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Shelf life extension of strawberry juice by equivalent ultrasound, high pressure, and pulsed electric fields processes

Semanur Yildiz [a,*], Prashant Raj Pokhrel [b], Sevcan Unluturk [c], Gustavo V. Barbosa-Cánovas [b]

[a] Department of Food Engineering, Sakarya University, Serdivan, 54050 Sakarya, Turkey
[b] Center for Nonthermal Processing of Food, Biological Systems Engineering Department, Washington State University, Pullman, WA 99164–6120, USA
[c] Department of Food Engineering, Izmir Institute of Technology, Urla, 35430 İzmir, Turkey

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ABSTRACT

Nonthermal processing technologies have focused on the production of safe, fresh-like and high quality food products very much in line with current consumer demands. It is a high priority to maintain the quality attributes of the food during its shelf life. In this study; microbial stability, physicochemical properties and phytochemical characteristics of strawberry juice (SJ) pasteurized by ultrasonication (US) (55 °C, 0.29 W/mL acoustic energy density, 120 μm amplitude, 3 min), high pressure processing (HPP) (300 MPa, 1 min), and pulsed electric fields (PEF) (35 kV/cm, 27 μs) were evaluated during 42 days of storage at 4 °C in comparison with conventional thermal pasteurization as a reference treatment (72 °C, 15 s). The nonthermal processes were equivalent in terms of *E. coli* inactivation since the selected processing conditions previously led to almost identical inactivation level (at least 5-log) of inoculated *E. coli*. Thus, the current study demonstrates how these equivalent US, HPP, and PEF treatments differ from each other in terms of their effect on SJ natural microbiota and quality characteristics during refrigerated storage. Results showed that US, HPP, and heat treatment ensured the microbial stability of SJ for at least 42 days while PEF extended the shelf life of SJ by at least 28 days based on the natural microbiota. No significant difference was found for the total soluble solids of the processed samples ($p > 0.05$) whereas acidity and pH of the samples varied during the storage period ($p < 0.05$). Immediately after processing, the total phenolic contents and antioxidant activities of SJ were better retained by HPP and PEF compared to thermal pasteurization. Furthermore, HPP and PEF significantly increased total anthocyanin content of SJ by 15 and 17% with respect to untreated SJ ($p < 0.05$). Phytochemical characteristics of processed SJ started to decrease after 7 days of storage irrespective of treatment type. HPP treated juices showed significantly higher levels of total anthocyanin and antioxidant activity at the final day of storage. Principal component and cluster analysis showed that the processed SJ samples had higher similarity to the untreated fresh SJ during storage up to 14 days, while the samples beyond this storage period clustered together and discriminated from the rest indicating a decreased similarity to the fresh juice. This study rendered simultaneous evaluation of several quality characteristics during storage of pasteurized strawberry juice based on the equivalent processing approach and multivariate data analysis. Under the selected processing conditions, HPP was the best option to extend the shelf life of SJ and enhance its phytochemical characteristics.

1. Introduction

Fruit juices have an important place in the human diet due to their nutritional and health related composition. Today's consumers prefer convenient fruit juices with fresh-like properties, reasonable cost, high nutritional and functional quality, and prolonged shelf life (Sanchez-Moreno, De Ancos, Plaza, Elez-Martinez, & Cano, 2009). However, several outbreaks caused by *E. coli* O157:H7 and *Salmonella* Typhimurium have been associated with fruit juices (Raybaudi-Massilia et al., 2009). Accordingly, the Food and Drug Administration (FDA) requests at least 5-log reduction of the microorganism of concern to establish the processing conditions, which can be identified by using *E. coli* O157:H7 as a target organism as recommended by the National Advisory Committee on Microbiological Criteria for Foods (FDA, 2001). Moreover,

* Corresponding author.
E-mail addresses: semanury@sakarya.edu.tr (S. Yildiz), prashant.pokhrel@outlook.com (P.R. Pokhrel), sevcanunluturk@iyte.edu.tr (S. Unluturk), barbosa@wsu.edu (G.V. Barbosa-Cánovas).

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since fruits are highly susceptible to spoilage due to their carbohydrate content and enzyme activity, shelf life of fruit juices can be limited by several microbial, chemical, physical, and enzymatic changes unless juice processing is properly handled (Bates, Morris, & Crandall, 2001). Although thermal processing has been widely used in order to meet FDA's requirement and avoid spoilage of fruit juices, negative effects of applied temperatures on nutritional content of products have become the motivation to investigate nonthermal food processing technologies. In this respect, nonthermal processes are becoming a sound alternative to preserve the nutritional and functional properties of the treated product, achieve required level of microbial inactivation, and extend product shelf life.

Many studies have contributed to the shelf life extension of fruit juices by ultrasonication (US), high pressure processing (HPP), and pulsed electric fields (PEF). In this context, sonication has been claimed to retard the microbial spoilage and preserve the quality attributes of different types of fruit juices (Guerrouj, Sanchez-Rubio, Taboada-Rodriguez, Cava-Rolla, & Marin-Iniesta, 2016; Martinez-Flores, Garnica-Romo, Bermudez-Aguirre, Pokhrel, & Barbosa-Canovas, 2015). HPP has been successfully applied in strawberry juice (Cao, Liu, Wu, Liao, & Hu, 2014), strawberry pulp (Cao et al., 2011), red grapefruit juice (Gao et al., 2015), pomegranate juice (Varela-Santos et al., 2012). It has also been reported that PEF extends the shelf life and retain the quality properties without causing any adverse alterations in several products such as orange juice (Agcam, Akyildiz, & Evrendilek, 2016; Plaza et al., 2006), pomegranate juice (Guo et al., 2014), peach nectar (Altuntas, Evrendilek, Sangun, & Zhang, 2011) compared to thermal pasteurization.

Furthermore, studies related to evaluation of changes in quality attributes of juices immediately after processing by different nonthermal technologies as well as during storage period have been encouraged to be carried out on the basis of the equivalent processing approach (Timmermans et al., 2011; Vervoort et al., 2011; Zulueta, Barba, Esteve, & Frigola, 2013). As stated by these authors equivalency refers to equivalent level of microbial inactivation under a given set of processing conditions. Considering this equivalent approach, Timmermans et al. (2011) concluded that HPP and PEF are the superior treatments to extend shelf life and retain quality characteristics of orange juice compared to thermal treatment at 72 °C. Vervoort et al. (2011) observed no significant differences among HPP, PEF, and mild heat-treated orange juices in terms of quality parameters such as Vitamin C and carotenoids during 2 months of storage when they applied equivalent processes in terms of microbial inactivation. Moreover, multivariate data analysis tools provide a comprehensive understanding of the shelf life stability of juice products by differentiating the processed products in terms of their quality parameters during the storage period (Kaya, Yildiz, & Unluturk, 2015). The changes in natural microbiota, physicochemical properties (pH, titratable acidity, soluble solid content) and phytochemical characteristics (total phenolic content, total anthocyanin content, antioxidant activity) are important to evaluate quality of fruit juices during storage.

Previous study by Yildiz, Pokhrel, Unluturk, and Barbosa-Cánovas (2019) identified the mild pasteurization conditions of US, HPP, and PEF processes for strawberry juice (SJ) considering equivalent acid-adapted *E. coli* inactivation in accordance with the FDA's 5-log reduction criteria. Afterwards, natural microbial quality, physicochemical and phytochemical characteristics of SJ were evaluated immediately after these processes in order to comprehend how natural microbiota and fresh-like attributes were affected by the identified processing conditions that are actually equivalent in terms of the targeted *E. coli* inactivation (Yildiz et al., 2020). Finally, the current study integrates equivalent fruit juice processing approach and multivariate data analysis to the comparative evaluation of the product shelf life. Thus, the objective of this work was to evaluate the microbial quality and retention of physicochemical properties and phytochemical characteristics of SJ equivalently processed by ultrasound, HPP, and PEF during refrigerated storage based on multivariate statistical analysis.

2. Materials and methods

2.1. Preparation of strawberry juice

Strawberries (*Fragaria* × *ananassa*) were purchased from a local grocery store (Walmart Inc., Pullman, USA) at their commercial maturity. Fruits stored at −30 °C were then defrosted overnight at ambient temperature in the dark. Then, the juice was extracted using a fruit juice extractor (Model K, Regal Ware, Inc., USA) followed by centrifugation (Beckman J2 HS centrifuge, GMI, MIC Group, Inc., Minnesota, US) at 6000 rpm and 4 °C for 5 min using a Fiberlite F14 6x250 rotor (Piramoon Technologies, Inc., US). The juice was subsequently filtered through a cheese cloth to remove the suspended particles.

2.2. Pasteurization of strawberry juice

Freshly squeezed SJ was pasteurized by ultrasound, high pressure, PEF and thermal treatments under equivalent processing conditions. The pasteurization conditions were previously determined based on equivalent inactivation (5 logarithmic cycles) of a non-pathogenic surrogate of *E. coli* O157:H7, i.e. *E. coli* (ATCC 11775) (Yildiz et al., 2019). Hereof, a summary of pasteurization procedures is given below, which were applied to further evaluate how these equivalent processes affected the natural microbiota and quality characteristics of SJ during storage.

Ultrasonication at 55 °C (US) was applied for 3 min in duplicate using an ultrasonic device (UP400S Hielscher USA Inc., Ringwood, NJ, USA) equipped with a probe of 22 mm diameter, double wall sample unit and a water circulator (Thermostat Physica Viscotherm VT 10, Germany). SJ was exposed to 0.29 W/mL acoustic energy density at 24 kHz frequency and 120 μm amplitude in continuous pulse mode. Based on preliminary tests, the temperature was kept at around 55 °C by pumping water at 25 °C through the jacketed vessel where the SJ was treated. The initial and the maximum temperatures recorded during sonication were 23.5 and 56.5 °C, respectively. The calorimetric method was used to estimate acoustic energy density (the amount of ultrasound energy per unit sample volume) by using the following equations (Tiwari, 2015).

$$P = mc_p\left[\frac{dT}{dt}\right]_{t=0} \quad (1)$$

$$AED = \frac{P}{V} \quad (2)$$

where dT/dt is the rate of change in temperature over time (°C/s), c_p is the SJ specific heat (3.7 kJ/kg °C), P is ultrasonic power (W), m is sample mass (kg), and V is sample volume (mL).

For the HPP treatment, a high hydrostatic pressure unit (Engineering Pressure Systems, Inc., Andover, USA) with a cylindrical chamber vessel (0.1 m internal diameter, 0.25 m internal height) was used. Freshly squeezed SJ was packed into Nylon/PE type plastic pouches (3 MIL, UltraSource, Kansas City, Missouri, USA), and subjected to 300 MPa for 1 min in duplicate. Come-up time required to reach the desired pressure was 0.5 s while the depressurizing time was recorded as <0.5 s. The initial temperature of the pressurizing liquid inside the chamber was 18.3 ± 1.0 °C, and temperature rise was 2.6 °C/100 MPa during processing. A typical time–temperature profile during treatment can be found in Yildiz et al. (2019).

The PEF equipment used in this study was a pilot plant scale Powermod™ PEF system (Diversified Technologies Inc., Bedford, MA, USA). This PEF system includes two processing chambers in line, where each one has two pairs of co-field electrodes having diameter of 0.50 cm and gap distance of 0.65 cm. The juice having an electrical conductivity of 4.09 ± 0.01 mS/cm was pumped through the PEF treatment chambers

by a peristaltic pump at a flow rate of 350 mL/min. SJ was subjected to PEF at 35 kV/cm of electrical field intensity (EFI), 155 Hz of frequency, 2 μs of pulse width in monopolar mode for 27 μs of treatment time. The temperature of SJ at the entrance and exit of treatment chamber were 22.7 and 46 °C, respectively.

Finally, conventional thermal pasteurization (T) was performed at 72 °C for 15 s as a reference treatment (Wibowo et al., 2019) in a double-walled beaker connected to a water bath (Thermostat Physica Viscotherm VT 10, Germany) which circulated hot water (74 °C) through the beaker walls. The sample vessel containing 400 mL of SJ was placed on a stirrer set at 250 rpm. SJ was heated to 72 °C, kept for 15 s, transferred to a previously sterilized bottle, and subsequently cooled down to ambient temperature by placing it into ice water (Yildiz et al., 2020). The SJ temperature was measured by a K-type thermocouples.

2.3. Storage study

Untreated, US, HPP, PEF, and heat-treated SJ samples, after being processed as described in the previous section, were immediately cooled down to 4 °C. Approximately 30 mL of SJ was transferred into previously sterilized borosilicate glass vials with screw caps (66012-066, VWR International, LLC, Radnor, PA) under aseptic conditions. Then the SJ samples were stored in dark inside the storage room at Center for Nonthermal Processing of Food (CNPF), Washington State University at refrigerated conditions (4 °C) for 42 days. Control samples were analyzed at 0-3-5-7-14th days of storage while processed samples were studied weekly to determine the microbial quality throughout storage period. For this purpose, SJ samples were plated on plate count agar (PCA) and potato dextrose agar (PDA) (BD Difco, Fisher Scientific, USA) to monitor total mesophilic aerobic bacteria count (TMAC), yeasts and mold (YM) count. Viable cells were counted after appropriate incubation at 37 °C for 48 h and 25 °C for 5 days for TMAC and YM count, respectively, where the results were expressed as log CFU/mL.

Physicochemical properties (pH, total soluble content (TSS), titratable acidity) and phytochemical characteristics (total phenolic content (TPC), total anthocyanin content (TAC), and radical scavenging activity (RSA)) of processed SJ samples were assessed during the storage period. pH measurement was performed by placing 10 mL of SJ on a bench top pH meter (Mettler Toledo™ FE20 FiveEasy) at 22 °C. A digital hand-held refractometer (PAL-α, Atago CO., LTD, Tokyo, Japan) was used to determine the TSS (°Brix) of the samples (3–4 drops). Titratable acidity was determined by titrating 10 mL of SJ against 0.1 N NaOH up to pH 8.1; and expressed as mg citric acid/100 mL (AOAC, 1995).

Phytochemical properties of the samples were determined according to the procedures detailed in Yildiz et al. (2019). TPC, TAC, and RSA of SJ samples were expressed as mg GAE/100 mL, mg pelargonidin-3-glucoside/L, and % DPPH inhibition, respectively. Retention of TPC, TAC, and RSA immediately after processing were calculated considering untreated SJ (control) as 100% at day 0; whereas those of processed SJ samples during storage from day 7 to day 42 were calculated with respect to the initial phytochemical content of corresponding SJ samples (considered as 100% on day 0) treated by heat, ultrasound, HPP, and PEF.

2.4. Data analysis

The experiments related to the microbiological quality and physicochemical properties of SJ samples were conducted in duplicate while phytochemical assays were performed in triplicate. All data were analyzed by using Excel worksheet (Microsoft Office 2010, USA) and Minitab 16 software (Minitab Inc., State College, PA, USA). The means of measured properties of SJ samples were compared by Analysis of Variance (ANOVA) considering Tukey's comparison test at 95% of confidence interval. Moreover, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to visualize the data structure and distinguish similarities/differences among treatments and storage days by simultaneous evaluation of physicochemical and phytochemical attributes of SJ. In this context, a data matrix was constructed using the physicochemical properties and phytochemical characteristics as columns and pasteurization technologies as rows; and subsequently introduced into Minitab 16. Correlation type of matrix and 5 components were computed for the generation of score and loading plots as PCA output. The score values, coefficients, and eigenvalues were saved as storage data; and PC-scores of interest were then used as new input data for HCA. The cluster analysis was implemented considering Ward's linkage as amalgamation method and Euclidean distance as similarity measurement. Thereby, the similarities/dissimilarities among all SJ samples were classified in terms of their physicochemical properties and phytochemical characteristics during refrigerated storage; and plotted on a tree-shaped map, i.e. dendrogram.

3. Results and discussion

3.1. Microbial quality

Logarithmic changes in natural microbiota, i.e. TMAC and YM count, of untreated (control), US, HPP, PEF, and heat-treated SJ during 42 days of refrigerated storage at 4 °C were evaluated. Untreated SJ initially contained 3.11 ± 0.12 log CFU/mL of TMAC, which increased to 4.01 ± 0.05 log CFU/mL after 14 days (Fig. 1). This is attributed to the naturally occurring microorganisms causing spoilage of fruit juices during refrigerated storage (Vergara, Marti, Mena, Saura, & Valero, 2013). YM count of untreated fresh SJ was 3.4 ± 0.1 log CFU/mL at the beginning and reached to 5.6 ± 0.0 log CFU/mL at the 14th day of storage (Fig. 2). Molds and yeasts have been reported to be the main microorganisms limiting the shelf life of unprocessed SJ (Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloso, 2012). As concluded by Yildiz et al (2019), the natural microbiota should also be taken into consideration while identifying the pasteurization conditions due to their high survival level in the fruit juices.

Microbial spoilage of SJ samples was evaluated following the recommendations of Santhirasegaram et al. (2015), and Pala & Toklucu (2013). The acceptable maximum microbial limits in terms of TMAC and YM count in fruit juices are 4-log and 3-log CFU/mL, respectively. In this regard, nonthermal and thermal pasteurization processes were able to retard the microbial growth during storage of juices (Fig. 1 & Fig. 2). Thermal pasteurization at 72 °C for 15 s, ultrasonication at 55 °C for 3 min, and HPP at 300 MPa for 1 min were able to keep the TMAC of SJ below 2 log CFU/mL during 42 days of refrigerated storage at 4 °C. The maximum counts of total aerobic bacteria in SJ treated with heat, US, and HPP were found as 1.8 ± 0.1, 1.8 ± 0.1, and 1.7 ± 0.0 log CFU/mL, respectively, at the end of storage period ($p > 0.05$). Besides, YM counts of heat-treated, ultrasonicated, and HPP treated SJ samples at the end of storage were found as 1.9 ± 0.1, 1.9 ± 0.0, and 1.97 ± 0.1 log CFU/mL,

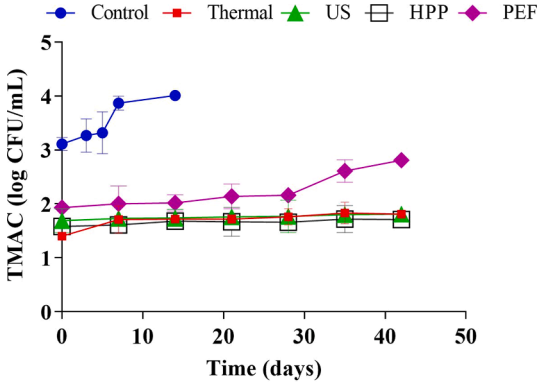

Fig. 1. Changes in total mesophilic aerobic bacteria counts during storage (4 °C for 42 days) of strawberry juice treated by heat, ultrasound, HPP, or PEF.

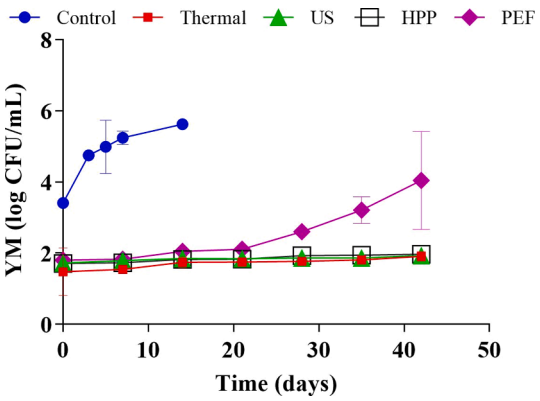

Fig. 2. Changes in yeast and molds counts during storage (4 °C for 42 days) of strawberry juice treated by heat, ultrasound, HPP, or PEF.

respectively. Since the counts of natural microbiota was below 2-log CFU/mL, equivalent processing conditions of ultrasonication, HPP, and thermal processes could be considered as efficient to extend the shelf life of SJ. Regarding PEF processing, TMAC of PEF-treated SJ reached to 2.8 ± 0.1 CFU/mL at the end of storage period (42 days) while YM count was recorded as 2.6 ± 0.0, 3.2 ± 0.4, 4.0 ± 1.4 log CFU/mL at 28th, 35th, and 42nd days, respectively. Based on the criteria mentioned above, it can be inferred that PEF extended the shelf life of SJ by at least 28 days at refrigerated conditions while ultrasonication, HPP, and thermal processes resulted in at least 42 days of shelf life.

Many studies have been mostly focused on the impact of sonication on the quality parameters of several fruit juices (Abid, Jabbar, Wu, et al., 2014; Bhat & Goh, 2017; Bhat, Kamaruddin, Min-Tze, & Karim, 2011; Rawson et al., 2011) rather than microbial stability during storage. This can be construed from the fact that sonication is more effective to reach the required amount of microbial reduction when it is combined with other technologies (Piyasena, Mohareb, & McKellar, 2003). Lethal effect of sonication has been increased when combined with other technologies such as PEF (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2009), pressure (Abid, Jabbar, Hu, et al., 2014), antimicrobials (Munoz et al., 2012), and moderate heat (Lee, Zhou, Liang, Feng, & Martin, 2009). In the current study, sonication at moderate temperature (55 °C) shortened the processing time (3 min) and extended the shelf life of SJ up to 42 days. This finding was in line with the study of Martinez-Flores et al. (2015) which presented shelf life extension of carrot juice up to 20 days by applying ultrasonication (24 kHz, 120 μm amplitude, 2.18 W/mL) at 58 °C. Since limited information exists on shelf life extension of fruit juices by thermosonication, this study revealed the potential of ultrasonication at mild temperature as an alternative pasteurization method for SJ.

The results of HPP treatment are in agreement with other studies such as those by Varela-Santos et al. (2012) that reported HPP at 350 MPa for 150 s extended the shelf life of pomegranate juice by >35 days under refrigerated conditions. The elevated pressures could reduce the microbial load below detection limits as reported by Aaby et al., (2018) for strawberry juice. The authors stated that HPP at ≥500 MPa extended shelf life of strawberry juice at least 49 days at 6 °C. In another study, the shelf life of turbid and clear strawberry juices were extended up to 6 months by subjecting the juices to 600 MPa for 4 min and subsequent storage at 4 °C (Cao et al., 2012).

The findings of PEF processing were partially in agreement with the study conducted by Guo et al. (2014) on pomegranate juice where PEF at 35 and 38 kV/cm and 55 °C for 281 μs kept total aerobic bacteria and yeast-mold counts below 2.5 and 3 log CFU/mL, respectively, during 12 weeks of refrigerated storage at 4 °C. Elez-Martinez, Soliva-Fortuny, and Martin-Belloso (2006) reported the reduction of the number of natural microbiota below 1 log CFU/mL and extended the shelf life of orange juice up to 56 days at 4 °C when the juice was process by high intensity PEF (35 kV/cm for 1,000 μs; bipolar 4-μs pulses at 200 Hz). The processing conditions, i.e. number of pulses, treatment time, type of pulse, etc., applied to orange juice in the study of Elez-Martinez et al. (2006) were more intense compared to the current study.

Thermal pasteurization (72 °C for 15 s) was able to ensure the microbial safety of SJ throughout the storage period (42 days) by keeping TMAC and YM count below 2-log CFU/mL. In the literature, different time and temperature parameters were applied for shelf life extension of fruit juices. For example, Bull et al. (2004) applied relatively low temperature (65 °C for 60 s) heat treatment to Valencia orange juice which had high initial load of aerobic bacteria and yeast-mold. They stated that the thermal treatment was able to reduce the aerobic bacteria population to 4.3 log CFU/mL while the yeast and mold count was reported to be 3 log CFU/mL after thermal pasteurization. Thus, the authors showed that moderate temperatures may not be sufficient when the initial microbial load is elevated. High temperatures were also applied as mentioned in the study of Elez-Martinez et al., (2006) where thermal pasteurization at 90 °C for 1 min rendered microbial counts below 1 log CFU/mL in orange juice and extended the shelf life of the juice up to 56 days. In our case, a moderate heat treatment was adopted for the thermal pasteurization of SJ.

In summary, the storage time (42 days) considered for SJ in the current study was relatively shorter than previously cited shelf life studies in the literature. Nonetheless, it is suggested that heat, ultrasonication, and HPP treated SJ samples might show good microbial stability for storage periods >42 days at refrigeration conditions. However, this suggestion may not be applied for PEF treated SJ since the yeast-mold count reached the critical limit (3-log CFU/mL) at the 35th day of storage.

3.2. Physicochemical properties

Influence of storage duration on the physicochemical properties, i.e. TSS, pH, titratable acidity, of SJ samples subjected to different processes was evaluated and the results are shown in Table 1. The initial TSS content of SJ was 7.9 ± 0.1 °Brix which was consistent with that of previously studied fresh SJ (7.8 ± 0.0 °Brix) (Odriozola-Serrano, Soliva-Fortuny, Gimeno-Añó, & Martin-Belloso, 2008). TSS of untreated SJ decreased to 7.7 ± 0.1 °Brix during 14 days of refrigerated storage. Even though the changes were not significant ($p > 0.05$), the decrease in TSS could be still correlated with the consumption of sugars by microorganisms growth during storage (Elez-Martinez et al., 2006). Besides, no significant changes were observed in the TSS of SJ samples ($p > 0.05$) after processing of SJ by ultrasonication, HPP, PEF, and thermal treatments (Table 1). These findings were in line with studies related to equivalently processed orange juice by thermal, HPP, and PEF (Timmermans et al., 2011), sonicated grapefruit juice (Aadil, Zeng, Han, & Sun, 2013), PEF treated grapefruit juice (Aadil, Zeng, Ali, et al., 2015). Irrespective of the treatment, no significant changes were observed in TSS of processed SJ samples during 42 days of refrigerated storage which was in agreement with other studies reported for different fruit juices subjected to heat, ultrasonication, HPP, and PEF (Elez-Martinez et al., 2006; Timmermans et al., 2011; Walkling-Ribeiro et al., 2009).

Untreated SJ had pH of 3.5 ± 0.0 and titratable acidity of 0.8 ± 0.0 g/100 mL (Table 1). Likewise, Tiwari, O'Donnell, Patras, and Cullen (2008) reported pH value of SJ as 3.14 and acidity as 0.73 g/100 mL which were close to the characteristics of SJ used in the present study. The changes in pH and acidity of SJ samples ranged between 3.39 and 3.51, and 0.79–0.88 g/100 mL, respectively, throughout refrigerated storage (Table 1). In accordance with this study, Tiwari, O'Donnell, Muthukumarappan, and Cullen (2009) observed some significant changes in pH and no significant changes in the titratable acidity of sonicated orange juice irrespective of amplitude level, treatment time or storage time. Martinez-Flores et al. (2015) attributed the change in pH of sonicated carrot juice during storage to the new chemical compounds generated in the media because of the ultrasound processing. It has been

Table 1
Total soluble solids (TSS), pH, and titratable acidity of fresh and equivalently pasteurized strawberry juice during storage at 4 °C.

Time (day)	Treatment	TSS (°Brix)	pH	Titratable acidity (g/100 mL)
0	Control	[a]7.85 ± 0.07**A**	[a]3.50 ± 0.01**A**	[ab]0.81 ± 0.00**A**
	T	[a]7.88 ± 0.04a	[ab]3.48 ± 0.02ab	[b]0.79 ± 0.00b
	US	[a]8.00 ± 0.14A	[b]3.45 ± 0.01B	[a]0.84 ± 0.02A
	HPP	[a]7.83 ± 0.04*a*	[ab]3.46 ± 0.00*a*	[ab]0.81 ± 0.00*c*
	PEF	[a]7.83 ± 0.04**a**	[ab]3.48 ± 0.00**a**	[ab]0.82 ± 0.00**a**
3	Control	7.83 ± 0.04**A**	3.48 ± 0.01**A**	0.82 ± 0.00**A**
5	Control	7.83 ± 0.04**A**	3.47 ± 0.05**A**	0.82 ± 0.00**A**
7	Control	7.80 ± 0.00**A**	3.46 ± 0.04**A**	0.84 ± 0.02**A**
	T	7.98 ± 0.04a	3.51 ± 0.01a	0.82 ± 0.00ab
	US	7.93 ± 0.04A	3.47 ± 0.00A	0.84 ± 0.00A
	HPP	7.90 ± 0.00*a*	3.47 ± 0.01*a*	0.82 ± 0.00*bc*
	PEF	7.90 ± 0.07**a**	3.45 ± 0.00**b**	0.82 ± 0.00**a**
14	Control	7.70 ± 0.07**A**	3.44 ± 0.00**A**	0.84 ± 0.02**A**
	T	7.95 ± 0.07a	3.46 ± 0.01ab	0.83 ± 0.01ab
	US	7.98 ± 0.04A	3.43 ± 0.00BC	0.85 ± 0.01A
	HPP	7.95 ± 0.07*a*	3.42 ± 0.01*b*	0.85 ± 0.01*ab*
	PEF	7.85 ± 0.07**a**	3.42 ± 0.00**c**	0.84 ± 0.04**a**
21	T	7.85 ± 0.07a	3.45 ± 0.05ab	0.81 ± 0.01ab
	US	7.80 ± 0.00A	3.40 ± 0.00D	0.84 ± 0.02A
	HPP	7.80 ± 0.00*a*	3.41 ± 0.00*bc*	0.86 ± 0.01*a*
	PEF	7.70 ± 0.00**a**	3.40 ± 0.00**d**	0.86 ± 0.00**a**
28	T	7.90 ± 0.00a	3.42 ± 0.00b	0.82 ± 0.00ab
	US	7.88 ± 0.04A	3.40 ± 0.01D	0.84 ± 0.03A
	HPP	7.88 ± 0.04*a*	3.39 ± 0.00*c*	0.86 ± 0.00*a*
	PEF	7.80 ± 0.00**a**	3.39 ± 0.00**d**	0.86 ± 0.02**a**
35	T	7.85 ± 0.07a	3.41 ± 0.00b	0.83 ± 0.00ab
	US	7.80 ± 0.00A	3.41 ± 0.00CD	0.85 ± 0.00A
	HPP	7.80 ± 0.00*a*	3.39 ± 0.00*c*	0.86 ± 0.01*a*
	PEF	7.60 ± 0.14**a**	3.40 ± 0.01**d**	0.86 ± 0.02**a**
42	T	7.85 ± 0.07a	3.47 ± 0.01ab	0.85 ± 0.02a
	US	7.90 ± 0.00A	3.43 ± 0.01B	0.85 ± 0.00A
	HPP	7.85 ± 0.07*a*	3.39 ± 0.00*c*	0.85 ± 0.00*ab*
	PEF	7.58 ± 0.18**a**	3.39 ± 0.00**d**	0.88 ± 0.02**a**

T, US, HPP, PEF refer to thermal pasteurization, ultrasonication, high pressure processing, and pulsed electric fields, respectively. Results were given as mean ± standard deviation. Different bold upper case, lower case, upper case, italic lower case and bold lower case letters indicate the significant differences during 42 days of storage of untreated SJ and SJ treated by thermal pasteurization, US, HPP, and PEF, respectively. With respect to day 0, different lower case letters given on the left side of the data as superscript show the significant differences among treatments ($p < 0.05$).

commonly reported that HPP and PEF showed insignificant change in pH and acidity of juice products during storage period (Barba, Esteve, & Frigola, 2012; Odriozola-Serrano, Aguilo-Aguayo, Soliva-Fortuny, & Martin-Belloso, 2013).

3.3. Phytochemical characteristics

3.3.1. Total phenolic content

Influence of storage on the phytochemical content of SJ treated by different technologies is shown in Table 2. Fresh SJ had TPC of 137.8 ± 0.9 mg/100 mL while TPCs of heat, ultrasound, HPP, and PEF treated samples were respectively found as 132.2 ± 1.7, 137.6 ± 1.9, 143.5 ± 2.8, 145 ± 1.5 mg/100 mL immediately after pasteurization. These values were similar to those previously reported by Varela-Santos et al. (2012). Although thermal pasteurization insignificantly affected the TPC compared to fresh juice ($p > 0.05$), heat-treated SJ samples contained significantly less phenolics than HPP and PEF treated SJ at day 0 ($p < 0.05$). HPP and PEF treatments resulted in 4% and 5% increase in TPC of SJ, respectively when compared to the control samples. Barba, Esteve, and Frigola (2013) also observed significant increase in TPC of blueberry juice in the range of 13–27% after subjecting the juice to varying treatment times and pressures up to 400 MPa, which may be attributed to an increased extractability of some of the antioxidant components after HPP (Barba, Esteve, & Frigola, 2013). The content and stability of total polyphenols in juices have been reported to be dependent on the storage conditions (Teleszko, Nowicka, & Wojdylo, 2016) as well. A remarkable increase in TPC of HPP and PEF treated SJ was observed at the 7th day of refrigerated storage (Fig. 3a); however only the effect of HPP was statistically significant (Table 2). The possible reason for such increments of phenolic compounds during storage has been related to the reactions between oxidized polyphenols and generation of new compounds that can show antioxidant characteristic (Kallithraka et al., 2009; Martinez-Flores et al., 2015). At this point, it is possible to speculate that this increment could be due to some formed reducing compounds that can react with the unspecific Folin–Ciocalteu reagent and significantly enhance the phenolic content (Barba, Jager, et al., 2012). In general, TPC remained higher in HPP and PEF treated SJ samples throughout storage. This result is consistent with the study conducted by Plaza et al. (2011). They indicated that HPP and PEF treatments were more effective for the preservation of bioactive compounds in orange juice compared to thermal pasteurization (70 °C for 30 min) during the storage period (Plaza et al., 2011). Sonicated samples maintained the initial TPC level at day 7 whereas thermal pasteurization caused 3% of loss in its initial amount of total phenolic compounds. Afterwards, a decreasing trend was observed in all SJ samples starting from the 7th day of storage irrespective of treatment (Fig. 3a). In fact, thermal processing resulted in the lowest content of total phenolics in SJ samples at the end of storage followed by ultrasonication, PEF, and HPP treatments. Nonetheless, the differences among TPC of processed SJ samples were not statistically significant at the 42nd day of refrigerated storage ($p > 0.05$) (Table 2). The initial TPCs of SJ samples treated by heat, ultrasound, HPP, and PEF were retained by 77, 76, 75, and 72, respectively, at the end of storage. Enzymes such as polyphenol oxidases have been involved in the degradation of phenolic compounds (Tomás-Barberán & Espín, 2001). Therefore, reduction in the content of phenolics may be explained by the possible residual enzyme activity, which is suggested as a future study.

Many studies demonstrated that sonication leads to significant increases in TPC of different juices (single strength or blends) such as orange juice (4.3–14.6%), strawberry-apple-lemon juice blend (7%), pear juice (up to 13.7%), and mango juice (up to 35%) (Feng et al., 2020; Ordóñez-Santos, Martínez-Girón, & Arias-Jaramillo, 2017; Saeeduddin et al., 2016; Santhirasegaram, Razali, & Somasundram, 2013). The

Table 2
Total phenolic content (TPC), total anthocyanin content (TAC), radical scavenging activity (RSA) of fresh and equivalently pasteurized SJ and their retention during storage at 4 °C.

Time (days)	Sample	Total phenolics		Total anthocyanins		Antioxidant activity	
		TPC (mg/100 mL)	Retention of TPC (%)	TAC (mg/L)	Retention of TAC (%)	RSA (% DPPH Inhibition)	Retention of RSA (%)
0	C	[ab] 137.8 ± 0.9	100[ab]	[b] 153.3 ± 2.6	100[b]	[ab] 33.7 ± 2.7	100[a]
	T	[b] 132.2 ± 1.7a	96[b]	[ab] 166.4 ± 2.4a	109[ab]	[b] 30.0 ± 2.2b	89[a]
	US	[ab] 137.6 ± 1.9A	100[ab]	[ab] 167 ± 0.4A	109[ab]	[ab] 39.6 ± 1.9B	117[a]
	HPP	[a] 143.5 ± 2.8*b*	104[a]	[a] 176.7 ± 1.7*a*	115[a]	[a] 40.0 ± 1.8*bc*	119[a]
	PEF	[a] 145 ± 1.5**ab**	105[a]	[a] 179.2 ± 8.5**a**	117[a]	[a] 40.3 ± 0.5**b**	119[a]
7	T	128.7 ± 20a	97	150.8 ± 2.8ab	91	50.6 ± 1.1a	169
	US	138.2 ± 5.2A	100	155.3 ± 10.6AB	93	54.2 ± 0.5A	137
	HPP	161.4 ± 4.1*a*	112	157.1 ± 3.3*b*	89	55.8 ± 1.2*a*	140
	PEF	153.8 ± 20.5**a**	106	161.2 ± 2.5**abc**	90	54.0 ± 1.0**a**	134
14	T	127.2 ± 11.4 a	96	144.3 ± 5.1bc	87	48.3 ± 2.3a	162
	US	131.5 ± 1.2A	96	154.4 ± 1.6ABC	92	48.8 ± 0.0A	123
	HPP	133.3 ± 1.7*bc*	93	156.9 ± 3.0*b*	89	49.6 ± 1.2*ab*	124
	PEF	132.7 ± 5.9**bcd**	92	159.6 ± 0.3**b**	89	49.9 ± 3.1**a**	124
21	T	116.9 ± 1.8a	88	133.4 ± 1.5bc	80	31.9 ± 0.9b	107
	US	122.9 ± 2.7AB	89	140.2 ± 3.4BCD	84	38.8 ± 0.1B	98
	HPP	123.5 ± 2.6*c*	86	152.4 ± 4.7*b*	86	39.7 ± 6.4*bc*	99
	PEF	123.3 ± 0.0**cde**	85	152.8 ± 0.2**bcd**	85	35.2 ± 1.1**b**	87
28	T	102.4 ± 1.7a	77	127.6 ± 3.7c	77	37.1 ± 2.0b	124
	US	115.9 ± 13.4AB	84	137.2 ± 1.2CD	82	37.4 ± 3.9B	95
	HPP	121.4 ± 7.9 *cd*	85	144.3 ± 8.3*b*	82	36.0 ± 0.8c	90
	PEF	114.8 ± 6.4**de**	79	143.0 ± 2.9 **cd**	80	35.0 ± 0.5**b**	87
35	T	102.2 ± 0.3a	77	125.4 ± 8.7c	75	34.3 ± 5.1b	115
	US	105.6 ± 2.5B	77	136.3 ± 1.6D	82	38.8 ± 0.4B	98
	HPP	108.1 ± 0.5*d*	75	143.3 ± 2.9*b*	81	39.0 ± 1.0*c*	98
	PEF	106.9 ± 6.4**e**	74	141.2 ± 1.0**d**	79	38.0 ± 0.8**b**	94
42	T	[a] 101.8 ± 0.3a	77	[c] 125.3 ± 5.6c	75	[b] 34.0 ± 1.0b	114
	US	[a] 104.4 ± 2.7B	76	[bc]128.3 ± 2.4D	77	[a] 38.8 ± 0.6B	98
	HPP	[a] 107.0 ± 0.1*d*	75	[a] 142.3 ± 0.34*b*	81	[a] 37.0 ± 0.3*c*	93
	PEF	[a] 104.5 ± 1.9**e**	72	[ab] 141.0 ± 2.4**d**	79	[a] 38.5 ± 0.7**b**	96

T, US, HPP, PEF refer to thermal pasteurization, ultrasonication at 55 °C, high pressure processing, pulsed electric fields, respectively. Results were given as mean ± standard deviation. Different letters in the same column show significant differences. To specify, different lower case, upper case, italic lower case and bold lower case letters indicate the significant differences during 42 days of storage of SJ treated by thermal pasteurization, US, HPP, and PEF, respectively. Regarding day 0 and day 42, different lower case letters given on the left side of the data as superscript show the significant differences among treatments ($p < 0.05$). Regarding the retention of phytochemical properties, the differences among SJ samples at day 0 were compared with respect to the untreated SJ. For the storage period between day 7 and day 42, retention of phytochemical properties was compared with respect to the initial TPC, TAC, and RSA of the corresponding processed SJ.

increase in TPC of sonicated samples can be attributed to the release of antioxidant compounds from the cell wall due to the collapse via cavitation in the surroundings of colloidal particles (Cheng et al., 2007). Another scenario has been associated with the formation of hydroxyl radicals that results in hydroxylation of aromatic ring of the phenolic compounds, increasing the antioxidant characteristics of the material subjected to sonication (Ashokkumar et al., 2008). On the other hand, gradual decreases in TPC through storage occur as reported by the study of Feng et al. (2020) where the authors demonstrated significant decreases in TPC of a sonicated strawberry-apple-lemon juice blend after 10 days of storage at 4 °C. In this case, sonication retained the TPC level similarly to control immediately after processing while the initial TPC of sonicated juice was gradually reduced to 76% throughout storage.

3.3.2. *Total anthocyanin content*

Monomeric pelargonidin-3-glucoside (Pg-3-glu), cyanidin-3-glucoside (Cy-3-glu), pelargonidin-3-rutinoside (Pg-3-rut) are the major anthocyanins widely analyzed in strawberry products (Cao et al., 2011; Cao et al., 2012; Teleszko et al., 2016). In the current study, total anthocyanin content of SJ samples were monitored during 42 days of storage as given in Fig. 3b. The concentration of total anthocyanins in untreated SJ was 153.3 ± 2.6 mg pelargonidin-3-glucoside equivalent/L. While thermal pasteurization at 72 °C for 15 s and ultrasonication at 55 °C did not significantly alter TAC of fresh SJ ($p > 0.05$), HPP and PEF treatment significantly increased TAC right after processing (day 0, $p < 0.05$) (Table 2). Heat, ultrasound, HPP and PEF treated SJ samples respectively contained total anthocyanins as 166.42 ± 2.42, 166.97 ± 0.39, 176.67 ± 1.73, and 179.21 ± 8.47 mg/L at the beginning of storage. Thus, HPP and PEF treatments increased initial content of total anthocyanins by 15 and 17% respectively. This finding was in agreement with a study conducted on blueberry juice where 109 and 105% retention of TPC were achieved by HPP and PEF, respectively (Barba, Jager, et al., 2012). Cao et al. (2012) reported total anthocyanins in HHP-treated cloudy and clear strawberry juices as 116.5 and 111.3 mg/L, respectively. Stability of anthocyanins depends on heat, light, pH, oxygen, and several enzymes, such as β-glucosidase, polyphenoloxidase and peroxidase (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009). During storage at 4 °C, TAC of SJ samples showed a decreasing trend regardless of processing type (Fig. 3b). HPP resulted in significantly higher content of total anthocyanins (142.3 ± 0.34 mg/L) compared to thermal treatment (125.3 ± 5.6 mg/L) at the end of the storage ($p < 0.05$). The initial TAC of processed samples at day 0 were retained by 75, 77, 81, and 79% for heat, ultrasound, HPP, and PEF treatments, respectively, at the end of their storage time. It is worth mentioning the role of storage time for each technology on the final content of total anthocyanins. This finding was in line with the studies conducted on anthocyanin retention during storage of sonicated strawberry juice (Tiwari, O'Donnell, et al., 2009) and HPP or PEF treated blueberry juice (Barba, Jager, et al., 2012). Loss of anthocyanins can be attributed to the oxidation as well as condensation of anthocyanins (Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009). It has been previously reported that condensation reactions of anthocyanins during storage occur due to the formation of complexes with other phenolics naturally occurred in juices such as strawberry and raspberry juices (Rein, Ollilainen, Vahermo, Yli-Kauhaluoma, & Heinonen, 2005). Degradation of anthocyanins during storage of sonicated strawberry juice may also be due to the residual enzymes such as polyphenoloxidase, peroxidase, and β-glucosidase (Tiwari, O'Donnell,

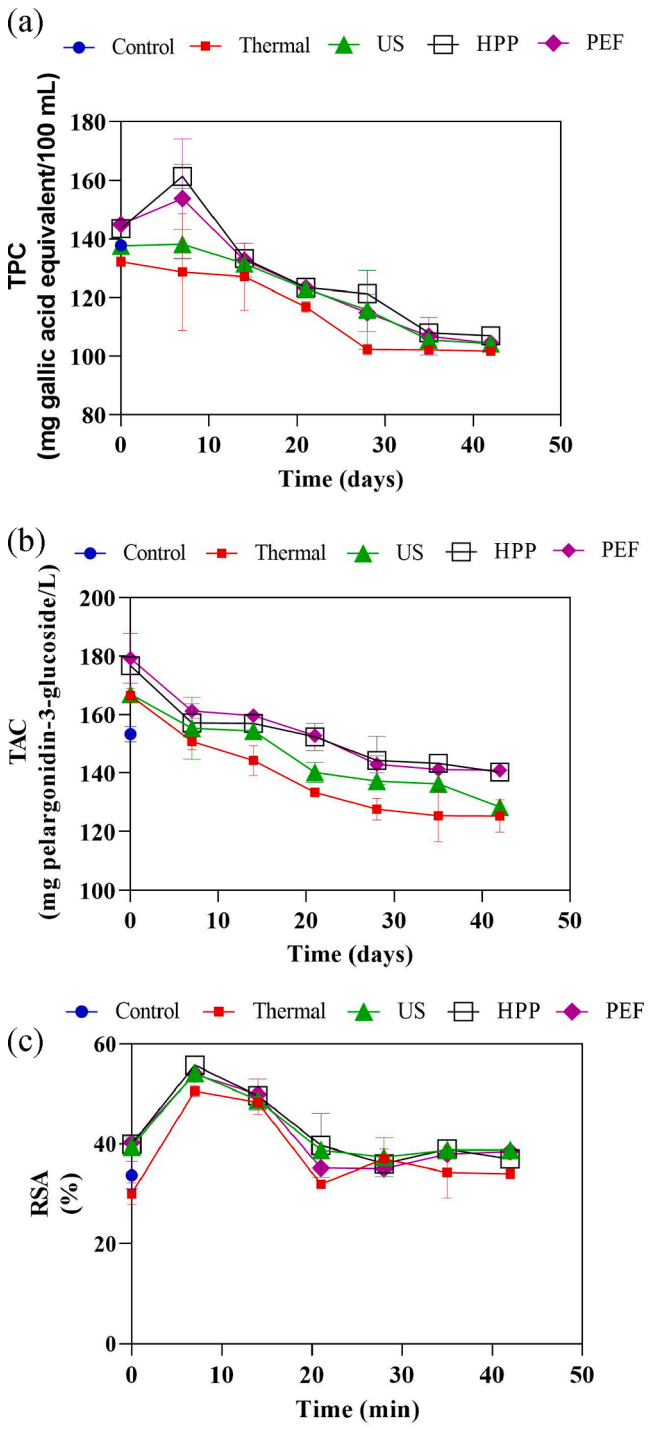

Fig. 3. Changes in phytochemical characteristics of strawberry juice samples during 42 days at refrigerated storage. (TPC: Total phenolic content, TAC: Total anthocyanin content, RSA: Radical scavenging activity, Control: untreated strawberry juice, T: Thermal pasteurization, US: Ultrasonication, HPP: High pressure processing, PEF: Pulsed electric fields).

et al., 2009).

3.3.3. Radical scavenging activity

The changes in RSA during storage of SJ samples are depicted in Fig. 3c in terms of % DPPH inhibition. The initial antioxidant activity of untreated SJ was 33.72 ± 2.74%. Irrespective of treatment type, RSA of processed samples was retained similarly to that of fresh SJ with insignificant changes ($p > 0.05$). In accordance with TPC results, HPP and PEF treated samples showed significantly higher antioxidant activity after processing compared to heat treated SJ ($p < 0.05$) (Table 2). Furthermore, it is noticeable to indicate that the effect of ultrasonication on RSA was statistically in between thermal pasteurization and other nonthermal processes at day 0. A significant increase was observed for RSA of all processed SJ samples after 7 days. Actually, this is in line with the results of TPC of SJ since phenolic compounds have been reported as important contributors to the antioxidant activity of berry fruits (Szajdek & Borowska, 2008). Jiang et al. (2015) have also reported increase in antioxidant activity at the early stages of storage of sonicated black mulberry juice. At the further stages of storage, antioxidant activities of processed SJ samples decreased in accordance with the reduced TPC and TAC levels. HPP, PEF, and US resulted in similar antioxidant activities at the final day of storage, which was statistically greater than that of the thermal treatment. Plaza et al. (2006) observed no significant differences among antioxidant activities of orange juice samples processed by HPP (400 MPa, 40 °C for 1 min), PEF (35 kV/cm for 750 μs), and mild heat treatment (70 °C for 30 s) after 40 days of storage at 4 °C, recommending HPP and PEF as alternative technologies (Plaza et al., 2006). Phenolic compounds and anthocyanins are natural antioxidants that can scavenge the free radicals in the media; thereby reduce the oxidative stress on human health (Giampieri et al., 2015). Martinez-Flores et al. (2015) pointed out that fruit juices processed by nonthermal technologies can exert higher radical scavenging activity due to better preservation or enhancement of bioactive compounds that contribute to the antioxidant activity of the product, supporting the results of this study. Reduction in antioxidant activity at the later stages of storage has been associated with polymerization reactions of phenolic compounds that would further reduce the availability of hydroxyl groups (Pinelo et al., 2004).

3.4. Multivariate analysis

PCA enabled visualization of the data structure for physicochemical properties and phytochemical characteristics of thermally pasteurized, ultrasonicated, high pressure processed and PEF-treated samples during storage. Control sample was only included at the beginning of storage (day 0) since the microbial load increased afterwards. Score plot in Fig. 4a discriminates the treatments by showing their location and distance from the center based on the physicochemical properties and phytochemical characteristics given in the loading plot (Fig. 4b). The loading plot demonstrates the distribution of physicochemical properties and phytochemical characteristics in space defined by the first and second PCA dimensions. Simultaneous evaluation of Fig. 4a and b indicates that samples located close to each other in score plot present similar attributes in terms of the quality characteristics given in the corresponding region of the loading plot. In this respect, the first principal component (PC 1) grouped the samples from the first 14 days of storage and separated from the rest due to the losses in mainly phytochemical characteristics of SJ during the continuation of the storage. According to the second principal component (PC 2), HPP and PEF treatments are located away from thermal pasteurization due to their higher content of phytochemical characteristics and titratable acidity as well as lower content of TSS and pH value compared to thermally pasteurized and untreated SJ. Thus, it is remarkable to state that processed samples stored up to the first 14 days fell close to each other except for the thermally pasteurized ones. This is because the HPP and PEF-treated samples from the first 14 days of storage showed greater phytochemical characteristics than control and heat-treated samples. Ultrasonicated samples are located in-between thermal pasteurization and HPP, PEF treatments (Fig. 4a).

The loadings of PCA are given in Table 3; it can be observed that all factors had loadings>0.3 for PC 1. However, TPC made the highest contribution (0.497). On the other hand, RSA and titratable acidity were the main contributors to PC 2 with values of 0.590 and 0.580, respectively. Eigen analysis shows how much variance could be explained by

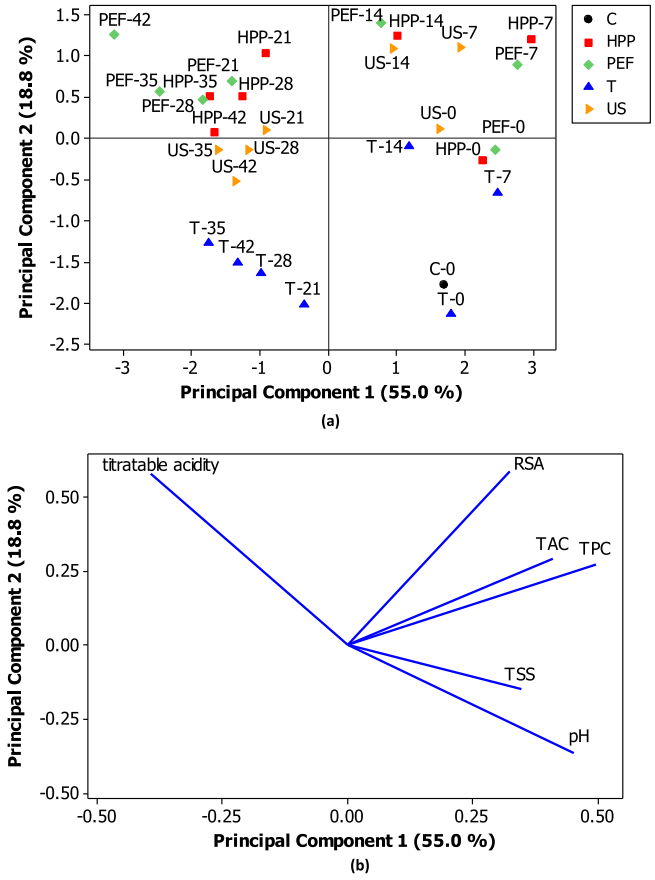

Fig. 4. PCA score (a) and loading (b) plots of principal component analysis for differentiation of mild pasteurization treatments based on physicochemical and phytochemical attributes of strawberry juice during storage. (C: untreated, US: ultrasonication, T: thermal, HPP: high pressure processing, PEF: Pulsed electric fields. The numbers given after hyphen indicates the storage day. For instance, HPP-7 represents the HPP treated sample on the 7th day of storage.)

Table 3
Loadings and eigen analysis for principal component analysis of storage study.

Factor loadings	PC 1	PC 2
TSS (°Brix)	0.35	−0.15
pH	0.45	−0.37
Titratable acidity	−0.39	0.58
TPC	0.50	0.27
TAC	0.41	0.29
RSA	0.32	0.59
Eigen analysis	**PC 1**	**PC 2**
Eigen value	3.29	1.13
Proportion of variance	0.55	0.19
Cumulative variance	0.55	0.74
% Cumulative variance	55	73.8

TSS, TPC, TAC, RSA refer to total soluble solids, total phenolic content, total anthocyanin content, radical scavenging activity, respectively.

each factor either in proportion or cumulative. PC 1 and PC 2 explained 55% and 18.8% of the total variance, respectively. Hence, PCA could cumulatively explain the 73.8% of total variance of this data set (Table 3). PCA has been applied by several researchers for the estimation of the relationships between different innovative processing technologies and quality parameters of the treated products including grapefruit juice (Aadil, Zeng, Zhang, et al., 2015), apple juice (Abid, Jabbar, Hu, et al., 2014), a lemon-melon juice blend (Kaya et al., 2015). Abid, Jabbar, Hu, et al. (2014) discriminated the combined US-HPP treated apple juice from the rest by explaining 84% of the total variance considering ascorbic acid, phenolic compounds, and radical scavenging activity. Kaya et al. (2015) were also able to distinguish untreated, UV-C or heat treated lemon-melon juice blends with respect to their physicochemical properties during storage of 30 days. Even though the level of explanation of total variance was lower (51.1%) compared to the current study, the authors were able to obtain a clear discrimination of the treatments.

The scores of PCA model were used as an input for HCA where Ward linkage and Euclidean distance were applied. Fig. 5 shows the similarities and differences among juice samples subjected to different processing technologies and subsequent refrigerated storage. Untreated SJ at the processing day and treated SJ stored up to the 14th day showed similar properties in terms of physicochemical properties and phytochemical characteristics by locating close to each other in the dendrogram. A deeper look at this region indicates that HPP and PEF treatments resulted in being close to each other due to their enhanced phytochemical characteristics (especially TPC and RSA) compared to untreated juice, while heat-treated and sonicated samples showed lower content of bioactives compared to HPP and PEF. Likewise, SJ samples collected after 21 days showed similar attributes by gathering together at the right-hand side of the graph.

4. Conclusions

This work focused on the comparative evaluation of the shelf life of SJ subjected to mild pasteurization by equivalent ultrasonication, high pressure, and pulsed electric fields processing. US, HPP, and PEF differed from each other in terms of their effect on SJ quality during storage while they were equivalent in terms of *E. coli* inactivation. It could be concluded that,

- Based on total aerobic bacteria and yeast-mold counts, the shelf life of fresh SJ was extended at least 42 days by thermal pasteurization (72 °C, 15 s), ultrasonication (55 °C, 3 min), and HPP (300 MPa, 1 min) by keeping the microbial counts around 2 log CFU/mL. PEF (35 kV/cm, 27 μs), on the other hand, prolonged the shelf life by at least 28 days since a significant microbial growth was observed at the 35th day.
- Considering both natural microbiota inactivation and quality retention, HPP was superior to PEF, US, and heat treatments in terms of SJ shelf life extension and enhanced phytochemical characteristics under the selected processing conditions.
- Phytochemical characteristics (TPC, TAC, and RSA) of SJ were significantly decreased by thermal pasteurization compared to HPP and PEF right after processing. However, irrespective of treatment types, a noticeable decrease was observed for the phytochemical content of SJ at the end of refrigerated storage. This could be a consequence of the residual enzymes acting on the degradation of antioxidant compounds. Still, HPP retained significantly higher levels of total anthocyanin and antioxidant activity at the final day of storage.
- Multivariate data analysis was a satisfactory tool for differentiation of the impact of different SJ pasteurization technologies and storage time while simultaneously evaluating several quality attributes.
- The equivalent processing approach avoids over or insufficient processing and establishes a baseline to make a relevant comparison among SJ samples treated by three different nonthermal technologies.

In summary, ultrasound at mild temperatures, HPP, and PEF processes can be considered as alternative methods to thermal pasteurization for the shelf life extension and bioactive compound retention of processed strawberry juice. Among these three nonthermal technologies, HPP is the best option to provide both extension of shelf life and better retention of phytochemicals under equivalent processing conditions as an alternative to the heat treatments.

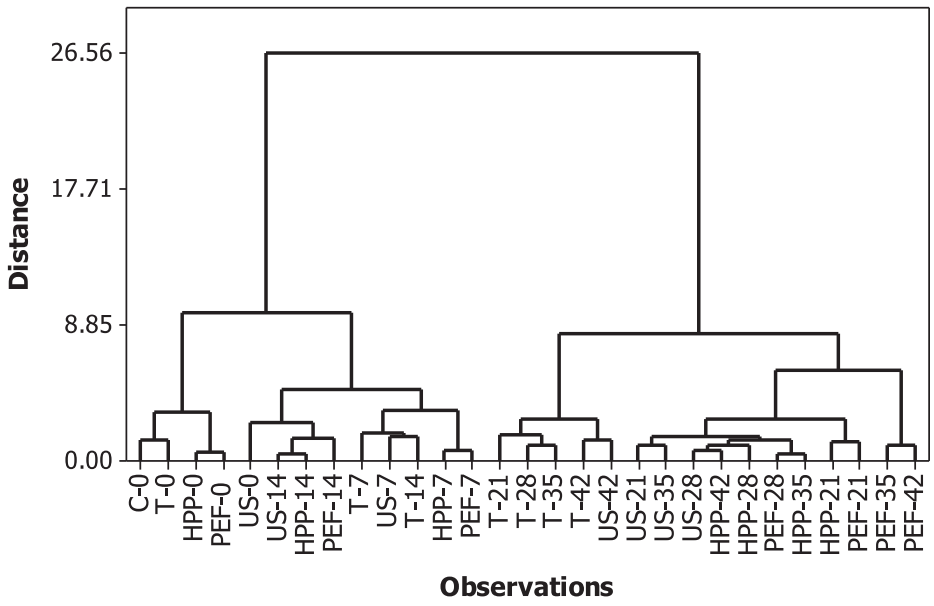

Fig. 5. Dendrogram as an output of hierarchical cluster analysis for classification of strawberry juice stored at 4 °C for 42 days. (C: untreated, US: ultrasonication, T: thermal, HPP: high pressure processing, PEF: Pulsed electric fields. The numbers given after hyphen indicates the storage day. For instance, HPP-7 represents the HPP treated sample on the 7th day of storage.)

CRediT authorship contribution statement

Semanur Yildiz: Methodology, Investigation, Formal analysis, Writing - original draft, Visualization, Writing - review & editing. **Prashant Raj Pokhrel:** Methodology, Investigation. **Sevcan Unluturk:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Gustavo V. Barbosa-Cánovas:** Conceptualization, Methodology, Investigation, Visualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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