other work has demonstrated improved color stability can be achieved during storage with the application of HHP (*13*).

Sensory Analysis. Sensory acceptability scores for fresh and thermally and HHP processed fruit smoothies assessed after 1, 10, 20, and 30 days of storage are presented in **Table 2**. No significant effect was observed between processing treatments. Thermally processed smoothies had the lowest score (3.45) followed by HHP5 samples (3.90). Fresh and high pressure treated samples (HHP1 and HHP3) scored well with taste panelists (ca. 4.2). Other studies suggest HHP retains more fresh-like characteristics than traditional thermal processing (*2*, *13*). The sensory acceptability of smoothies was significantly affected by storage ($p < 0.01$). As expected, all samples received lower scores than day 1 (mean value = 3.99). However, a significant difference for acceptability was observed only between days 1 and 30. Most of the smoothie samples were deemed acceptable (> 3), with the exception of fresh samples on day 30, which were unacceptable (1.84). This could be attributed to a buildup of oxidative or enzymatic biproducts and an increased microbiological load that could lead to off-flavors developing within the nonprocessed controls, which is important for the extension of the storage life of products. In general, whereas sensory acceptability significantly decreased between days 1 and 30, the difference overall was small in practical terms (3.99 vs 3.36 mean scores).

Dynamic Oscillatory Measurements. Frequency sweeps were carried out on all smoothies. These properties were measured within the linear viscolelastic (LVE) region by amplitude sweeps at a frequency of 0.1 Hz. Parameters describing the LVE characteristics of the smoothies are the storage modulus (G', a measure of the deformation energy stored by the material), the loss modulus (G'', a measure of the deformation energy lost by the material), and the complex modulus (G'', related to the overall viscoelastic rigidity) corresponding to $(G'^2 + G''^2)^{1/2}$. All smoothies had higher storage moduli (G') than loss moduli (G''). This indicates that the smoothies had a solid, elastic-like behavior. The complex (G^*) modulus of smoothies (at a frequency of 1 Hz) is presented in **Table 2**. The complex modulus is a measure of the rigidity of the test material (*39*). Whereas no significant difference was observed for the type of process, a significant difference ($p < 0.01$) was observed at all data points of the frequency sweep in the rheological properties of the smoothies during storage (**Table 2**). Complex moduli of the smoothies decreased over the storage period, with day 30 shown to have the lowest values ($p < 0.001$). This may be attributed to the various smoothie constituents settling out due to gravity over time. A visual assessment of the smoothies revealed distinct layers forming after day 10. However, all smoothies were vigorously shaken prior to testing to ensure a homogeneous sample.

In conclusion, the effect of HHP processing and storage on individual polyphenol compounds and some quality attributes of fruit smoothies were compared against fresh and thermal (mild conventional pasteurization) products. Although HHP processing requires significant capital investment, the perceived improvements in product quality may justify the extra investment and may offer potential benefits to the beverage industry. However, levels of phenolic compounds in all fruit smoothie samples were lower than expected. This view is based on literature data which suggest that higher levels of the compounds should be present as well as a broader range of compounds than reported here given the individual components of the smoothie, that is, apple, strawberry, banana, and orange. Despite literature evidence to the contrary, no significant improvement in color or bioactive stability was observed for HHP treated samples over their traditional thermally processed counterparts. Both processing and storage affected color variables as expected. In fact, deleterious effects on color appeared more pronounced in high pressure treated samples stored for 30 days compared to thermally treated samples. This may be due to a number of factors including the possibility that enzymatic degradation systems were not inactivated by the HHP conditions applied and matrix effects may have further contributed to polyphenol degradation and color change during storage. Whereas enzymes within the thermally processed smoothies would have been sufficiently inactivated, heat-sensitive phenolic compounds may have been degraded, implying no net gain for either processing methodology. There were less pronounced affects for some of the other quality attributes with rheological properties unaffected by the type of processing (although some product settling occurred), and smoothies had a good sensory acceptability (> 3) overall. Further optimization of the applied processing conditions is required to ensure complete inactivation of enzymes and to increase color and polyphenolic stability during storage.

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