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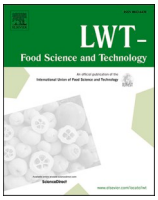

Impact of blanching and high hydrostatic pressure combined treatments on the physico-chemical and microbiological properties and bioactive-compound profile of an industrial strawberry smoothie

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ABSTRACT

Enhancing the shelf life of fruit and vegetable products is an important research field in the food industry. Smoothies are an alternative to filtered juices and highly processed beverages. High-pressure processing (HPP) has recently gained increasing attention as a non-thermal technology to reduce microbiological load while preserving juice quality-related properties. Herein, the combination of blanching and HPP to treat strawberry smoothies at industrial scale was compared with conventional thermally pasteurized (TP) and untreated (NT) smoothies. Analysis focused on physicochemical (pH, °Brix, colour, and viscosity) and microbiological properties, and bioactive compound contents. Additionally, phenolic compounds were analysed by HPLC-ESI-TOF-MS, vitamin C content was analysed by HPLC-UV-Vis, and antioxidant activity was determined by DPPH and ABTS assays. HPP samples treated at 600 MPa for 3 min at 4 °C showed increased viscosity and a significant increase in phenolic compounds (particularly *p*-coumaroyl hexose) related to TP and untreated samples (26.8 and 17.6%, respectively) and maintained colour stability compared to untreated samples. Anthocyanidins retention was better in HPP- than in TP- treated samples. Vitamin C content increased significantly by 15% in HPP-treated samples, contributing to enhanced antioxidant potential (12%), as shown by the minimal microbiological load observed, in comparison with untreated samples. These findings suggest that HPP is an effective alternative to TP for improving the overall quality of strawberry smoothies.

1. Introduction

Strawberries (*Fragaria x ananassa*) are globally renowned as one of the most popular fruits because of their appealing colour, delightful flavour, and abundance in a range of bioactive compounds. Strawberries are consumed both fresh and as various processed products, such as juices and jams (Buvé et al., 2018). In 2021, a total of 9.18 million metric tons of strawberries were produced globally, which made them the most produced red fruit in the world (Statista, 2022).

Fruits and vegetables are important products for human health, as their consumption is associated with reduced incidence of chronic diseases such as obesity, diabetes, cancer, and heart diseases (Andrés, Villanueva, & Tenorio, 2016; Picouet et al., 2016). Further, consumers around the world have an increasing preference for processed food products with high nutritional value, similar to that of their fresh counterparts, with the natural flavour, health-related properties, and a prolonged shelf-life, but without additives (Andrés et al., 2016; Yordanov & Angelova, 2010). In recent years, fruit-based smoothies have gained popularity worldwide and are currently a major segment of the global soft-drink market (Picouet et al., 2016). The main problem with manufacturing ready-to-eat fruits and vegetables is their short shelf life span. Heat treatments can extend shelf life but negatively affect their nutritional and organoleptic characteristics, as well as their physico-chemical properties (Wu et al., 2021).

Strawberries are a remarkable reservoir of nutritional compounds. Particularly, they are one of the fruits that are richest in ascorbic acid

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content (Derossi, De Pilli, & Fiore, 2010). However, the susceptibility of strawberry smoothies to temperature variations during transportation and storage poses a challenge that may potentially and significantly compromise its shelf life. Therefore, different methods have been tested for their efficacy to help the end product retain its original nutritional quality and bioactive contents, thereby improving shelf life. Thus, several alternative technologies to conventional thermal treatment have been tested, including ultrasound (Tiwari, O';Donnell, Patras, & Cullen, 2008; Yildiz & Aadil, 2020) and high-intensity ultrasound (Wang, Wang, Ye, Vanga, & Raghavan, 2019), high-intensity pulsed electric fields (Aguiló-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009, 2010), or their application in mixed systems such as strawberry-kale (Stübler et al., 2020) or strawberry-juçara-banana (de Oliveira Ribeiro et al., 2018).

In particular, high-pressure processing (HPP) has emerged as a promising alternative to conventional heat preservation methods, demonstrating effective microbial inactivation while preserving sensory quality related characteristics (Huang, Ye, & Chen, 2013). Research evidence has indicated that HPP can be a viable alternative technology for inactivating various harmful pathogenic and spoilage microbes without adversely impacting product quality and nutritional attributes. However, the main limitation of HPP is its ability to inactivate the endogenous oxidative enzyme-activities present in fruit tissues. Hence, if enzyme inactivation is desired in pressure-pasteurized products, a mild thermal treatment may be given prior to pressure pasteurization (Balasubramaniam, Martínez-Monteagudo, & Gupta, 2015). For example, Terefe et al. showed that the extent of inactivation of polyphenol oxidase (PPO) and peroxidase (POD) by HPP treatment varied between 15%–38% and 20%–33%, respectively, depending on cultivar. Whereas, thermal processing (TP) reportedly results in almost complete inactivation of these enzymes (Terefe et al., 2013). In turn, Sulaiman et al. showed that HPP at 600 MPa for 15 min was equivalent to treating strawberry puree thermally at 65 °C during 15 min, in which case both treatments resulted in a 15% inactivation of PPO activity (Sulaiman, Farid, & Silva, 2017). Other enzymes, such as polygalacturonase (PG) and lipoxygenase (LOX), are relatively more pressure sensitive and can be substantially inactivated by HPP (Nawawi et al., 2023). Previously, some authors reported that the highest inactivation of peroxidases, pectinesterases and polygalacturonases was recorded when a higher pressure (600 MPa) was used (Marszałek, Woźniak, & Skąpska, 2016). The enzyme inactivation is important to produce fruits-mix smoothies. The process required two steps: the preparation of the fruits puree which is normally thermal treated to be stabilized and stored for a long period before use it for the second step which involve the mixing of the purees following by an additional thermal treatment.

Liu et al. (2016) studied the effects of HPP, with and without blanching, on the quality attributes of mango pulp. The results showed that the total phenol content and antioxidant activity significantly increased in mango pulp after blanching, whereas the changes in L-ascorbic acid, carotenoids, sugars, and colour were not significant. Given its long-term shelf life, blanching + HPP was considered a promising alternative to HPP to preserve the quality of mango pulp, thereby eliminating the possible effects produced by the residual activity of endogenous enzymes.

To our knowledge, the effect of the HPP process with blanching on the strawberry smoothies/puree has not been evaluated before. Therefore, the objective of this work was to study the impact of the HPP on strawberry smoothies preliminary processed by blanching (70 °C for 2 min) and then treated with HPP at 600 MPa for 3 min at 20 °C and to compare it with conventional thermal treatment smoothies and untreated samples. Specifically, some of the most significant physicochemical characteristics of the smoothies were measured, including their colour, viscosity, pH, °Brix, and titratable acidity. Besides, it was evaluated the phenolic compounds and anthocyanidins content by HPLC-ESI-TOF-MS in negative and positive ionization mode, the antioxidant activity by DPPH and ABTS, and the total vitamin C and its oxidation rate by HPLC-UV. In addition, the microbial count was also carried out.

2. Materials and methods

2.1. Chemicals and samples

High-performance liquid chromatography, HPLC-grade water and other reagents and solvents were purchased from Merck KGaA (Darmstadt, Germany).

Strawberry puree was obtained after the blanching phase (70 °C for 2 min) and immediately frozen at −18 °C. The samples were then thawed, packaged in 250-ml polyethylene terephthalate pouches, and sealed. The bags were refrigerated until high-pressure processing (HPP) was performed. Untreated (NT) and post-pasteurization phase (heat-treated TP) samples were used to compare the effects of HPP treatment on the end product. After processing, all samples were freeze-dried prior to analysis. Freeze-drying was performed on strawberry smoothies by using a Lyovapor L-300 (Buchi, Milano, Italia). The samples were put in a tray, with a thickness of 2 cm. The temperature of the condenser was set to −104 °C and the pressure of the chamber was 0.1 mbar. The product reached a final temperature of about 20 °C at the end of the drying process. The drying time to reduce moisture content to equilibrium was 120 h.

2.2. Thermal processing (TP)

Samples were pasteurized at Dodaco s.r.l. company (Scafati, Naples, Italy). Samples were heated at 100 °C for 1 min in a shell and tube heat-exchange with a mass flow rate of 1.6 kg/s. In the same way, samples were cooled at 35 °C for 1 min.

2.3. High pressure processing (HPP)

The HPP treatment was performed using an HPP equipment (Hiperbaric 420, Burgos, Spain) at the Masseria Fruttirossi Srl company located in Taranto. The samples were then placed in specific vessels. Water at 4 °C was used as a pressure-transmitting medium. A pressure level of 600 MPa was applied for 3 min (holding time) to the smoothie samples.

2.4. Physico-chemical determinations

2.4.1. Colour

Sample colour was determined using a colorimeter (Minolta Chroma Meter, CR 300, Japan), with the light source set on D65 and an observation angle of 10 °C. The instrument was calibrated using a plate (L* = 96.64; a* = 0.23; b* = 1.85), and Hunter parameters were measured for each sample: L* (from 0 = black to 100 = white), a* (-a* = greenness to + a* = redness), and b* (-b* = blueness to + b* = yellowness). The procedure reported by Guazi, Lago-Vanzela, and Conti-Silva (2019) was followed. Five readings were recorded for each sample.

2.4.2. Viscosity

Sample viscosity was measured using a stress-controlled rheometer (HAAKE MARS 40 Rheometer, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a parallel plate (80 mm). Steady shear tests were performed at 25 °C by increasing the shear rate from 1 to 100 s^{-1} (Ribeiro et al., 2019).

2.4.3. pH, total soluble solids (TSS) and titratable acidity (TA)

These determinations were performed according to the methodologies proposed by the AOAC (1984). For pH measurement, 10 ml of the smoothies was analysed using a pH meter (pH 8 Series-Vers.1.1_04/2015-Eutech Instruments Pte Ltd. Ayer Rajah Crescent, Singapore). A few drops of the smoothie were used to measure the

TSS content with a digital refractometer (Atago PR32- Palette, Tokyo, Japan). Three measurements were performed for each sample. Lastly, 5 mL of each smoothie was used to measure the TA by titration with 0.01 N NaOH to an endpoint at pH 8.1 (AOAC, 1984) and expressed as g of citric acid•L^{-1}.

2.5. Determination of phenolic compounds

Freeze-dried (Lyovapor L-300, Buchi) strawberry smoothie (0.2 g) was submitted to triple extraction during 15 min with ethanol: water 80/20 (v/v), in a 1:30 sample: solvent (w/v) ratio in an ultrasonic bath (Bandelin, Sonorex, RK52, Berlin, Germany) at a frequency of 35 kHz (Razola-Díaz, Gómez-Caravaca et al., 2022). The extractions were conducted in duplicate for all samples. Subsequently, the samples underwent centrifugation for 10 min at 8603×*g*, and the resulting supernatants were evaporated (Buchi R-205 rotavapor) at a maximum temperature of 40 °C to prevent the degradation of phenolic compounds. The last step involved reconstituting the samples with 1 mL of methanol/water 1:1 (v/v) and filtering them through a 0.2 μm nylon syringe filter. The prepared samples were then stored in a freezer at −32 °C until analysis. Phenolic and anthocyanidins composition analysis via HPLC-ESI-TOF-MS.

Phenolic compounds were analysed using an ACQUITY Ultra Performance LC system (Waters Corporation, Milford, MA, USA) coupled to an electrospray ionization (ESI) source operating in negative or positive mode and a time-of-flight (TOF) mass detector (Waters Corporation, Milford, MA, USA). The target compounds were separated using an ACQUITY UPLC BEH Shield RP18 column (1.7 μm, 2.1 mm × 100 mm; Waters Corporation, Milford, MA, USA) at a temperature of 40 °C. A gradient previously described (Verni et al., 2020), using water with 1% acetic acid as mobile phase A and acetonitrile as mobile phase B was used. Data analysis was performed using the MassLynx 4.1 software (Waters Corporation, Milford, MA, USA). Phenolic compounds were identified by comparing the *m/z* and predicted molecular formulas with those reported in previous studies. For quantification, standard curves of chlorogenic acid, vanillic acid, ferulic acid, rutin, quercetin, catechin and cyanidin-chloride were obtained, and the results were expressed as μg/g d.w (dry weight). Standard curve construction involved using the peak areas of each standard measured by HPLC at varying concentrations. Each extract for each sample was injected two times (n = 6).

2.6. Vitamin C content by HPLC-UV-Vis

Ascorbic acid (AA) extraction was conducted according to the procedure reported by Razola-Díaz, del, Guerra-Hernández, García-Villanova, and Verardo (2022). Briefly, each smoothie sample (0.5 mL) was combined with a 10% metaphosphoric acid solution (2.5 mL) and diluted to a final volume of 25 mL in a glass volumetric flask with demineralised water. After homogenisation and centrifugation, the supernatant was filtered through 0.20 μm Millex filters. The resulting samples were injected into the HPLC system.

For total vitamin C determination, the reduction of dehydroascorbic acid (DHAA) to ascorbic acid (AA) was necessary. Briefly, the reducing agent dithiothreitol (DTT) was added to the filtered sample for AA analysis. After a 30-min dark incubation period at 20 °C, reduction was stopped with phosphoric acid and the samples were injected into the HPLC system.

The DHAA content was calculated as the difference between vitamin C levels after DHAA reduction and the initial AA levels before reduction. Both AA and vitamin C determinations were conducted in triplicate, and the results were expressed as μg AA/mL in the smoothie.

The HPLC system used a 325 ultraviolet detector model (Varian Prostar, California, USA), and samples were introduced through an automatic injector with a sample loop (20 μL). Separations occurred on a Gemini 5 μm C18 Phenomenex column (150 × 4.6 mm) under isocratic conditions, using demineralised water acidified with sulfuric acid to pH 2.2 as the mobile phase at a flow rate of 0.6 mL/min, with detection at a wavelength of 245 nm. Each sample was extracted 3 times.

2.7. Determination of antioxidant activity

The antioxidant capacity was evaluated by two different methods. The ABTS assay was developed according to Re et al. (1999). For each extract, it was added 1 mL of this ABTS monocation $^{\bullet+}$ solution to 0.01 mL of extract and it was measured the detriment of absorbance during 10 min at 734 nm. A Trolox calibration curve (1, 10, 20, 50, 80, 100, 150, 200 μg/g) was used as a reference to express the results as μg/g of Trolox equivalent (TE).

DPPH radical scavenging activity was assayed with a method proposed by Parejo, Codina, Petrakis, & Kefalas, 2000. Then, 100 μL of each extract was added of 2.9 mL of DPPH• solution (100 μM), and after rapid stirring, the bleaching power of the extract was observed in a time interval from 0 to 30 min at 517 nm. The results were compared with a standard curve of Trolox in methanol/water (4:1, v/v) (1, 10, 20, 50, 80, 100, 150, 200 μg/g). Results are expressed as μg Trolox equivalents (TE)/g d.w.

2.8. Microbiological characterization

Samples were serially decimal diluted and aliquoted in plates of suitable media for each microbial group researched: plates of Plate count Agar (PCA) for the enumeration of psychotropic bacteria incubated at 20 °C for 3 days; Violet Red Bile Glucose agar (VRBGA) incubated at 37 °C for 48 h to count Enterobacteriaceae; Dichloran Rose-Bengal Cholramphenicol agar (DRBC) incubated at 28 °C for 4 days to count yeasts and moulds; Tryptone Bile X-glucuronide agar (TBX) incubated at 44 °C for 48 h for *E. coli*. To research *Clostridium* species, the samples were heated at 80 °C for 15 min and then plated in Reinforced Clostridial Agar (RC Agar) and incubated under anaerobiosis at 37 °C for 72 h. The results were expressed and in CFU/ml.

2.9. Statistical analysis

These tests were repeated thrice. Analysis of variance (ANOVA) and Tukey's test were performed to determine significant differences among samples with STATISTICA 7.0 (OK, USA). Pearson's correlation analysis was performed to identify significant associations between the various parameters under study using SPSS, 23.0 (IL, USA).

3. Results and discussion

3.1. Physico-chemical analysis

The physicochemical characteristics of juices and smoothies largely determine consumer acceptance and are used as markers of possible fraud and polyphenol content. Viscosity, colour, pH, °Brix, and TA were evaluated for all the treated and untreated strawberry smoothies.

The viscosity of strawberry smoothies as a function of shear rate is shown in Fig. 1.

Strawberry smoothies showed a pseudoplastic behaviour. Samples differed in viscosity, with HPP-treated samples having the highest viscosity, whereas heat-treated (TP) ones showed the lowest. These results are in agreement with Lacey et al. (2023) who reported that, when strawberry nectar was treated with HPP at 600 MPa, it showed a significant 52.1% increase in viscosity compared to the TP treatment. Such significant increase in the viscosity of the HPP-treated samples is likely due to the inactivation of pectin methylesterase (Karacam, Sahin, & Oztop, 2015). A characteristic property of pectin is its ability to form a gel in the presence of Ca^{2+} ions, sugars, or acids. Fruit smoothies are a blend of carbohydrates and proteins whose interactions can influence the viscosity of the end product and, ultimately, the texture and flavour of the smoothies prepared from such blend (Xu, 2023).

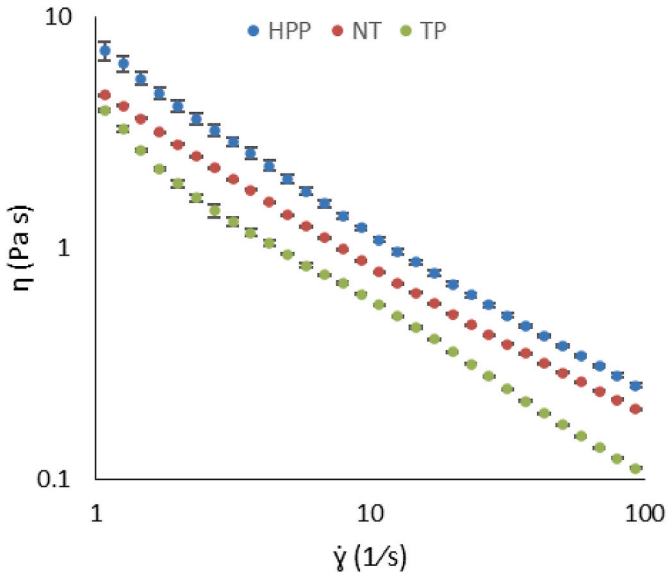

Fig. 1. Viscosity curves of strawberry smoothies.

In addition, a reduction of viscosity is expected during storage (Cao et al., 2012); thus, the slight improvement (20%) in viscosity achieved upon HPP, compared to TP treatment, will allow for a longer lasting stability of the smoothie and hence, product acceptance.

However, a 12.40% decrease in viscosity in HPP-treated sample compared to untreated strawberry juice was previously reported (Cao, Liu, Wu, Liao, & Hu, 2014). In our study, such reduction in viscosity was observed for TP-treated smoothie samples and is presumably explained by the fact that at higher temperatures, the thermal energy of the molecules increases with increasing intermolecular distances, causing a reduction in intermolecular forces and consequently a decrease in viscosity (Ortega Quintana, Salcedo Galván, Arrieta Rivero, & Torres Gallo, 2015).

The colour properties and appearance of the strawberry smoothie samples are shown in Table 1. Heat treatment (TP) significantly ($p < 0.05$) affected the colour of the strawberry smoothies, in particular the a* parameter (Table 1). When comparing the chromatic a* parameter of TP-treated with that of untreated, NT, and HPP-treated smoothies, a significant ($p < 0.05$) decrease of approximately 50% was observed. Other researchers have reported that TP leads to an increment of the a* parameter due to thermal treatment (Lacey et al., 2023), causing the visual colour to change from red to brown. Non-enzymatic browning following TP treatment can result from several reactions, including Maillard's condensation, caramelization, and destruction of pigments (Ibarz, Pagán, & Garza, 2000). In contrast, HPP treatment did not affect colour and no differences were observed between NT and HPP-treated smoothies for any colour parameter. These findings were in agreement with those reported by Patras et al. who reported minor colour changes between HPP-treated (400, 500 and 600 MPa/10–30 °C/15 min) and untreated strawberry and blackberry purees, thereby better retaining redness, with higher a* values than those of TP-treated samples (Patras, Brunton, Da Pieve, & Butler, 2009). Conversely, higher values for L*, a*, and b* of HPP-treated (600 MPa/50 °C/15 min) strawberry purees compared to untreated ones have been reported (Marszałek et al., 2016). Nonetheless, this similarity or even increase in colour parameter values might be due to the mechanical extraction of anthocyanins and other pigments from tissues after HPP treatment (Nawawi et al., 2023). In fact, a positive correlation was reported between anthocyanidin content and a* values for strawberry juices (Wang, Zhang, & Wu, 2015).

Table 1
Impact of HPP treatment on pH, TSS (°Brix), TA%, and colour of strawberry smoothies.

Sample	pH	°Brix	TA%	L*	a*	b*
HPP	3.57 ± 0.03 [b]	9.0 ± 0.3 [a]	0.777 ± 0.009 [a]	29.6 ± 0.5 [a]	25.5 ± 0.3 [a]	13.1 ± 0.8 [a]
TP	3.633 ± 0.005 [a]	8.83 ± 0.05 [a]	0.687 ± 0.007 [b]	29.4 ± 0.1 [a]	12.51 ± 0.05 [b]	12.6 ± 0.2 [a]
NT	3.60 ± 0.01 [ab]	9.0 ± 0.1 [a]	0.75 ± 0.02 [a]	28.18 ± 0.03 [b]	25.4 ± 0.1 [a]	12.4 ± 0.1 [a]

Different letters (a,b) in the same column indicate significant differences ($p < 0.05$).

The pH, °Brix, and TA% values for all strawberry smoothies under study are shown in Table 1. The pH values of treated and untreated smoothies ranged from 3.57 to 3.63. Further, TP-treated samples showed a slightly higher pH than HPP-treated samples neither of them significantly ($p < 0.05$) different from untreated, NT, samples. Furthermore, no differences were found among samples for °Brix, with values ranging between 8.8 and 9.1. Meanwhile, TA% of the smoothies ranged between 0.68 and 0.78, with TP having a significantly lower TA % than either HPP-treated or NT samples. The reduction in pH and subsequentially the increase in TA% for HPP smoothies, could be affected by the imposed pressure of HPP on the food matrix. Indeed, depending on the nature of the food product, the pressure could cause an ionization of small molecules, such as H_2O and weak acids, which in turn increase the concentration of H+ ions (Kaushik, Kaur, Rao, & Mishra, 2014; Ravichandran, Jayachandran, Kothakota, Pandiselvam, & Balasubramaniam, 2023). All corresponding values were in the ranges previously reported strawberry smoothies. For example, Yildiz et al. reported values of 7.83–8.00 °Brix, 0.79%–0.84% titratable acidity, and pH values of 3.45–3.50 for strawberry juices (Yildiz, Pokhrel, Unluturk, & Barbosa-Cánovas, 2021a).

3.2. Identification and quantification of phenolic compounds by HPLC-ESI-TOF-MS

Polyphenol composition of strawberry smoothies was determined using HPLC-ESI-TOF-MS, and the identified compounds are listed in Table 2. A total of 18 compounds were identified, of which three were identified as hydroxycinnamic acids derivatives, three as chalcone derivatives, seven as flavonols derivatives, and five as phenolic precursors.

Previously, Aaby et al. described the hydroxycinnamic acid derivative *p*-coumaroyl hexose with *m/z* 325 (Aaby, Ekeberg, & Skrede, 2007). Consistently, here, coumaric acid was detected at 5.17 min. In turn, 3-*O*-caffeoylquinic acid was detected as corresponding to peak number 2 with the predicted formula $C_{16}H_{18}O_9$, in agreement with Mattila et al., who found a similar compound (5-*O*-Caffeoylquinic acid) in strawberries (Mattila & Kumpulainen, 2002).

Three flavonoids were detected in our study, which were categorized as chalcone derivatives, the first of which was identified as dihydrochalcone phloridzin, corresponding to peak number 9, at 9.24 min with *m/z* 435, and first described in strawberries by Hilt et al. (Hilt et al., 2003). Another dihydrochalcone, named phloretin, was detected with predicted molecular formula $C_{15}H_{14}O_5$ and *m/z* 273. Additionally, a phloretin derivative, phloretin 2′-xyloglucoside, was also observed at 8.76 min with *m/z* 567 and its *m/z* in source fragment 273. Both compounds detected were in agreement with Nakhate et al., who reviewed some of the studies in which these compounds were extracted from strawberry sources (Nakhate et al., 2022).

As for the remaining flavonoids, they were identified as two quercetin derivatives named quercetin 3-*O*-beta-D-glucofuranoside (peak 5) and quercetin-3-glucuronide (peak 10), the flavonols kaempferol (peak 13) and four derivatives named kaempferol-3-glucoside (peak 11), kaempferol-7-glucuronide (peak 12), kaempferol 3-*O*-beta-(2″-acetyl) galactopyranoside (peak 16), and kaempferol-3-coumaroylglucoside (peak 18), all previously described from strawberry sources by Aaby et al. (2007).

In addition, some phenolic precursors were detected in the

Table 2
Phenolic compounds identified in the strawberry smoothies by HPLC-ESI-TOF-MS in negative mode.

Peak	Time (min)	*m/z* experimental	*m/z* calculated	Error (ppm)	Score (%)	Molecular formula $[M-H]^-$	*m/z* in source fragments	Compound	Ref.
1	3.860	325.0909	325.0923	−4.3	100	$C_{15}H_{17}O_8$	163.0368	*p*-coumaroyl hexose	Aaby et al. (2007)
2	4.406	353.0876	353.0873	0.8	100	$C_{16}H_{17}O_9$	–	3-*O*-Caffeoylquinic acid	Mattila and Kumpulainen (2002)
3	5.169	163.0390	163.0395	−3.1	100	$C_9H_7O_3$	–	Coumaric acid	
4	8.403	371.1331	371.1342	−3	100	$C_{17}H_{23}O_9$	209.0822	Syringin isomer a	Sun et al. (2020)
5	8.718	463.0862	463.0877	−3.2	91.94	$C_{21}H_{19}O_{12}$	300.026; 273.0811	Quercetin 3-*O*-beta-D-glucofuranoside	Aaby et al. (2007)
6	8.755	567.1753	567.1714	5.9	94.69	$C_{26}H_{31}O_{14}$	273.077	Phloretin 2′-xyloglucoside	Nakhate et al. (2022)
7	8.934	371.1368	371.1342	7	100	$C_{17}H_{23}O_9$	209.0822	Syringin isomer b	Sun et al. (2020)
8	9.227	273.0771	273.0763	2.9	100	$C_{15}H_{13}O_5$	167.0317; 217.0013	Phloretin	Nakhate et al. (2022)
9	9.242	435.1297	435.1291	1.4	100	$C_{21}H_{23}O_{10}$	273.0773	Phloridzin	Hilt et al. (2003)
10	9.361	477.063	477.0651	4.4	90.23	$C_{21}H_{17}O_{13}$	301.032	Quercetin-3-glucuronide	Aaby et al. (2007)
11	10.088	447.0906	447.0927	−4.7	94.14	$C_{21}H_{19}O_{11}$	285.0434	Kaempferol-3-glucoside	Aaby et al. (2007)
12	10.208	461.0731	461.072	2.4	92.86	$C_{21}H_{17}O_{12}$	285.0424	Kaempferol-7-glucuronide	Aaby et al. (2007)
13	10.244	285.0405	285.0399	2.1	100	$C_{15}H_9O_6$	–	Kaempferol	Aaby et al. (2007)
14	10.873	371.1335	371.1342	−1.9	100	$C_{17}H_{23}O_9$	209.083	Syringin isomer c	Sun et al. (2020)
15	11.383	385.1498	385.1499	−0.3	100	$C_{18}H_{25}O_9$	223.0975	Methylsyringin isomer a	(Antunes et al., 2019; Sun et al., 2020)
16	11.524	489.1046	489.1033	2.7	596.21	$C_{23}H_{21}O_{12}$	284.033; 255.0325; 227.0317	Kaempferol 3-*O*-beta-(2″-acetyl)galactopyranoside	Aaby et al. (2007)
17	13.268	385.1490	385.1499	−2.3	100	$C_{18}H_{25}O_9$	223.0961	Methylsyringin isomer b	(Antunes et al., 2019; Sun et al., 2020)
18	13.554	593.1318	593.1295	3.9	94.3	$C_{30}H_{25}O_{13}$	285.0417; 255.0307; 227.0365	Kaempferol-3-coumaroylglucoside	Aaby et al. (2007)

strawberry smoothies under analysis herein. Specifically, three isomers of syringin (a, b, and c) were identified with *m/z* 371 and molecular formula $C_{17}H_{24}O_9$ (peak number 4, 7, and 14, respectively), as previously reported for strawberries by Sun et al. (Sun et al., 2020). Syringin reportedly has potential health benefits, including anti-inflammatory (Zhang et al., 2020), hypoglycaemic (H. S. Niu, Liu, Cheng, Lin, & Hsu, 2008), antiadipogenic (Hossin, Inafuku, Takara, Nugara, & Oku, 2021), and cardioprotective effects (Yao et al., 2021). Moreover, syringin is reportedly metabolised to bioactive sinapyl alcohol and (+)-syringaresinol in rat intestines (Yang et al., 2015). Additionally, two methylsyringin derivatives, named isomers a and b, were detected at 11.38 and 13.27 min, respectively, as previously reported for strawberries by Antunes et al. (2019).

The quantified phenolic acid and precursors, and the differences among treatments, are summarised in Table 3. The sum of phenolic compounds increased significantly ($p < 0.05$) for the HPP-treated smoothie samples, whereas it decreased significantly in the TP-treated ones, compared to NT samples. Phenolic acids accounted for 75%, 67%, and 77% of the total phenolic compounds in NT, TP-, and HPP-treated smoothies, respectively. This increase in the HPP-treated samples was due to an increase in *p*-coumaroyl hexose content. In contrast, TP treatment may have caused the degradation of *p*-coumaroyl hexose to coumaric acid, a compound whose content increased significantly ($p < 0.05$) in TP compared to that in NT and HPP-treated samples. Total flavonoid content in both TP and HPP treatments was significantly higher than that in the NT group. Additionally, phloretin and phloridzin levels increased significantly when HPP was used, and decreased with TP. Further, significant increases in quercetin 3-*O*-beta-D-glucofuranoside, quercetin-3-glucuronide, and kaempferol-3-glucoside contents were observed under both treatments, but the increase was higher for the TP treatment. As for the remaining flavonoids, no significant differences or changes were observed between the TP and NT groups. In contrast, the HPP treatment caused a significant increase in kaempferol, kaempferol 3-*O*-beta-(2″-acetyl) galactopyranoside, and kaempferol-3-coumaroylglucoside.

Thus, an overall increase of 17.6% and 26.8% in total phenolic compounds was observed in HPP-treated smoothie samples relative to that in untreated and TP-treated samples, respectively. According to a Donda Zbinden, Schmidt, Vignatti, Pirovani, and Böhm (2024) HPP is able to influence the cell structure favouring the release of bioactive compounds. In fact, the high pressure disrupts the cell walls increasing the release of the phenolic compounds (Salazar-Orbea et al., 2023). These hypotheses has been corroborated by other authors; Similarly, Patras et al. reported an increase of 8%–10% in the total phenols in HPP-treated strawberry and blackberry juice samples compared to untreated controls (Patras et al., 2009). In turn, Yildiz et al. reported an increase of 4%–5% in total phenolic compounds in strawberry juices treated with HPP compared to NT samples (Yildiz et al., 2021a). Lastly, Cao et al. reported that HPP treatment of cloudy strawberry juice resulted in a significant increase in phenol content compared to NT samples (Cao et al., 2014).

The phenolic precursors syringin and methylsyringin were reduced significantly by 9.1% and 4.7%, respectively, in TP-treated, compared to NT samples, and increased significantly by 22% and 17.7% in HPP-treated smoothie samples compared to NT ones.

After HPP processing, the samples showed increased levels of phenolic components derived from strawberries compared to the NT samples. This increase was attributed to the enhanced solubilisation of specific phenolic constituents or their precursors, a phenomenon linked to the ability of HPP to increase cell permeability by deprotonating charged groups and disrupting salt bridges and hydrophobic bonds within cell membranes. These effects are believed to contribute to enhanced matrix disruption and extractability of phenolic compounds from the matrix (Stübler et al., 2020).

3.3. Identification and quantification of anthocyanidins by HPLC-ESI-TOF-MS

Considering the potential content of anthocyanins and/or anthocyanidins, these were also analysed by HPLC-ESI-TOF-MS in the positive mode, and the identified compounds are listed in Table 4. Previously, several authors have identified pelargonidin (Marszałek, Mitek, & Skąpska, 2015; Stübler et al., 2020; Terefe et al., 2013; Zabetakis, Leclerc, & Kajda, 2000), cyanidin (Marszałek et al., 2015; Stübler et al.,

Table 3
Phenolic compounds and precursors quantified in the strawberry smoothies expressed in μg/g d.w. with the average and the standard deviation.

Compound	NT	TP	HPP
p-coumaroyl hexose	583.32 ± 31.51b	238.22 ± 15.18c	722.82 ± 22.15a
3-*O*-Caffeoylquinic acid	75.63 ± 3.49a	73.22 ± 3.05a	76.03 ± 3.50a
Coumaric acid	< LOD	156.54 ± 4.27a	< LOD
Syringin isomer a	229.63 ± 20.56a	175.08 ± 11.63b	253.39 ± 14.91a
Quercetin 3-*O*-beta-D-glucofuranoside	5.77 ± 0.01c	7.19 ± 0.11a	6.14 ± 0.15b
Phloretin 2′-xyloglucoside	18.18 ± 0.84a	17.60 ± 0.73a	18.27 ± 0.84a
Syringin isomer b	54.32 ± 10.67b	55.45 ± 7.64b	80.14 ± 7.12a
Phloretin	32.40 ± 0.54b	30.57 ± 0.47c	34.46 ± 0.78a
Phloridzin	27.38 ± 0.18b	24.84 ± 0.09c	30.01 ± 0.27a
Quercetin-3-glucuronide	18.68 ± 0.66b	22.17 ± 1.01a	20.40 ± 0.67a
Kaempferol-3-glucoside	47.00 ± 0.77c	71.71 ± 1.01a	50.58 ± 0.11b
Kaempferol-7-glucuronide	9.05 ± 0.12a	8.22 ± 0.03b	8.98 ± 0.00a
Kaempferol	8.36 ± 0.01b	7.87 ± 0.05c	8.86 ± 0.01a
Syringin isomer c	< LOD	27.57 ± 5.52a	13.03 ± 5.67b
Methylsyringin isomer a	1032.66 ± 38.82b	863.11 ± 23.07c	1194.75 ± 22.11a
Kaempferol 3-*O*-beta-(2″-acetyl) galactopyranoside	24.24 ± 0.75b	11.87 ± 0.05c	29.00 ± 0.38a
Methylsyringin isomer b	446.65 ± 17.74b	546.80 ± 10.61a	546.70 ± 10.44a
Kaempferol-3-coumaroylglucoside	20.17 ± 0.68b	21.80 ± 0.51b	30.74 ± 0.91a
Sum of phenolic compounds	870.18 ± 47.84b	691.82 ± 29.54c	1036.29 ± 39.65a
Sum of phenolic acids	658.95 ± 39.47b	467.98 ± 22.50c	798.85 ± 31.58a
Sum of flavonoids	211.22 ± 3.78c	223.84 ± 3.02b	237.44 ± 3.45a
Sum of phenolic precursors	1763.25 ± 87.80b	1640.44 ± 52.95b	2074.97 ± 54.58a

Different letters in the same line (a-c) indicate significant differences ($p < 0.05$).

2020; Terefe et al., 2013), and delphidin (Taghavi, Patel, Akande, & Galam, 2022) in aglycone or glycoside forms in strawberry samples. Therefore, these were also quantified here, and the results are shown in Fig. 2.

The major compounds among the three anthocyanidins varied depending on solvent, pH, and sample type. In this case, they were extracted from freeze-dried smoothie samples with ethanol-water at pH 2.6, which allowed obtaining an anthocyanidin profile that agreed well with a previous report (Taghavi et al., 2022). Cyanidin was detected as the major anthocyanidin, accounting for 61% in NT and HPP-treated smoothie samples and 80% in TP-treated ones. In turn, pelargonidin was the second major compound in NT and HPP-treated smoothies at 25% and 26%, respectively. However, it was reduced to 1% of the total anthocyanidin content in TP-treated samples. Also, minor amounts of delphinidin were observed, with 12% in NT and HPP-, and 17% in TP-treated samples. In general, although the extent of reduction in anthocyanidin content was significant ($p < 0.05$) under both treatments, the lesser reduction was recorded for the HPP treatment (32.5%), which thereby maintained an anthocyanidin profile more similar to that of NT smoothies, whereas anthocyanidins were significantly reduced in the TP-treated smoothies by more than 55%.

During TP treatment, anthocyanidins undergo degradation through non-enzymatic oxidation and covalent bond cleavage following a consistent pathway. This involves various transformations, such as hydrolysis of sugar moieties and formation of aglycones, ring-opening leading to the creation of a chalcone, and subsequent breakdown into carboxylic acids and carboxyaldehydes under acidic conditions. The stability of anthocyanins is predominantly influenced by their chemical structure, with enhanced stability observed in cases of higher glycosylation, acylation, and methoxylation (Nawawi et al., 2023). Despite being a non-thermal technology, HPP also causes the breakdown of anthocyanins, although to a much lesser extent. However, anthocyanin degradation might be due to insufficient enzyme inactivation and high residual enzyme activity after HPP treatment (Nawawi et al., 2023). Further, previously reported results of HPP use in strawberry juice are controversial. Thus, for example, Terefe et al. observed a significant reduction in total anthocyanin content of strawberry samples both upon TP (22–25%) and HPP (20–28%) treatment, compared to NT samples, with no significant difference between TP- and HPP-treated ones (Terefe et al., 2013). Similarly, Marszalek et al. reported anthocyanins losses of 43% and 14% when treating strawberry puree with TP and HPP, respectively, compared to untreated puree (Marszałek et al., 2015, 2017). Consistently, Zabetakis et al. observed a lower reduction in anthocyanins when strawberry juice was treated with HPP at 800 MPa compared to lower treatment intensities or sterilisation (Zabetakis et al., 2000). In contrast, Patras et al. reported no significant differences in anthocyanins between pressure-treated and unprocessed strawberry and blackberry purées ($p < 0.05$), whereas conventional TP significantly reduced these levels (Patras et al., 2009). Similarly, Stübler et al. did not report significant differences in anthocyanin content between HPP and NT strawberry smoothies (Stübler et al., 2020). Conversely, Yildiz et al.

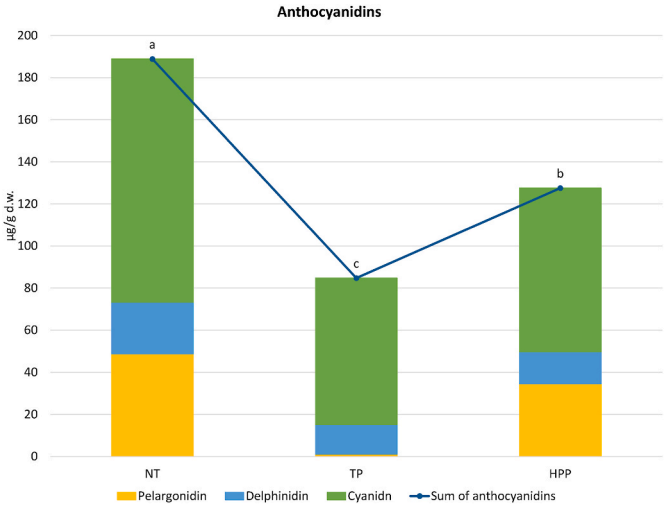

Fig. 2. Anthocyanidin content of the analysed strawberry smoothies with different treatments. Different letters (a–c) indicate significant differences ($p < 0.05$).

Table 4
Anthocyanidins detected in strawberry smoothies by HPLC-ESI-TOF-MS.

N	Time (min)	*m/z* experimental	*m/z* calculated	Error (ppm)	Score (%)	Molecular formula $[M]^+$	Compound
1	4.78	271.0619	271.0606	4.8	100	$C_{15}H_{11}O_5$	Pelargonidin
2	9.331	303.0493	303.0505	−4	100	$C_{15}H_{11}O_7$	Delphinidin
3	10.848	287.0561	287.0556	1.7	100	$C_{15}H_{11}O_6$	Cyanidin

reported a 15%–17% increase of anthocyanin content in strawberry juices treated for 1 min by HPP at 300 MPa, compared to untreated samples (Yildiz et al., 2021a; Yildiz, Pokhrel, Unluturk, & Barbosa-Cánovas, 2021b).

3.4. Vitamin C content of strawberry smoothies by HPLC-UV-Vis

Vitamin C was also analysed, and the results are illustrated in Fig. 3, which shows the AA and DHAA contents of strawberry smoothies treated with TP or HPP and those in NT smoothies. Total AA content was within the range (40–70 mg/100 g) previously reported (Nawawi et al., 2023). According to Derossi et al., strawberry juices can show wide variability in AA concentrations, ranging from 48.78 to 87.86 mg/100 g due to differences in degree of ripening, handling, and thermal treatment (Derossi et al., 2010). In turn, DHAA was 13%, 28%, and 10% of the total vitamin C content in NT, TP- and HPP-treated smoothies, respectively, in agreement to Marszalek et al., who reported DHAA contents <55% of the total vitamin C content in strawberry purees (Marszałek et al., 2015). The DHAA percentage between NT and HPP samples is quite similar according to other authors that reported as HPP has a minimal impact on covalent bonds because of that is unable to directly damage small molecules, such as vitamin C (Niu et al., 2022). Furthermore, a significant reduction of 9.3% in DHAA content was observed in HPP-treated smoothies compared to NT samples. Previously, no increase or decrease in DHAA content was observed in strawberry samples treated with HPP at 600 MPa (Hurtado et al., 2017; Nawawi et al., 2023). In our study, the observed reduction in DHAA, which is the reversible oxidation product of AA, was better for smoothie shelf-life and its vitamin C content. Meanwhile, the total vitamin C content increased significantly ($p < 0.05$) in HPP-treated samples compared to the controls or TP-treated samples. This finding is in agreement with previous reports in which, in comparison to TP-treated samples, all HPP-treated strawberry juices had significantly higher AA levels (Nawawi et al., 2023).

In addition, the AA/DHAA ratio increased from 6.9 in NT to 9.2 in HPP-treated samples. In contrast, Marszalek et al. reported a reduction in the AA/DHAA ratio from 2.3 in NT to 1 in HPP-treated strawberry puree. Moreover, they observed that HPP at 600 MPa reduced AA content by more than 50% (Marszałek et al., 2016). In contrast, Patras et al. reported that different pressure treatments did not significantly change AA content of strawberry or blackberry purees, and found a significant reduction of 21% in AA upon TP treatment, compared to NT and between 5% and 10% upon HPP treatment (Patras et al., 2009). Consistently, Cao et al. reported a decrease between 7.8% and 12.6% in the AA content in HPP strawberry juices compared to that in untreated samples. They reported that the reduction in AA was smaller in cloudy juices compared to that in clear juices, because of the protection provided by higher antioxidative compound content in the former case (Cao et al., 2014). Smoothies considered "cloudy" juices likely do not experience a significant reduction in vitamin C owing to the presence of whole fruit in them.

In our study, vitamin C content decreased significantly by 54% in TP-treated smoothies in comparison to NT samples. Similarly, Marszalek et al. reported that TP treatment reduced the vitamin C content of strawberry puree by 62% compared to that in NT samples (Marszałek et al., 2015). The stability of AA in strawberry purees and fruit smoothies, including those made from strawberries, poses a significant challenge for preservation. The inherently low stability of AA during storage makes its degradation a major concern. Notably, the impact on AA degradation becomes particularly evident within the temperature range of 40–60 °C, reaching a maximum effect at 60–80 °C (Nawawi et al., 2023). Specifically, the pathway for degradation of AA involves a reaction with hydroxyl radicals, resulting in the formation of oxidation products (Tiwari, O'Donnell, Patras, & Cullen, 2008).

3.5. Antioxidant activity of strawberry smoothies using DPPH and ABTS assays

The antioxidant activities of untreated, and HPP- and TP-treated smoothies were analysed using the 2,2-diphenyl-1-picrylhydrazyl, (DPPH), and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The results are shown in Fig. 4.

The antioxidant activities in NT smoothies were 15175 ± 56 and 1498 ± 39 μg TE/g d.w. for DPPH and ABTS, respectively. Meanwhile, a significant ($p < 0.05$) reduction of 11.1% and 64.6% in the antioxidant activities as per DPPH and ABTS assays were observed, respectively, when treated with TP. In contrast, with HPP treatment, a non-significant increase of 4.3% and a significant increase of 13.2% were recorded during the DPPH and ABTS assays, respectively, compared to the controls. This finding implies that the antioxidant activity of the strawberry smoothies was not only preserved but resulted in a better release of antioxidant compounds upon HPP treatment than that promoted by TP treatment. Some authors have reported significantly higher antioxidant activity in strawberry juices and purees treated with HPP at different pressures (120–550 MPa) compared to that observed upon TP treatment (Nawawi et al., 2023). Thus, for example, Stübler et al. reported a significant increase in the antioxidant activity of strawberry smoothies treated with HPP compared to untreated samples (Stübler et al., 2020).

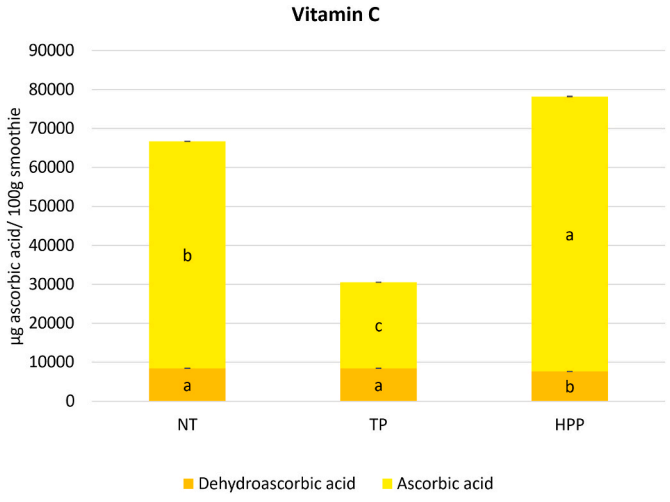

Fig. 3. Ascorbic and dehydroascorbic acid of the strawberry smoothies. Different letters (a–c) indicate significant differences ($p < 0.05$).

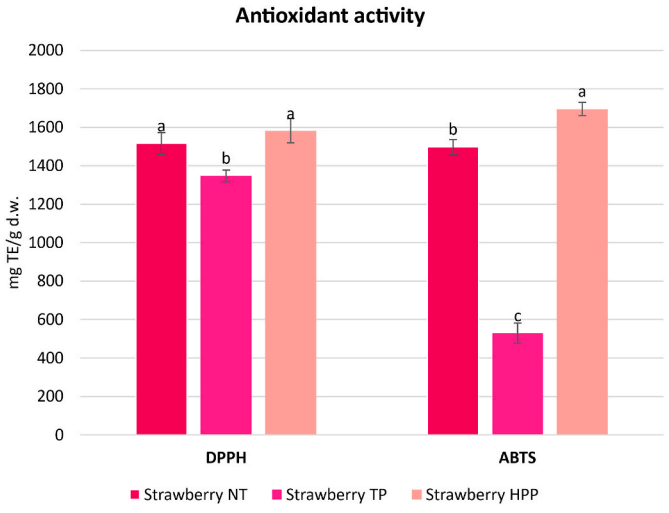

Fig. 4. Antioxidant activity of strawberry smoothies analysed by DPPH and ABTS assays. Different letters (a–c) indicate significant differences ($p < 0.05$).

Similarly, Yildiz et al. observed an 18%–19% increase in the radical scavenging activity of HPP-treated strawberry juices compared with NT ones (Yildiz et al., 2021a). In turn, Patras et al. reported significantly higher antioxidant activity in HPP-treated strawberry and blackberry purées than in TP-treated samples (Patras et al., 2009). In contrast, Sulaiman et al. reported that HPP and ultrasound processing technologies caused a significant decreases in the antioxidant activity content, but smaller than that observed in TP-treated strawberry puree samples, compared to NT controls (Sulaiman et al., 2017). Furthermore, Terefe et al. observed significant decreases in antioxidant activity measured by ORAC and FRAP during processing and storage of both HPP- and TP-treated strawberry juices compared to NT samples, with no statistically significant difference between HPP and TP treatments in terms of FRAP retention (Terefe et al., 2013).

Pearson's correlation coefficients (Supplementary Fig. 1) revealed a significant ($p < 0.001$) positive correlation between the antioxidant activity measured by the DPPH and ABTS assays and the sum of phenolic compounds (r = 0.9602 and 0.9320, respectively), with the sum of phenolic acids (r = 0.9636 and 0.9547, respectively) being the main compounds responsible *p*-coumaroyl hexose (r = 0.9508 and 0.9914, respectively). Other phenolic compounds, such as phloretin, phloridzin, kaempferol, and kaempferol 3-*O*-beta-(2″-acetyl)galactopyranoside, showed a significant ($p < 0.001$) positive correlation with antioxidant activity. In addition, total vitamin C exhibited a strong positive correlation ($p < 0.001$) with antioxidant activities as per the DPPH and ABTS assays, with r values of 0.9185 and 0.9951, respectively, which was mainly attributable to AA content (r = 0.9185 and 0.9940, respectively). At a significance level of $p < 0.01$, also phenolic precursors showed a significant strong correlation with the antioxidant activity by DPPH (r = 0.8823) and ABTS (r = 0.8015) assays. Furthermore, anthocyanidins showed positive but non-significant ($p > 0.05$) correlation with DPPH (r = 0.5738) and ABTS (r = 0.7061) assays. Consistently, Cao et al. recorded significant positive correlations of the antioxidant activity measured by DPPH and FRAP with anthocyanidin and phenolic contents but negative with AA content in strawberry juice (Cao et al., 2014).

3.6. Antimicrobial activity

After processing, the counts of all microbial populations searched were below detection limit (1 CFU/ml) for all samples. However, after 30 days of storage, the untreated samples were not analysed because the packs exploded, probably owing to high contamination levels with gas-producing Clostridia. As for the HPP- and TP-treated samples, the counts of all populations searched were also below the detection limit (1 CFU/ml). Furthermore, HPP treatment stabilized the product compared to the untreated packs. Thus, effective antimicrobial activity of the HPP treatment was confirmed. Previously, TP treatment was reported to inactivate *Escherichia coli* O157:H7, yeast, and moulds in strawberry puree. However, pasteurising above 55 °C reduces the anthocyanidin and antioxidant contents, and increases the browning index >10% (Hsu, Huang, & Wu, 2014). Furthermore, Cao et al. previously reported that HPP at 600 MPa/4 min/ambient temperature inactivated total aerobic bacteria, coliform bacteria, yeasts, and moulds in strawberry juice (Cao et al., 2014). Similarly, Hurtado et al. reported that HPP at 350 MPa/10 °C/5 min showed slightly lower efficacy in microbial inactivation (mesophilic and psychrophilic bacteria, coliforms, yeasts, and moulds) than TP processing at 85 °C/7 min, in smoothies stored at 4 °C for up to 28 days (Hurtado et al., 2017). In turn, Aaby et al. observed that following processing, TP- and HPP-treated strawberry purees exhibited total aerobic bacteria levels of 2.2 and 2.2–2.7 log CFU/g, respectively, with an absence of yeast and mould (Aaby, Grimsbo, Hovda, & Rode, 2018). Consistently, Yildiz et al. reported a reduction of 5-log CFU/mL of *Escherichia coli* and 2-log CFU/mL in total aerobic bacteria and yeast mould counts in strawberry juice with HPP at 300 MPa for 1 min (Yildiz, Pokhrel, Unluturk, & Barbosa-Cánovas, 2019), while Vallejo et al. reported minimum bactericide concentrations (MBC) between 750 and 950 g/mL against *Salmonella typhimurium* and *Listeria monocytogenes* (Vallejo, Minahk, Rollán, & Rodríguez-Vaquero, 2021).

4. Conclusions

The efficacy of the combination of blanching and high-pressure processing (HPP) at industrial scale was evaluated as an alternative treatment for strawberry smoothies' quality preservation and it was compared with traditional thermal processed (TP) and untreated samples. Besides the HPP coupled with blanching has widely been used for juices, the effects on smoothies have scarcely been investigated. All samples were analysed, and several physicochemical characteristics were determined, including viscosity, colour stability, chemical characteristics, phenolic compounds and anthocyanidins by HPLC-ESI-TOF-MS, vitamin C contents, antioxidant activity, and microbiological load. To our knowledge, it is the first time that all these parameters have been evaluated at the same time for strawberry smoothies. HPP-treated samples exhibited higher viscosity than TP and untreated samples, maintained colour stability respect to untreated samples, and increased levels of phenolic compounds, particularly *p*-coumaroyl hexose, compared to TP and untreated samples. Anthocyanidins retention was also better in HPP- than in TP-treated samples, and vitamin C content increased significantly compared to untreated samples contributing to the enhancement of antioxidant potential, whereas in TP samples a decrease was observed. Moreover, also the ratio AA/DHAA was enhanced by HPP treatment confirming a low oxidation rate of vitamin C compared to the thermal treatment. HPP effectively inactivated microbes, demonstrating its potential as a preservation method for strawberry smoothies. Overall, HPP has emerged as a promising technology, offering significant advantages over conventional TP processing for preserving colour and bioactive compounds, while effectively preventing quality loss due to microbial activity in strawberry smoothies.

However, it is important to acknowledge some limitations of the study, including the lack of sensory evaluation, consumer acceptability testing, and long-term stability assessments, which could provide further insights into the practical implications of adopting HPP technology for strawberry smoothie processing. Additionally, the study focused solely on strawberry smoothies, and extrapolation of the findings to other fruit-based beverages may require further investigation.

CRediT authorship contribution statement

María del Carmen Razola-Díaz: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Stefania Volpe:** Writing – review & editing, Methodology, Investigation. **Ana M. Gómez-Caravaca:** Writing – review & editing, Methodology. **Elena Torrieri:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Vito Verardo:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Elena Torrieri reports financial support was provided by European Commission. Vito Verardo reports financial support was provided by European Commission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116612.

References

Aaby, K., Ekeberg, D., & Skrede, G. (2007). Characterization of phenolic compounds in strawberry (*Fragaria x ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. *Journal of Agricultural and Food Chemistry, 55*(11), 4395–4406. https://doi.org/10.1021/jf0702592

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 Chemistry, 260*, 115–123. https://doi.org/10.1016/J.FOODCHEM.2018.03.100

Aguiló-Aguayo, I., Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. *LWT - Food Science and Technology, 42*(4), 813–818. https://doi.org/10.1016/j.lwt.2008.11.008

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2010). High-intensity pulsed electric fields processing parameters affecting polyphenoloxidase activity of strawberry juice. *Journal of Food Science, 75*(7). https://doi.org/10.1111/j.1750-3841.2010.01735.x

Andrés, V., Villanueva, M. J., & Tenorio, M. D. (2016). The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry, 192*, 328–335. https://doi.org/10.1016/j.foodchem.2015.07.031

Antunes, A. C. N., Acunha, T., dos, S., Perin, E. C., Rombaldi, C. V., Galli, V., et al. (2019). Untargeted metabolomics of strawberry (*Fragaria x ananassa 'Camarosa'*) fruit from plants grown under osmotic stress conditions. *Journal of the Science of Food and Agriculture, 99*(15), 6973–6980. https://doi.org/10.1002/jsfa.9986

AOAC. (1984). *Official methods of analysis*. Association of Official Analytical Chemist.

Balasubramaniam, V. M. B., Martínez-Monteagudo, S. I., & Gupta, R. (2015). Principles and application of high pressure-based technologies in the food industry. *Annual Review of Food Science and Technology, 6*, 435–462. https://doi.org/10.1146/annurev-food-022814-015539

Buvé, C., Kebede, B. T., De Batselier, C., Carrillo, C., Pham, H. T. T., Hendrickx, M., et al. (2018). Kinetics of colour changes in pasteurised strawberry juice during storage. *Journal of Food Engineering, 216*, 42–51. https://doi.org/10.1016/J.JFOODENG.2017.08.002

Cao, X., Bi, X., Huang, W., Wu, J., Hu, X., & Liao, X. (2012). Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innovative Food Science and Emerging Technologies, 16*, 181–190. https://doi.org/10.1016/J.IFSET.2012.05.008

Cao, X., Liu, F., Wu, J., Liao, X., & Hu, X. (2014). Effects of high hydrostatic pressure combined with blanching on microorganisms and quality attributes of cloudy and clear strawberry juices. *International Journal of Food Properties, 17*(9), 1900–1920. https://doi.org/10.1080/10942912.2013.766887

de Oliveira Ribeiro, L., Almeida, A. C. S., de Carvalho, C. W. P., Borguini, R. G., Ferreira, J. C. S., Freitas, S. P., et al. (2018). Effect of processing on bioactive compounds, physicochemical and rheological characteristics of juçara, banana and strawberry smoothie. *Plant Foods for Human Nutrition, 73*(3), 222–227. https://doi.org/10.1007/s11130-018-0681-2

Derossi, A., De Pilli, T., & Fiore, A. G. (2010). Vitamin C kinetic degradation of strawberry juice stored under non-isothermal conditions. *LWT-Food Science & Technology, 43*(4), 590–595. https://doi.org/10.1016/J.LWT.2009.10.006

Donda Zbinden, M., Schmidt, M., Vignatti, C. I., Pirovani, M.É., & Böhm, V. (2024). High-pressure processing of fruit smoothies enriched with dietary fiber from carrot discards: Effects on the contents and bioaccessibilities of carotenoids and vitamin E †. *Molecules, 29*(6). https://doi.org/10.3390/molecules29061259

Guazi, J. S., Lago-Vanzela, E. S., & Conti-Silva, A. C. (2019). Development of smoothies from dehydrated products of strawberry and banana pulps obtained through foam-mat drying. *International Journal of Food Science and Technology, 54*(1), 54–61. https://doi.org/10.1111/ijfs.13900

Hilt, P., Schieber, A., Yildirim, C., Arnold, G., Klaiber, I., Conrad, J., et al. (2003). Detection of phloridzin in strawberries (*Fragaria x ananassa Duch.*) by HPLC-PDA-MS/MS and NMR spectroscopy. *Journal of Agricultural and Food Chemistry, 51*(10), 2896–2899. https://doi.org/10.1021/JF021115K

Hossin, A. Y., Inafuku, M., Takara, K., Nugara, R. N., & Oku, H. (2021). Syringin: A phenylpropanoid glycoside compound in Cirsium brevicaule A. GRAY root modulates adipogenesis. *Molecules, 26*(6). https://doi.org/10.3390/MOLECULES26061531

Hsu, H. Y., Huang, L., & Wu, J. S. B. (2014). Thermal inactivation of Escherichia coli O157: H7 in strawberry puree and its effect on anthocyanins and color. *Journal of Food Science, 79*(1). https://doi.org/10.1111/1750-3841.12327

Huang, Y., Ye, M., & Chen, H. (2013). Inactivation of Escherichia coli O157: H7 and Salmonella spp. in strawberry puree by high hydrostatic pressure with/without subsequent frozen storage. *International Journal of Food Microbiology, 160*(3), 337–343. https://doi.org/10.1016/j.ijfoodmicro.2012.11.008

Hurtado, A., Guàrdia, M. D., Picouet, P., Jofré, A., Ros, J. M., & Bañón, S. (2017). Stabilization of red fruit-based smoothies by high-pressure processing. Part A. Effects on microbial growth, enzyme activity, antioxidant capacity and physical stability. *Journal of the Science of Food and Agriculture, 97*(3), 770–776. https://doi.org/10.1002/JSFA.7796

Ibarz, A., Pagán, J., & Garza, S. (2000). Kinetic models of non-enzymatic browning in apple puree. *Journal of the Science of Food and Agriculture, 80*(8), 1162–1168. https://doi.org/10.1002/1097-0010(200006)80:8<1162::AID-JSFA613>3.0.CO;2-Z

Karacam, C. H., Sahin, S., & Oztop, M. H. (2015). Effect of high pressure homogenization (microfluidization) on the quality of Ottoman Strawberry (*F. Ananassa*) juice. *Lebensmittel-Wissenschaft und -Technologie, 64*(2), 932–937. https://doi.org/10.1016/J.LWT.2015.06.064

Kaushik, N., Kaur, B. P., Rao, P. S., & Mishra, H. N. (2014). Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (*Mangifera indica cv. Amrapali*). *Innovative Food Science and Emerging Technologies, 22*, 40–50. https://doi.org/10.1016/j.ifset.2013.12.011

Lacey, K. L., Moreno-Barreto, A., Pavón-Vargas, D., Cattani, L., Rinaldi, M., Rainieri, S., et al. (2023). A quality assessment of strawberry nectar stabilized by thermal and high-pressure processing conditions. *Journal of Food Processing and Preservation, 2023*, 1–13. https://doi.org/10.1155/2023/5481142

Liu, S., Xu, Q., Li, X., Wang, Y., Zhu, J., Ning, C., et al. (2016). Effects of high hydrostatic pressure on physicochemical properties, enzymes activity, and antioxidant capacities of anthocyanins extracts of wild *Lonicera caerulea* berry. *Innovative Food Science and Emerging Technologies, 36*, 48–58. https://doi.org/10.1016/j.ifset.2016.06.001

Marszałek, K., Mitek, M., & Skąpska, S. (2015). The effect of thermal pasteurization and high pressure processing at cold and mild temperatures on the chemical composition, microbial and enzyme activity in strawberry purée. *Innovative Food Science and Emerging Technologies, 27*, 48–56. https://doi.org/10.1016/J.IFSET.2014.10.009

Marszałek, K., Woźniak, Ł., & Skąpska, S. (2016). The application of high pressure–mild temperature processing for prolonging the shelf-life of strawberry purée. *High Pressure Research, 36*(2), 220–234. https://doi.org/10.1080/08957959.2016.1172072

Marszałek, K., Woźniak, Ł., Skąpska, S., & Mitek, M. (2017). High pressure processing and thermal pasteurization of strawberry purée: Quality parameters and shelf life evaluation during cold storage. *Journal of Food Science and Technology, 54*(3), 832–841. https://doi.org/10.1007/S13197-017-2529-4

Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food Chemistry, 50*(13), 3660–3667. https://doi.org/10.1021/jf020028p

Nakhate, K. T., Badwaik, H., Choudhary, R., Sakure, K., Agrawal, Y. O., Sharma, C., et al. (2022). Therapeutic potential and pharmaceutical development of a multitargeted flavonoid phloretin. *Nutrients, 14*(17), 3638. https://doi.org/10.3390/NU14173638, 2022, Vol. 14, Page 3638.

Nawawi, N. I. M., Ijod, G., Senevirathna, S. S. J., Aadil, R. M., Yusof, N. L., Yusoff, M. M., et al. (2023). Comparison of high pressure and thermal pasteurization on the quality parameters of strawberry products: A review. *Food Science and Biotechnology, 32*(6), 729–747. https://doi.org/10.1007/s10068-023-01276-3

Niu, H. S., Liu, I. M., Cheng, J. T., Lin, C. L., & Hsu, F. L. (2008). Hypoglycemic effect of syringin from Eleutherococcus senticosus in streptozotocin-induced diabetic rats. *Planta Medica, 74*(2), 109–113. https://doi.org/10.1055/S-2008-1034275

Niu, H., Yuan, L., Zhou, H., Yun, Y., Li, J., Tian, J., et al. (2022). Comparison of the effects of high pressure processing, pasteurization and high temperature short time on the physicochemical attributes, nutritional quality, aroma profile and sensory characteristics of passion fruit purée. *Foods, 11*(5), 632. https://doi.org/10.3390/foods11050632

Ortega Quintana, F. A., Salcedo Galván, E., Arrieta Rivero, R., & Torres Gallo, R. (2015). Efecto de la temperatura y concentración sobre las propiedades reológicas de la pulpa de mango variedad Tommy Atkins. *Revista ION, 28*(2), 79–92. https://doi.org/10.18273/REVION.V28N2-2015007

Parejo, I., Codina, C., Petrakis, C., & Kefalas, P. (2000). Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH(2,2-diphenyl-1-picrylhydrazyl) free radical assay. *Journal of Pharmacological and Toxicological Methods, 44*, 507–512.

Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science and Emerging Technologies, 10*(3), 308–313. https://doi.org/10.1016/J.IFSET.2008.12.004

Picouet, P. A., Hurtado, A., Jofré, A., Bañon, S., Ros, J. M., & Guàrdia, M. D. (2016). Effects of thermal and high-pressure treatments on the microbiological, nutritional and sensory quality of a multi-fruit smoothie. *Food and Bioprocess Technology, 9*(7), 1219–1232. https://doi.org/10.1007/s11947-016-1705-2

Ravichandran, C., Jayachandran, L. E., Kothakota, A., Pandiselvam, R., & Balasubramaniam, V. M. (2023). Influence of high pressure pasteurization on nutritional, functional and rheological characteristics of fruit and vegetable juices and purees-an updated review. *Food Control, 146*(May 2022), Article 109516. https://doi.org/10.1016/j.foodcont.2022.109516

Razola-Díaz, M., del, C., Guerra-Hernández, E. J., García-Villanova, B., & Verardo, V. (2022b). New insight on phenolic composition and evaluation of the vitamin C and

nutritional value of smoothies sold on the Spanish market. *Molecules, 27*(23), 8229. https://doi.org/10.3390/molecules27238229

Razola-Díaz, M. del C., Gómez-Caravaca, A. M., López de Andrés, J., Voltes-Martínez, A., Zamora, A., Pérez-Molina, G. M., et al. (2022a). Evaluation of phenolic compounds and pigments content in yellow bell pepper wastes. *Antioxidants, 11*(3), 557. https://doi.org/10.3390/antiox11030557

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Ribeiro, L. O., Brígida, A. I. S., Sá, D. D. G. C. F., Carvalho, C. W. P., Silva, J. P. L., Matta, V. M., et al. (2019). Effect of sonication on the quality attributes of juçara, banana and strawberry smoothie. *Journal of Food Science and Technology, 56*(12), 5531–5537. https://doi.org/10.1007/s13197-019-03998-x

Salazar-Orbea, G. L., García-Villalba, R., Bernal, M. J., Hernández, A., Tomás-Barberán, F. A., & Sánchez-Siles, L. M. (2023). Stability of phenolic compounds in apple and strawberry: Effect of different processing techniques in industrial set up. *Food Chemistry, 401*(February 2022). https://doi.org/10.1016/j.foodchem.2022.134099

Statista. (n.d.). The Statistics Portal for Market Data, Market Research and Market Studies. Retrieved May 4, 2022, from https://www.statista.com/.

Stübler, A. S., Lesmes, U., Juadjur, A., Heinz, V., Rauh, C., Shpigelman, A., et al. (2020). Impact of pilot-scale processing (thermal, PEF, HPP) on the stability and bioaccessibility of polyphenols and proteins in mixed protein- and polyphenol-rich juice systems. *Innovative Food Science and Emerging Technologies, 64*. https://doi.org/10.1016/J.IFSET.2020.102426

Sulaiman, A., Farid, M., & Silva, F. V. M. (2017). Strawberry puree processed by thermal, high pressure, or power ultrasound: Process energy requirements and quality modeling during storage. *Food Science and Technology International, 23*(4), 293–309. https://doi.org/10.1177/1082013216685485

Sun, J., Janisiewicz, W. J., Takeda, F., Evans, B., Jurick, W. M., Zhang, M., et al. (2020). Effect of nighttime UV-C irradiation of strawberry plants on phenolic content of fruit: Targeted and non-targeted metabolomic analysis. *Journal of Berry Research, 10*(3), 365–380. https://doi.org/10.3233/JBR-190482

Taghavi, T., Patel, H., Akande, O. E., & Galam, D. C. A. (2022). Total anthocyanin content of strawberry and the profile changes by extraction methods and sample processing. *Foods, 11*(8). https://doi.org/10.3390/foods11081072

Terefe, N. S., Kleintschek, T., Gamage, T., Fanning, K. J., Netzel, G., Versteeg, C., et al. (2013). Comparative effects of thermal and high pressure processing on phenolic phytochemicals in different strawberry cultivars. *Innovative Food Science and Emerging Technologies, 19*, 57–65. https://doi.org/10.1016/J.IFSET.2013.05.003

Tiwari, B. K., O'Donnell, C. P., Patras, A., & Cullen, P. J. (2008). Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. *Journal of Agricultural and Food Chemistry, 56*(21), 10071–10077. https://doi.org/10.1021/jf801824v

Vallejo, C. V., Minahk, C. J., Rollán, G. C., & Rodríguez-Vaquero, M. J. (2021). Inactivation of Listeria monocytogenes and Salmonella Typhimurium in strawberry juice enriched with strawberry polyphenols. *Journal of the Science of Food and Agriculture, 101*(2), 441–448. https://doi.org/10.1002/JSFA.10653

Verni, M., Pontonio, E., Krona, A., Jacob, S., Pinto, D., Rinaldi, F., et al. (2020). Bioprocessing of brewers' spent grain enhances its antioxidant activity: Characterization of phenolic compounds and bioactive peptides. *Frontiers in Microbiology, 11*(July), 1–15. https://doi.org/10.3389/fmicb.2020.01831

Wang, J., Wang, J., Ye, J., Vanga, S. K., & Raghavan, V. (2019). Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control, 96*, 128–136. https://doi.org/10.1016/J.FOODCONT.2018.09.007

Wang, Z., Zhang, M., & Wu, Q. (2015). Effects of temperature, pH, and sunlight exposure on the color stability of strawberry juice during processing and storage. *Lebensmittel-Wissenschaft und -Technologie, 60*(2), 1174–1178. https://doi.org/10.1016/J.LWT.2014.09.015

Wu, W., Xiao, G., Yu, Y., Xu, Y., Wu, J., Peng, J., et al. (2021). Effects of high pressure and thermal processing on quality properties and volatile compounds of pineapple fruit juice. *Food Control, 130*(June). https://doi.org/10.1016/j.foodcont.2021.108293

Xu, F. (2023). The role of pectin in the food industry. *Enhancing texture and shelf life, 7*(5), 2022–2023. https://doi.org/10.35841/2591-796X-7.5.197

Yang, B., Fan, Z., Zhou, L., Zhu, J. P., Yang, T., Qiu, M. S., et al. (2015). In vitro metabolic transformation of syringin by rat intestinal flora. *Chinese Traditional and Herbal Drugs, 46*(9), 1333–1337. https://doi.org/10.7501/J.ISSN.0253-2670.2015.09.014

Yao, J., Li, Y., Jin, Y., Chen, Y., Tian, L., & He, W. (2021). Synergistic cardioptotection by tilianin and syringin in diabetic cardiomyopathy involves interaction of TLR4/NF-κB/NLRP3 and PGC1a/SIRT3 pathways. *International Immunopharmacology, 96*. https://doi.org/10.1016/J.INTIMP.2021.107728

Yildiz, G., & Aadil, R. M. (2020). Comparison of high temperature-short time and sonication on selected parameters of strawberry juice during room temperature storage. *Journal of Food Science and Technology, 57*(4), 1462–1468. https://doi.org/10.1007/S13197-019-04181-Y

Yildiz, S., Pokhrel, P. R., Unluturk, S., & Barbosa-Cánovas, G. V. (2019). Identification of equivalent processing conditions for pasteurization of strawberry juice by high pressure, ultrasound, and pulsed electric fields processing. *Innovative Food Science and Emerging Technologies, 57*. https://doi.org/10.1016/J.IFSET.2019.102195

Yildiz, S., Pokhrel, P. R., Unluturk, S., & Barbosa-Cánovas, G. V. (2021a). Shelf life extension of strawberry juice by equivalent ultrasound, high pressure, and pulsed electric fields processes. *Food Research International, 140*. https://doi.org/10.1016/J.FOODRES.2020.110040

Yildiz, S., Pokhrel, P. R., Unluturk, S., & Barbosa-Cánovas, G. V. (2021b). Changes in quality characteristics of strawberry juice after equivalent high pressure, ultrasound, and pulsed electric fields processes. *Food Engineering Reviews, 13*(3), 601–612. https://doi.org/10.1007/s12393-020-09250-z

Yordanov, D. G., & Angelova, G. V. (2010). High pressure processing for foods preserving. *Biotechnology & Biotechnological Equipment, 24*(3), 1940–1945. https://doi.org/10.2478/V10133-010-0057-8

Zabetakis, I., Leclerc, D., & Kajda, P. (2000). The effect of high hydrostatic pressure on the strawberry anthocyanins. *Journal of Agricultural and Food Chemistry, 48*(7), 2749–2754. https://doi.org/10.1021/JF9911085

Zhang, H., Gu, H., Jia, Q., Zhao, Y., Li, H., Shen, S., et al. (2020). Syringin protects against colitis by ameliorating inflammation. *Archives of Biochemistry and Biophysics, 680*. https://doi.org/10.1016/J.ABB.2019.108242