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Determining the effect of high hydrostatic pressure on the refrigerated stability of avocado puree

Sinan Uzunlu[1*]

Abstract

The present study aimed to extend refrigerated stability of locally grown avocado fruit by applying High Hydrostatic Pressure (HHP) treatment. HHP was applied at 600 MPa for 3 min to avocado puree and stored at 4 °C for 28 days. Physicochemical (colour CIE *L**, *a**, *b**, total oil amount, chlorophyll, pH, moisture) and microbiological (mesophilic, yeast-mould) analyses were performed at seven-day intervals on both control (untreated) and HHP-treated sample packs. It could be judged that HHP treatment effectively controlled the colour indices, preventing undesired changes during the cold storage period of avocado puree. In conclusion, this study demonstrated that HHP-processed avocado puree exhibited stability for 28 days compared to unprocessed puree at 4 °C. The shelf-life stability of avocado puree under chilled conditions was extended from 7 to 28 days.

Keywords Avocado, Cold pasteurisation, High hydrostatic pressure, Puree

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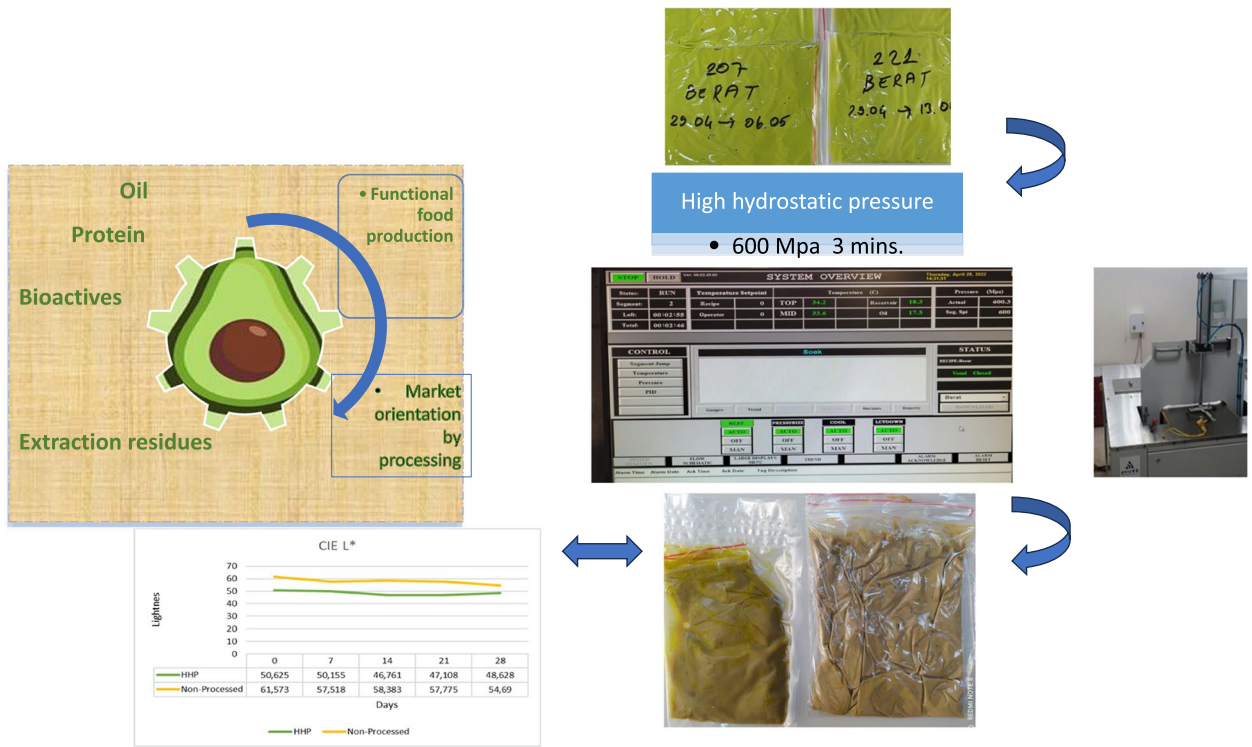
Graphical Abstract



Introduction

Avocado is a popular tropical fruit known for its numerous health-benefiting nutritional attributes (Rodríguez López et al. 2020). Native to South and Central America, the avocado (*Persea americana* Mill.) is widely cultivated and consumed worldwide (Lara-Flores et al. 2018). In the case of Türkiye, 2021 statistical data reveals that avocado cultivation in Türkiye amounts to 9,081 tons annually, steadily increasing each year due to high demand for local consumption and import potential (Er et al. 2023). Türkiye, a producer of various tropical fruits in its Mediterranean region, consistently increases its avocado production volumes. Avocado is locally cultivated in the Alanya district of Antalya Province, taking the lead across the Mediterranean region. The most grown varieties of the fruit in the Alanya district include Hass, Bacon, Fuerte and Zutano.

Avocado fruit is rich in protein, fibre, and various beneficial substances (Anusha & Bishnoi 2023). Maintaining a healthy lifestyle has become a global challenge for many consumer groups; therefore, avocado consumption has increased throughout the world (Rahmani et al. 2017). Unfortunately, fruits and vegetables have a short shelf life due to intrinsic enzyme degradation, temperature fluctuations, microbial growth, and physical stresses (Elez-Martínez et al. 2005). Controlling enzymatic browning during avocado fruit preparation and storage is critical to preserve the fresh appearance of the fruit. Furthermore, quality degradation is accelerated by enzyme-mediated oxidative processes in the aqueous fraction, where phenolic substrates are hydroxylated and then oxidised, resulting in the generation of brown compounds (Robards et al. 1999; Pourcel et al. 2007; Higuera-Rubio et al. 2022; Makboriboon et al. 2023).

Meanwhile, low-quality fruits face challenges in establishing a presence in local, national, and international markets. Therefore, the need for producing a marketable product from the fruit is much fulfilled by processing the fruit to puree form. Avocado puree has a limited shelf life (approximately 5 days) compared to whole fruit. The oxidative processes, affecting both lipid and aqueous components, play a significant role in causing quality losses (Purroy Balda et al. 2011; Rosenthal et al. 2018).

High Hydrostatic Pressure (HHP), as a cold pasteurization technique, has been widely utilized and approved by many countries for several years among non-thermal technologies. Since the early eighties, meat, orange juice, and avocado paste (guacamole) have

been successfully processed using HHP treatment (Ma & Ledward 2013). The application of HHP processing for avocado pulp paste, guacamole and other products is implemented by many leading producers globally. It is reported that more than forty avocado paste producers utilize HHP machines in the USA, Mexico and Spain (Houška et al. 2022).

Several researchers have treated avocados at 600 MPa including Tabilo-Munizaga et al. (2005), Jacobo-Velázquez & Hernández-Brenes (2010), Woolf et al. (2013), Rodríguez López et al. (2020), and Sarantakou et al. (2023). The most advantageous aspect of this treatment was reported as no change in the colour, flavour, and nutritional quality of the subjected foods. For instance, Ala˜n´on et al. (2022) found that HHP processed guacamole sauce exhibited good sensorial quality in their comparative study between canned (sterilized) and HHP processed guacamoles.

To retard enzymatic browning and inactivate indigenous microbial flora, avocado fruit is treated to HHP for 3 min at 600 MPa at ambient temperature as an industrial norm globally (Jacobo-Velázquez & Hernández-Brenes 2012).

Aligned with the worldwide processing of avocado fruit, this study aimed to assess the refrigerated stability of high-pressure-treated avocado puree. Physicochemical parameters (colour CIE L^*, a^*, b^*, total oil amount, chlorophyll, pH, moisture) and microbiological (aerobic mesophilic, yeast-mould) parameters were monitored at seven-day intervals for four weeks. To the best of our knowledge, this will be the first study conducted in the Alanya region on locally grown avocados (*Persea americana* Mill.) of the Hass cultivar.

Materials and methods

Materials

Avocado (*Persea americana* Mill.) cv. Hass fruits were supplied from local producers, with a total of twenty fruits designated as samples. Ten of these samples were used for HHP treatment, while the remaining ten were allocated to the control group. Plate count agar (PCA), maximum recovery diluent (MRD) and yeast extract glucose chloramphenicol agar (YGC) were purchased from Merck (Darmstadt, Germany). Citric acid (E 330), acetone and petroleum ether were purchased from Sigma–Aldrich (United Kingdom).

Methods

High hydrostatic pressure (HHP) treatment

Ripened fruits were initially disinfected with 70% v/v ethanol. Fruits were then peeled and mashed by using a kitchen-type grinder, and the pH value was adjusted to 4.0 with citric acid. The obtained puree samples were immediately sealed in hundred-gram polyethylene (PE) bags and stored at 4 °C. Samples were then vacuum-packed and transported under a cold chain to Bolu, a province in Türkiye. The HHP treatment was applied at room temperature (22 °C) for 3 min at a pressure level of 600 MPa in the facilities of Bolu Abant İzzet Baysal University of Yenilikçi Gıda Teknolojileri Geliştirme Uygulama ve Araştırma Merkezi (YENİGIDAM). The pilot-scale (high hydrostatic pressure) HHP system (Avure, Middletown, OH, USA) with the treatment chamber unit of 2-L capacity, was employed. In each batch five individual sample packs, each containing a hundred grams, underwent HHP treatment and were coded as 100, 107, 114, 121, and 128. Meanwhile, control group samples (untreated) were coded as 200, 207, 214, 221 and 228 for periodic analyses. All individual sample packs were stored at 4 °C until the day of sampling.

Physicochemical analyses

A Konica Minolta (Japan) device was used for colour (CIE L^*, a^*, b^*) determinations. To determine the total oil amount, samples were dried on an aluminium sheet at 55 °C for 48 h. Samples were then processed in a Gerhard Oil Extraction (Germany) instrument, with petroleum ether employed as a solvent. To evaporate the solvent from the sample, the bottles were left in an oven (Memmert, Germany) at 55 °C. The remaining amount was calculated and expressed as a percentage. For chlorophyll analysis, the Arnon (1949) method, as referenced in Kumar et al. (2015), was employed. In brief, 0.1 g of each sample was gently mixed in 5 mL of 80% acetone. After centrifugation at 3000 rpm for 5 min, the supernatant was transferred to a blank tube. The UV- VIS spectrophotometer (Shimadzu UV-1280) was filled with 80% acetone. In another tube, the same amount of acetone and 6 drops of pigment extract were added and mixed. Absorbance data at 645 and 663 nm were collected and substituted into the formula given below:

$$\Sigma chlorophyll(mg/1000mL) = 20.2A645 + 8.02A663$$

To determine the pH of the samples, ten grams of each sample were homogenized in 90 mL of distilled water using a pH meter (Milwaukee Mİ 151). An average of three measurements was taken, following the Association of Official Analytical Chemists (AOAC) guidelines (Anonymous 2000). For moisture analyses, five grams of each sample were dried in an oven at 105 °C until the samples reached a constant weight (Cemeroğlu 2007).

Microbiological analyses

For serial decimal dilutions, ten grams of puree samples were aseptically weighed in sterile bottles containing 90 mL of Maximum Recovery Diluent (MRD) and

gently homogenized. Appropriate dilutions were then immediately spread onto Plate Count Agar (PCA) plates. Incubations were carried out at 35 °C for mesophilic microorganisms for 24–48 h. Yeast Extract Glucose Chloramphenicol Agar (YGC) plates were utilized for yeast-mould analyses, with the incubation period set at 4 to 5 days at 25 °C (Halkman 2019).

Statistical analyses

Analysis of variance (ANOVA) was conducted using SPSS (Statistical Package for Social Sciences) software, version 21 (IBM, United States). Subsequently, Duncan's post hoc test was applied at a significance level of $P<0.05$, following the methodology outlined by Steel & Torrie 1980.

Results and discussion

Physicochemical analyses

Lightness is the most determinative parameter in the enzymatic browning of avocados (Ramos-Villarroel et al. 2011). In the colour analysis, the initial CIE L^* values of untreated fresh avocado puree samples (control group) were 61.57 ± 1.85. These CIE L^* values decreased to 54,69 ± 1.09 at the end of cold storage, and this difference was found to be statistically significantly ($P<0.05$), indicating a darkening of the samples. In contrast, in the HHP-treated group samples, CIE L^* values showed a slight decrease, and this decrease was statistically insignificant ($P>0.05$).

Palou et al (2000) recorded the lightness of guacamole samples (Hass cultivar) at a pH of 4.3 as 64.35 in control samples, while HHP treatment at 689 MPa for 5 min yielded a lightness of 63.19. López-Malo et al (1998) found the lightness of the control group of avocado samples (Hass cultivar) at a pH of 4.1 as 62.41 and 59.99 in HHP-treated samples at 689 MPa for 10 min. In our samples (at a pH of 4.0), however, we found a lightness value of 61.57, which is very similar to earlier findings. Nevertheless, a significant decrease was observed after HHP treatment at 600 MPa for 3 min in our Hass cultivar puree samples (Table 1). The decrease might have arisen from the exceeded holding time (8 h) of puree samples under cold chain until the HHP treatment in our study, where Palou et al (2000) held no longer than 5 h at 5 ± 0.5 °C. Additionally, a study documented that among different avocado varieties (Bacon, Zutano, Fuerte, Hass), the lightness index of the Hass cultivar was the lowest, grown in Antalya, the primary avocado-growing province in Türkiye (Gölükcü 2006).

As the most significant indices of avocado colour, the negative CIE a^* values serve as indicators of greenness in avocado. The initial CIE a^* values of untreated fresh avocado puree samples (control group) were found to be -9.44 ± 0.04. Similarly, Gölükcü (2006) observed CIE

Table 1 Physicochemical changes of avocado puree as affected by HHP [a]

DAYS	0	7	14	21	28
Total oil (%)					
HHP	20,98 ± 0.15a	19,95 ± 0.93ab	20,48 ± 0.83ab	18,38 ± 0.51ab	17,81 ± 1.18b
Control	21,36 ± 0.53ab	22,98 ± 0.39a	21,21 ± 0.63ab	21,98 ± 0.16ab	20,77 ± 0.69b
Chlorophyll (mg/1000 mL)					
HHP	67,04 ± 0,04a	62,15 ± 0,95b	62,79 ± 0,41b	58,16 ± 0,66c	53,31 ± 0,19d
Control	67,05 ± 0,05bc	72,69 ± 2,51a	71,14 ± 0,84ab	70,65 ± 0,45ab	64,96 ± 0,36c
pH					
HHP	4,00 ± 0,00a	3,99 ± 0,03a	4,06 ± 0,10a	4,07 ± 0,11a	3,96 ± 0,00a
Control	4,06 ± 0,06a	4,08 ± 0,08a	4,09 ± 0,11a	3,98 ± 0,02a	3,95 ± 0,01a
L^*					
HHP	50,75 ± 0.60a	50,74 ± 0.60a	48,11 ± 1.35a	49,05 ± 1.95a	52,18 ± 3.60a
Control	63,41 ± 1.85a	58,82 ± 1.32ab	60,10 ± 1.72ab	59,01 ± 1.24ab	55,77 ± 1.09b
a^*					
HHP	-2,79 ± 0.14a	-2,76 ± 0.11a	-1,62 ± 0.69a	-1,99 ± 0.57a	-2,25 ± 0.52a
Control	-9,48 ± 0.04d	-0,49 ± 0.11c	0,99 ± 0.12b	1,19 ± 0.03b	1,93 ± 0.14a
b^*					
HHP	25,75 ± 0.56a	25,66 ± 0.47a	22,27 ± 0.29b	22,43 ± 0.29b	23,10 ± 0.37b
Control	31,44 ± 0.08a	23,77 ± 0.77b	21,00 ± 0.43c	20,82 ± 0.30c	18,66 ± 0.52d

[a] Values are means ± SD

Values in rows with different letters differ significantly ($P<0.05$)

HHP High hydrostatic pressure treated samples

a^* values in puree samples of the Hass cultivar as -9.92. However, during the cold storage period, CIE a^* values were increased significantly ($P < 0.05$) in our control group samples, indicating a shift towards redness. Gölükcü (2006) also noted an increase in CIE a^* values in puree samples from -9.92 to -5.40 on the thirtieth day of 4 °C storage. Conversely, in our HHP-treated group samples, CIE a^* values showed a slight increase during 4 °C storage, but this increase was statistically insignificant ($P > 0.05$).

The findings of López-Malo et al. (1998) and Palou et al. (2000) are consistent with our results regarding CIE a^* values, both in untreated fruit (control) samples and HHP-treated samples. HHP-treated samples exhibited variations by increasing the a^* values depending on the storage temperature. Storage at 5 °C resulted in a slight increase compared to 25 °C during a storage period of more than 30 days (López-Malo et al. 1998).

The initial CIE b^* value of untreated fresh avocado puree samples (control group) was 31.36 ± 0.08. CIE b^* values exhibited a significant ($P < 0.05$) decrease during the cold storage period in the control group samples. This signifies that the samples became darker due to a reduction in their yellowness, reaching an unmarketable colour by day three (data not shown), rendering the product unattractive. Even HHP-treated avocado puree samples stored at 25 °C displayed severe browning reactions within a few days, as reported by López-Malo et al. (1998). Additionally, in our HHP-treated group samples, CIE b^* values showed a slight decrease. The data from the first week (0 and 7th day) were significantly ($P < 0.05$) different from the data from the last three storage weeks (2nd, 3rd and 4th weeks). However, the CIE b^* values for the last three weeks remained similar and were found to be statistically insignificant ($P > 0.05$).

López-Malo et al. (1998) and Palou et al. (2000) identified CIE b^* values of 35.18 and 36.24, respectively, similar to our findings in untreated control samples. Both research groups highlighted that the yellow component of the fruit remained relatively constant during the storage period, which aligns with our findings (data not shown). Palou et al. (2000) further stated that applying HHP at 517 or 689 MPa when the pH of the puree is 4.1 or 3.9 maintains an acceptable colour during chilled storage for one month. Woolf et al. (2013) determined that high pressure, but not the duration of the treatment had a significant effect on the tissue colour of the fruit. Additionally, Houśka et al. (2022) documented that HHP processing retains the green colour of avocado paste, while traditional heat treatments result in browning. In summary, it can be inferred that HHP treatment effectively controlled the colour indices, preventing an undesired change during the cold storage period of avocado puree.

In the case of the chlorophyll analyses, there were decreases observed during the cold storage period in both sample sets (control and HHP-treated), and these decreases were found to be significant ($P < 0.05$). Palou et al. (2000) attributed the decline in greenness to chlorophyll degradation in their work. Additionally, Cox et al. (2004) found that the chlorophyll content of avocado fruit skin decreased initially in the postharvest period, but later in ripening, there was little change.

The total oil amounts of the control group samples remained relatively stable during the cold storage period, with only slight and statistically significant ($P < 0.05$) changes observed among the control group data (Table 1). In contrast, HHP-treated samples exhibited significant ($P < 0.05$) changes between the first and last days of storage. This change might be attributed to the high-pressure treatment. Zulkurnain et al. (2016) documented that high pressure treatment results in a reduction of approximately 17 to 30% in lipids, identified as one of the most pressure-sensitive biological components. Additionally, high pressure treatment is reported to induce phase transformation behaviour, alter the structure and physiology of biomolecules, and lead to lipid oxidation.

Throughout the world, the most cultivated avocado varieties include Hass, Bacon, Fuerte, Zutano, and Pinkerton. Hass, with an approximate 20% oil content, is the richest oil-containing variety and the most extensively grown globally, accounting for over 80% of cultivation due to its high lipid content and sensorial quality (Ferreyra et al. 2016; Ramírez-Gíl et al. 2019; Tan 2019; Sarantakou et al. 2023). Our findings (Table 1) align with the literature.

Regarding pH values, control samples were initially measured as 4.0 on day 0. Over 21 days of cold storage, the pH values of the control samples decreased to 3.94. For the HHP-treated samples, the pH values were initially 4.0 on day 0, decreased to 3.96 on day 7, and remained stable until the end of the storage period (Table 1). Therefore, no significant ($P > 0.05$) changes were observed for either sample set during cold storage.

Microbiological analyses

The total aerobic mesophilic microbial load of the control group samples was 5.7×10^6 (6.75 log) CFU/g on day zero. The findings of microbial load on day zero for a fresh fruit was evaluated as higher than expected. After a week of cold storage, the microbial load decreased by 2 logs (Table 2). This decrease is highly likely dependent on the acidification of puree to pH 4.0, considering the initial pH was 5.7 in the fresh fruit.

However, on day 14, the microbial load increased by 2 logs, exceeding 8 logs. It is well-known that if a food

Table 2 Microbial changes of avocado puree as affected by HHP [a]

DAYS	0	7	14	21	28
TAMB (CFU/g)					
HHP	6,75 ± 0,00a	4,64 ± 0,04c	5,12 ± 0,01b	5,14 ± 0,03b	5,23 ± 0,09b
Control	6,75 ± 0,00c	4,57 ± 0,07d	8,52 ± 0,05b	10,30 ± 0,19a	-
Yeast and Mould (CFU/g)					
HHP	4,97 ± 0,01a	4,49 ± 0,01b	1,15 ± 0,01d	1,21 ± 0,10 cd	1,42 ± 0,08c
Control	5,00 ± 0,04b	4,49 ± 0,08b	4,52 ± 0,02b	4,54 ± 0,01b	7,33 ± 0,37a

[a] Values are means ± SD

Values in rows with different letters differ significantly ($P < 0.05$)

HHP High hydrostatic pressure treated samples

TAMB Total aerobic mesophilic bacteria

exceeds 7 logs of microbial load, it is considered spoiled. For instance, Mastromatteo et al. (2012) studied packaging strategies to prolong the shelf life of fresh carrots (*Daucus carota* L.), highlighting a microbial load of 5×10^7 CFU/g as the threshold for total mesophilic bacteria, as imposed by French Regulation. This implies that the product is considered spoiled on day 14. Therefore, subsequent storage day analyses were not performed due to the microbial load exceeding 7 logs on day 14.

HHP-treated samples exhibited significant ($P < 0.05$) decreases starting from day seven, and the treatment stabilized the growth of microbial cells at approximately 5 logs (Table 2). From a food safety standpoint, it is evident that industrial production of avocado puree should benefit from HHP treatment.

López-Malo et al. (1998) reported low standard plate and yeast and mould counts on the order of 5.2×10^2 – 3.5×10^3 CFU/g on avocado (Hass cultivar) puree samples before HHP treatment. However, they observed spoilage within the first 5 days of storage at 5 °C in avocado purees without HHP treatment. High pressure yielded inactivation, reducing counts to lower than 10 CFU/g in acidified and sodium chloride-added avocado puree samples.

Similarly, Palou et al. (2000) found that the standard viable count of guacamole samples before processing was 1.2 to 1.3×10^4 CFU/g. They reported that HHP treatment at 689 MPa for 5 min reduced both total viable counts and yeast and mould to lower than 10 CFU/g.

Jacobo-Velázquez and Hernández-Brenes (2010) similarly found indigenous mesophilic aerobic bacterial counts in fresh unprocessed avocado fruit (Hass variety) of $2.4 \times 10^6 \pm 1.0 \times 10^6$ CFU/g. Applying HHP treatment at 600 MPa for 3 min reduced four logs of the aerobic plate counts. They also stabilized the microbial counts during 30 days of storage at 4 °C. However, due to the reparability of microbial cells, beyond day 40, a sudden increase was observed by researchers. More recently, Rodríguez López et al. (2020) determined a reduction of 3.28 log CFU/g (from 6.70 log in control sample, to 3.42 log in the pressurized sample) in total aerobic mesophilic counts of avocado paste (containing seasoning ingredients to meet Mediterranean guacamole standard) when processed at 600 MPa for 3 min and stored at 4 °C for 30 days.

In terms of yeast and mould analyses, on day 0, the microbial load was 5.02 log CFU/g. On subsequent storage days, the yeast and mould counts remained similar to those on day 0, with no significant ($P > 0.05$) change. However, on day 28, the growth exceeded 7 logs (Table 2). In contrast to the control group, HHP treatment reduced yeast and mould counts by 0.47 log CFU/g on day seven; however, the lethality increased with acidification, and the counts were further reduced to 1.15 log CFU/g on day fourteen and remained consistent on subsequent storage days (Table 2). Similar to our findings, Rodríguez López et al. (2020) found yeast and moulds in avocado paste (containing seasoning ingredients to meet Mediterranean guacamole standard) on day 30 at 4 °C storage. The control samples exhibited 6.46 log CFU/g, while HHP-treated samples, processed at 600 MPa for 3 min, showed less than 1.48 log CFU/g.

Conclusion

In conclusion, avocado puree samples achieved microbial stability for 28 days, while unprocessed (control) puree samples exceeded 8 logs on day 14 during refrigerated storage. Control samples exhibited unattractive sensory characteristics on day 3, whereas HHP-treated samples remained stable for 28 days. These results clearly demonstrate that avocado puree gained stability, both in terms of microbial and physicochemical aspects, through the application of HHP processing at 600 MPa for 3 min under ambient conditions.

Abbreviation

HHP High Hydrostatic Pressure

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