Effect of high pressure and thermal processing on quality changes of aloe vera-litchi mixed beverage (ALMB) during storage

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Abstract The effect of high pressure processing (HPP) (600 MPa/15 min/56 °C) and thermal processing (TP) (95 °C/5 min) on the quality characteristics of aloe vera-litchi mixed beverage samples (ALMB) stored at 4, 15 and 25 °C were studied. The total color difference and browning index of ALMB samples increased with the storage period for both HPP and TP treated samples under all storage conditions. HPP of ALMB resulted in inactivation of pectinmethylesterase (PME), polyphenoloxidase (PPO) and peroxidase (POD) to 34, 65 and 62 %, respectively after immediate processing, whereas, TP treatment lead to 83, 79 and 78 %, respectively. The residual activity of all the studied enzymes decreased with storage period. The ascorbic acid loss of up to 22 % was observed after HPP treatment and up to 31 % for thermally treated samples. Minimal changes were noted for phenolics content after HPP as well as thermal processing. The natural microbiota present in samples was below the detection limit (1 log CFU/mL) throughout the storage period. The shelf life of HPP and TP treated samples stored at 4 °C was estimated to be 100 and 80 days, respectively, based on the sensory quality, ascorbic acid degradation and instrumental color difference.

Research Highlights

- HPP treatment minimally affected ascorbic acid, phenolics and antioxidants.
- Microbial population in HPP treated ALMB was below detection limit (1 log CFU/mL).
- HPP of ALMB led to partial inactivation of indigenous enzymes.
- Extended shelf life of up to 100 days achieved for HPP treated ALMB.

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Introduction

Aloe vera (*Aloe barbadensis* Miller) is well known for its health benefits and is being used in cosmetics, medicines, ayurvedic formulations and also as a health supplement in food products. The major constituents in aloe vera are aloe polysaccharides, phenolics, minerals, vitamins and other compounds present in small amounts (Ahlawat and Khatkar 2011). Litchi (*Litchi chinensis*) is a sub-tropical fruit originated from China; however it is grown in many countries. China and India account collectively for more than 90 % of the world litchi production. Litchi is a non-climacteric fruit and is highly perishable with a very short shelf life (Nagar 1994).

Thermal processing (TP) is commonly used for the preservation of the fruit based products, however, it causes considerable reduction in the nutritional quality as well as sensory attributes which may not be acceptable from consumer point of view (Rattanathanalerk et al. 2005). Non-thermal technologies have been developed ensuring retention of nutritional and sensory characteristics of the food products. These different non-thermal technologies include irradiation, electromagnetic radiation, ultrasound, pulse light, pulsed electric field and high pressure processing. These technologies alone or in combination can be used to process the food products following the hurdle technology approach (Raso and Barbosa-Canovas 2003). Among the above mentioned non-thermal processing technologies, high pressure processing (HPP) has been studied extensively, and also being commercialised due to its effectiveness in addressing microbial safety, high nutritional and sensory quality attributes (Huang et al. 2014). It has been widely studied for fluid as well as semi-solid food

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products in diverse areas such as seafood, meat and poultry, dairy, fruit and vegetable based products. Unique advantage of HPP includes uniform intensity of pressure treatment throughout the food product irrespective of its shape, size and weight (Jolie et al. 2012).

The advantages of HPP in fruit based products have been reported in terms of complete inactivation of viable microorganisms (Raso and Barbosa-Canovas 2003), minimal changes in color, retention of vitamins, antioxidants and phenolic compounds and so forth (Rastogi et al. 2007). It also partially inactivates enzymes like polyphenoloxidase (PPO), peroxidase (POD), pectinmethylesterase (PME) etc. (Chakraborty et al. 2014a). Vega-Galvez et al. (2014) studied the effect of HPP (300–500 MPa/3 min) on quality of aloe vera gel and reported minimal loss of vitamin C after 35 days of storage. Swami Hulle and Rao (2015) reported minimal changes in physicochemical and textural properties of aloe gel with pressure application. In another study with HPP of aloe vera gel (350 MPa/30 s), enhanced antioxidant activity was reported and compared to blanching and microwave treatments (Vega-Galvez et al. 2011). Reyes et al. (2012) studied the HPP effect (300–500 MPa/1–3 min/20 °C) on microbial quality of aloe gel and reported that HPP at 400 MPa/3 min was able to keep the microbial population below detection limit for 90 days of storage period at 4 °C. Swami Hulle et al. (2014) studied the effect of HPP (60–700 MPa/3–40 min) on rheological, microbiological and PME enzyme activity of aloe vera gel and reported the reduction of natural microbiota below the detection limit (1 log CFU/mL) along with inactivation of PME up to 30 %.

The HPP effects on litchi have been reported in terms of the inactivation of PPO activity up to 51 % along with minimal color changes and anthocyanin content for samples processed at 600 MPa/30 min and 50 °C/20 min (Dajanta et al. 2012). Jayachandran et al. (2015) studied the effect of HPP (200–600 MPa/30–70 °C/0–20 min) on litchi based mixed beverage. They reported retention of ascorbic acid (up to 73 %), increased phenolics content (up to 12 %) and antioxidant capacity (up to 19 %). Phunchaisri and Apichartsrangkoon (2005) studied the varying conditions of HPP (200–600 MPa/20–60 °C/10 or 20 min) on PPO and POD enzyme activities. They reported increased POD activity at low pressures (200 MPa) whereas, samples treated at 600 MPa/60 °C resulted in PPO and POD inactivation of 90 and 50 %, respectively, indicating POD as the most baro-resistant compared PPO.

The objective of the present work was to study the effect of HPP on different quality attributes and shelf life of an aloe vera-litchi mixed beverage (ALMB) under different storage and packaging conditions. The quality attributes considered were microbial quality, enzyme activity, ascorbic acid, phenolics content, instrumental color and sensory attributes.

Material and methods

Sample preparation

Fresh litchi (*Litchi chinensis* cv. Bombai) fruits and aloe vera (*Aloe barbadensis* Miller) leaves of uniform size and maturity were obtained from the research farm of Agricultural and Food Engineering Department, IIT Kharagpur. They were washed and disinfected with hypochlorite solution (100 ppm) in water to remove surface dirt and microbial load. Litchi juice was extracted from the aril portion of the fruit by grinding in domestic blender followed by filtration through the muslin cloth to get the litchi juice. In case of aloe vera juice extraction the leaf after washing, was trimmed at the bottom and kept vertically for about one hour to drain yellow liquid from the aloe vera leaf. The side edges along with upper and lower rind portion were removed to get the inner fillet portion. The fillets obtained were crushed and filtered to get the aloe vera juice.

The optimum formulation with aloe vera: litchi juice as (15: 85 %, v/v) was used to prepare an aloe vera-litchi mixed beverage (ALMB). The optimum concentration was selected based on a preliminary formulation study considering the sensory acceptability (data not presented here). Freshly prepared ALMB (100 mL) was packed in EVOH (ethyl vinyl alcohol polymeric film), and multilayer metalized polyester film (co-extrudate of LD, HD & LLDPE) pouch of 105 µm thickness. The sample packets were stored in chilled condition (4 °C) until subjected to high pressure and thermal processing treatments.

Processing and storage conditions

The HPP of ALMB samples was carried out using lab-scale HPP unit (Model: S-IL-100-250-09-W; Make: Stansted Fluid Power Ltd., UK). The double packed samples were inserted into the pressure vessel and treated at 600 MPa/15 min/56 °C. The rate of pressurization was fixed 300 MPa/min, whereas the decompression was instantaneous. Aqueous monopropylene glycol 30 % (v/v) was used as pressure transmitting fluid. Temperature control during each test run was controlled using circulating system around the pressure vessel.

The thermal processing (TP) of ALMB samples was carried out in a precision water bath with digital temperature controller, in which, the sample pouches were heated at 95 ± 1 °C for 10 min. After TP treatment, ALMB samples were immediately cooled down to room temperature by dipping in cold water, and kept in refrigerated condition (4 °C) until further use. A thermocouple was inserted into one specimen sample pouch to estimate the time required to achieve the target temperature. The untreated, HPP and thermally treated ALMB samples were stored in dark at different temperature conditions of 4, 15 and 25 °C. The different quality

attributes of ALMB samples were analysed periodically. The ALMB samples were stored at 4, 15 and 25 °C for the period of 120, 60 and 30 days, respectively.

Physicochemical analysis

The physicochemical analysis of beverage samples was carried out in terms of pH, TSS, ascorbic acid, and color. The total soluble solids content was measured with digital refractometer (Model: PAL-1; Make: Atago, Japan). The pH of the beverage sample was measured using pH meter (Model: CL 46+; Make: Toshcon, India). The ascorbic acid (AA) content of ALMB samples was measured using 2,6-dichlorophenol-indophenol visual titration method as described in detail by Ranganna (2007) and expressed as mg/100 mL.

Color measurement of all the ALMB samples was carried out at room temperature (25 ± 2 °C) using a portable colorimeter (Model: Spectro-guide 45/0; Make: BYK Gardner, Germany). The color values were measured in terms of the CIE color parameters L^* (lightness), a^* (red to green) and b^* (yellow to blue). The total color difference was calculated using formula:

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{0.5} \quad (1)$$

Where, $\Delta L^* = (L_1^* - L_0^*)$, $\Delta a^* = (a_1^* - a_0^*)$ and $\Delta b^* = (b_1^* - b_0^*)$. The subscript '1' indicates color values of treated samples and '0' for the color values of untreated/reference samples. All the measurements were carried out in triplicate, and average values were reported. In addition to the color difference, browning index was also calculated using a formula described by Picouet et al. (2014):

$$BI = \left(\frac{100}{0.172} \times \left(\frac{a^* + 1.75 \times L^*}{5.645 \times L^* + a^* - 3.012} - 0.31 \right) \right) \quad (2)$$

where L^* , a^* and b^* values are independent color parameters of the sample.

Total phenolics

The total phenolics content was estimated using Folin-Ciocalteu method as described by Loots et al. (2007). The absorbance of the reaction solution was measured using UV-Vis spectrophotometer (Model: UV 1700; Make: Shimadzu, Japan). The total phenolics content is expressed as mg gallic acid equivalent/100 mL of ALMB juice.

Enzyme activity measurement

The pectinmethylesterase activity (PME) assay was carried out according to the method described in detail by Swami Hulle et al. (2014). The crude enzyme extract for

polyphenoloxidase (PPO) and peroxidase (POD) assay was prepared according to the method described by Chakraborty et al. (2014b) with some modification. In the PPO assay, 0.2 mL of the crude extract was mixed with 2.8 mL of 0.1 M catechol in 0.2 M SPSS buffer (pH 6.5) solution. Whereas, in the POD assay, 0.1 mL of crude extract was mixed 0.16 mL of (1 %, v/v) hydrogen peroxide solution and 0.32 mL of 5 % catechol in 2.42 mL 0.2 M SPSS buffer (pH 6.5). The absorbance of PPO and POD reaction mixtures was monitored at a wavelength of 420 nm for 15 min, at 25 °C in the UV-Vis spectrophotometer.

The protein concentration of the enzyme extract was estimated using Bradford assay with bovine serum albumin as standard for quantification (Bradford 1976). The enzyme activity is expressed as change in absorbance/s-mg of protein extracted. The assay for each sample was performed in triplicate and mean value is reported. The percent residual activity (RA) was calculated as:

$$RA = \frac{A}{A_0} \times 100 \quad (3)$$

where A and A_0 are the activity of treated and untreated samples, respectively.

Microbiological analysis

Microbiological quality of ALMB during storage was assessed in terms of aerobic mesophiles (AM) and yeast and molds (YM) based on their sensitivity to treatment and survival during storage. The detailed methodology for enumeration and incubation was followed as described by (Swami Hulle et al. 2014) with slight modification. In case of YM enumeration, the yeast and malt agar was used. The detection limit of the analysis was 1 log CFU/mL. All the samples were analysed in duplicate, and the mean values are reported.

Sensory Evaluation

Sensory acceptability of ALMB samples was carried out by trained sensory panel consisting of ten members. Before tasting the samples, panellists were briefed about the criteria for the assessment of quality attributes of ALMB samples. The sensory score were given on 9-point hedonic scale ranging from 1 ("Dislike extremely") to 9 ("Like extremely") (Raj et al. 2011). The quality attributes considered were color, aroma, consistency, taste and aftertaste.

Results and discussion

The effects of HPP and TP treatment on different quality attributes were assessed during storage at 4, 15 and 25 °C. Each

of the quality parameters is discussed in the respective section along with the comparison of the HPP and TP treated ALMB samples.

Effect of high pressure and thermal processing on color of ALMB during storage

The change in color of ALMB samples during storage at 4, 15 and 25 °C was measured; the total color difference and browning index values were calculated as represented in Table 1. The instrumental color values of fresh ALMB samples in terms of L^* , a^* and b^* values were 43.27, -2.18 and 0.37 respectively and immediately after processing an increase in all the color coordinates was observed. Similar observations have been reported earlier for litchi (Kaushik et al. 2014; Phunchaisri and Apichartsrangkoon 2005). As storage progressed L^* values decreased, however, a^* and b^* values increased significantly ($p < 0.05$). The values of L^* , a^* and b^* for HPP treated ALMB samples stored at 4 °C varied from

41.44 to 46.42, -2.33 to -0.1 and 1.08 to 4.85 , respectively. Whereas, for TP treated ALMB samples the L^* , a^* and b^* values ranged from 43.12 to 47.76, -1.72 to -0.36 and 2.14 to 5.39 , respectively. Figure 1 represents the changes in visual color of the ALMB samples at different days of the storage. Initially after 30 days of storage, minimal changes in colour were observed in HPP treated samples stored at 4 °C compared to TP treatment, whereas, samples stored at 15 and 25 °C showed browning. This indicated that temperature during storage can play an important role in preventing the browning reaction in HPP treated samples. The higher browning rates along with the increase in storage temperatures have been previously reported for Naval orange juice (Polydera et al. 2005b). They observed higher activation energy in case of HPP treated orange juice samples indicating greater temperature dependence of the browning rate compared to TP treated ones. Similar observation in the case of ALMB was noted, where, at the end of the storage period HPP treated samples stored at 4 °C appeared to be more brownish

Table 1 Effect of HPP and Thermal treatments on color difference (ΔE^*) and browning index (BI)

Treatments	HPP - EVOH - 4 °C		HPP - MetP - 4 °C		Thermal - EVOH - 4 °C		Thermal - MetP - 4 °C	
Storage (days)	ΔE^*	BI	ΔE^*	BI	ΔE^*	BI	ΔE^*	BI
0		1.25 ^a		1.25 ^a		1.88 ^a		1.88 ^a
7	0.41 ^a	1.52 ^b	0.80 ^a	1.37 ^b	0.30 ^a	2.59 ^b	1.17 ^a	3.80 ^b
15	0.72 ^b	2.34 ^c	0.93 ^b	1.75 ^c	0.56 ^b	3.26 ^c	1.94 ^b	5.83 ^c
30	1.3 ^c	3.41 ^d	1.68 ^c	1.80 ^d	0.97 ^c	3.62 ^d	2.21 ^c	6.22 ^d
45	1.91 ^d	4.54 ^e	1.74 ^d	3.91 ^e	0.99 ^c	3.58 ^d	2.46 ^d	7.00 ^e
60	2.35 ^e	5.28 ^f	2.24 ^e	4.78 ^f	1.83 ^d	3.92 ^e	2.59 ^e	7.30 ^f
70	2.77 ^f	6.38 ^g	2.49 ^f	5.34 ^g	2.02 ^e	5.94 ^f	2.90 ^f	8.33 ^g
80	3.15 ^g	6.85 ^h	3.25 ^g	7.74 ^h	2.35 ^f	7.27 ^g	3.35 ^g	9.33 ^h
90	3.68 ^h	7.86 ⁱ	3.53 ^h	8.57 ⁱ	2.61 ^g	7.85 ^h	3.49 ^h	9.53 ⁱ
100	3.61 ⁱ	9.05 ^j	3.95 ⁱ	9.68 ^j	2.66 ^h	7.68 ⁱ	3.88 ⁱ	10.20 ^j
110	4.48 ^j	10.01 ^k	4.45 ^j	11.12 ^k	3.95 ⁱ	9.32 ^j	4.13 ^j	10.92 ^j
120	5.33 ^k	11.3 ^l	4.71 ^k	11.08 ^k	3.85 ^j	10.93 ^k	4.42 ^k	11.73 ^k
Treatments	HPP - EVOH - 15 °C		HPP - MetP - 15 °C		Thermal - EVOH - 15 °C		Thermal - MetP - 15 °C	
0		1.25 ^b		1.25 ^b		1.88 ^a		1.88 ^a
10	0.77 ^a	1.00 ^a	0.63 ^a	0.74 ^a	2.25 ^a	7.16 ^c	2.55 ^a	4.93 ^b
20	1.72 ^b	5.11 ^c	1.71 ^b	3.75 ^c	2.37 ^b	6.83 ^b	2.92 ^b	8.95 ^c
30	3.64 ^c	9.35 ^d	3.52 ^c	6.33 ^d	3.12 ^c	9.17 ^d	3.38 ^c	10.59 ^d
40	7.24 ^d	18.18 ^e	6.99 ^d	15.28 ^e	3.79 ^d	10.68 ^e	4.40 ^d	13.34 ^e
50	7.68 ^e	19.16 ^f	7.16 ^e	15.51 ^e	5.17 ^e	13.93 ^f	5.97 ^e	18.06 ^f
60	8.10 ^f	22.11 ^g	9.51 ^f	22.90 ^f	6.04 ^f	13.82 ^g	6.39 ^f	16.45 ^g
Treatments	HPP - EVOH - 25 °C		HPP - MetP - 25 °C		Thermal - EVOH - 25 °C		Thermal - MetP - 25 °C	
0		1.25 ^a		1.25 ^a		1.88 ^a		1.88 ^b
5	3.48 ^a	6.64 ^b	3.21 ^a	7.88 ^b	2.37 ^a	7.94 ^b	1.83 ^a	6.34 ^b
10	6.65 ^b	15.26 ^c	7.25 ^b	15.06 ^c	3.04 ^b	9.65 ^c	4.08 ^b	12.08 ^c
20	8.86 ^c	18.09 ^d	9.70 ^c	22.19 ^d	4.63 ^d	12.33 ^d	4.95 ^c	15.37 ^d
30	11.81 ^d	32.06 ^e	11.88 ^d	33.11 ^e	6.06 ^e	15.52 ^e	5.61 ^d	17.02 ^e

Different letters in the same column indicate significantly different mean values ($p < 0.05$)

Fig. 1 The HPP and TP treated ALMB samples stored at different conditions

compared to TP treated sample as shown in Fig. 1. This was possibly due to residual enzyme activity in HPP samples which was less in TP treated samples compared to HPP treated ALMB. Total color difference (ΔE^*) values were calculated using eq. 1 and are presented in Table 1. The color values of HPP and TP treated samples on the first day of storage study were taken as reference. Increasing trend of ΔE^* values was observed in all the samples, as expected. Effects of packaging material on color values of ALMB during the storage period are shown in Table 1. The ΔE^* values of ALMB samples stored at 4 °C up to 60 days were minimal as observed from Fig. 1, and it was also indicated by small color difference values ($\Delta E^* < 2.5$). It was noted that the ΔE^* values were slightly higher for the samples packed in EVOH compared to metalized polyester (MetP) for HPP and TP treated samples stored at 4 °C, whereas, this difference was opposite for HPP treated samples stored at 15 and 25 °C. The observed variation in two packaging materials might be attributed to their difference in water vapor or oxygen permeability which might change after HPP and TP processing. It can be interpreted from Table 1 that, the change in ΔE^* values for the thermally treated samples with storage period was less compared to the HPP treated ALMB samples, packed in both the packaging material.

The change in browning index (*BI*) values followed the trend similar to that of color difference. The *BI* values for

HPP treated samples stored at 4 °C ranged from 1.25 to 11.3 for EVOH packaged samples, whereas for MetP it varied from 1.25 to 11.12, indicating small variation between both packaging materials. However, for thermally treated ALMB samples the *BI* ranged from 1.88 to 10.93 for EVOH packages samples and 1.88 to 11.73 for MetP packaged samples. The *BI* values for TP samples stored in MetP were higher than the samples stored in EVOH packaging material. The HPP treated ALMB samples stored at 15 and 25 °C showed significant increase in the *BI* values ($p < 0.05$), and were higher than TP treated ones. At 15 °C the *BI* values reached up to 22.9 compared to 16.5 for TP treated samples. Whereas, for samples stored at 25 °C *BI* values for HPP treated ALMB samples reached up to 33.1 compared to up to 17.2 for their TP counterparts. The increase in *BI* values is possibly due to residual enzyme activity, and similar observations are reported earlier for HPP treated whole litchi fruits by Kaushik et al. (2014).

Pectinmethylesterase activity

The residual activity of PME enzyme in ALMB samples stored at different temperatures and in two different packaging materials is represented in Fig. 2. The PME activity of fresh untreated ALMB was found to be 1.2 PE units/mL. It was observed in general that PME activity decreased with storage period in all ALMB samples. A similar observation has been previously reported by Vervoort et al. (2011) for orange juice. The residual PME activity of HPP treated ALMB samples stored at different temperatures varied from 66 to 33 %, whereas, for TP treated samples it ranged between 22 and 8 %. It indicated that PME was more temperature sensitive in thermal treatment compared to HPP. The presence of residual PME activity after processing is mainly attributed to heat-stable fraction of PME (Vervoort et al. 2011). The PME activity was found to be more for ALMB samples in EVOH package compared to those in MetP at 4 °C. However, this difference reduced with storage period. For the samples stored at 15 and 25 °C the residual activity of samples stored in EVOH packaging material was more than samples stored in MetP. However, this difference was less than 10 % for HPP and TP treated samples.

Polyphenol oxidase activity

Inactivation of PPO in fruit based products is essential to minimize the quality changes and extend the shelf life. The residual PPO activity of the ALMB samples stored at different temperatures and in different packaging materials is given in Fig. 2. The PPO activity showed a decreasing trend with an increase in storage period for all the treatments. The residual PPO activity for ALMB samples varied from 35 to 8 % for HPP treated samples and 25 to 10 % for TP treated samples stored at 4 °C. It was observed that PPO was more sensitive to

Fig. 2 The % residual activity of PPO, POD and PME activity of ALMB samples during storage at 4 (a), 15 (b) and 25 °C(c)

TP treatment than HPP. Within the HPP treated samples, the PPO enzyme was less baro-resistant compared to PME. The PPO activity of ALMB samples stored at 4 and 15 °C was found to be more or less similar in the samples packed in MetP packaging material, however, the activity was higher for samples stored at 25 °C. It was noticed that the residual PPO activity of ALMB samples stored at 4 °C reduced to the values similar to the TP ones. Whereas, for samples stored at 15 and 25 °C, the PPO activity of HPP treated samples remained higher (18 to 35 %) compared to TP treated ones (12 to 25 %). After HPP treatment, the cell wall bound PPO gets released into the surrounding media and reacts with it leading to oxidation of phenolic compounds. The temperature application (up to 60 °C) during HPP helped to increase the inactivation of PPO present in the substrate leading to 43 % inactivation of HPP treated ALMB samples. Rapeanu et al. (2005) reported a synergistic effect of the pressure and temperature on PPO inactivation in grape.

Peroxidase activity

The residual POD activity of HPP treated ALMB samples varied from 38 to 13 %, whereas, for TP treated samples it ranged from 25 to 10 %. POD was found to be the most sensitive to HPP when compared to the PME and PPO residual activities. For all the stored samples, a decreasing trend of POD activity was observed with storage period, which was similar to the PPO and PME. A similar trend for POD activity during storage has been reported by Wang et al. (2013). For samples stored at 4 °C, the POD activity for the ALMB samples was found to be less in EVOH pouches compared to MetP pouches during the storage period. However, for thermally treated samples, no such trend was observed. The POD activity of ALMB samples stored at 25 °C remained unchanged from 10 to 30 day of storage.

The presence of POD activity in fruit based products is not desirable as it leads to the oxidative reactions affecting its natural flavour and aroma (Vervoort et al. 2011). In the present study, it was witnessed that HPP could not completely inactivate the POD activity. However, the residual activity can be delayed by keeping the HPP treated samples in refrigerated conditions and retain the fresh like characteristics of ALMB as witnessed in samples stored at 4 °C.

Ascorbic acid

The ascorbic acid content of ALMB samples was found to decrease linearly with an increase in storage period at all the temperature conditions. The ascorbic acid content of fresh ALMB sample was found to be 56 mg/100 mL. The loss of ascorbic acid during storage was found to follow first-order degradation kinetics as observed from the good correlation between experimental and predicted data. The degradation rates for ascorbic acid were higher for TP treated samples stored at 4 and 15 °C compared to HPP treated samples. However, for samples stored at 25 °C, the degradation rates for HPP treated samples were found to be higher than for thermally treated ones (Table 2). This may be attributed to the higher oxidative enzymatic reactions at 25 °C.

Ascorbic acid is an essential nutrient in fruit based products and considered as a marker of quality as well as of shelf life (Fernandez-Sestelo et al. 2013). The retention of ascorbic acid for ALMB samples stored at 4 °C varied from 78 to 47 % for HPP treated samples, whereas, for TP treated samples it ranged from 69 to 42 % (Fig. 3). A similar observation of minimal ascorbic acid losses compared to pasteurized orange juice has been reported by Polydera et al. (2005a). The HPP treated ALMB stored at 15 and 25 °C showed a higher ascorbic acid degradation rate as represented in Fig. 3. Ascorbic acid loss for samples stored at 15 °C was up to 54 % after 60 days and 58 % for those stored at 25 °C after 30 days. It was clear from the storage study that ascorbic acid degradation

Table 2 Ascorbic acid degradation rates (days^{-1}) of HPP and TP treated ALMB samples stored at different temperatures

Storage temperature($^{\circ}\text{C}$)	HPP treated ALMB			
	EVOH		MeTP	
	k (days^{-1})	R^2	k (days^{-1})	R^2
4	0.0032 ± 0.0004	0.97	0.0028 ± 0.0002	0.96
15	0.0088 ± 0.0003	0.84	0.0072 ± 0.0002	0.88
25	0.0267 ± 0.0010	0.91	0.0297 ± 0.0011	0.85
Storage temperature($^{\circ}\text{C}$)	TP treated ALMB			
	EVOH		MeTP	
	k (days^{-1})	R^2	k (days^{-1})	R^2
4	0.0037 ± 0.0002	0.96	0.0039 ± 0.0001	0.95
15	0.0108 ± 0.0005	0.83	0.0086 ± 0.0004	0.86
25	0.0153 ± 0.0009	0.85	0.0181 ± 0.0013	0.72

increases due to increase in storage temperature. Hence, refrigerated storage is essential for HPP treated ALMB samples. Another possible reason for the retention of ascorbic acid in HPP treated ALMB during storage might be non-availability of metal ions due to the formation of complexes with chelating agents. The partial inactivation of oxidative enzymes might have also helped in retaining ascorbic acid content (Kaushik et al. 2014; Polydera et al. 2005a).

Total Phenolics

The changes in total phenolics content of ALMB samples during storage is shown in Fig. 4. The phenolics content of fresh ALMB sample was 55 mg GAE/100 mL. The ALMB samples stored at 4 $^{\circ}\text{C}$ showed minimal changes in phenolics content up to 60 days and then decreased thereafter. However, for samples stored at 15 and 25 $^{\circ}\text{C}$, the decrease was found to be more compared to

Fig. 3 The ascorbic acid retention (%) of HPP and thermally treated ALMB samples during storage

Fig. 4 Changes in total phenolics content during storage

samples stored at 4 °C. The phenolics content for ALMB samples processed using HPP ranged between 31 and 54 mg GAE/100 mL. Whereas, for TP treated samples it ranged between 25 and 52 mg GAE/100 mL ALMB for samples stored at 4 °C. Similar observations have been reported earlier by Chen et al. (2013), wherein, they observed a lower rate of phenolics degradation during storage compared to thermally treated cloudy pomegranate juice. At the end of 4 °C storage, up to 42 % loss of phenolics content was observed for the HPP treated samples and for TP treated samples it was up to 55 %. Changes in packaging materials had no noticeable effect on the phenolics content of ALMB samples for all the treatment and storage conditions studied. The decrease in phenolics content during storage in both treatments can be attributed to the residual PPO and POD enzyme activity, which might have acted synergistically in degrading phenolics present in ALMB over the course of storage period. The degradation of phenolics in thermally treated samples was higher compared to HPP treated samples, which may be possibly due to non-enzymatic browning in addition to enzymatic browning. The autoxidation of phenolics in thermally processed products during storage was reported earlier (Terefe et al. 2013).

Microbial characteristics

The microbial characteristics in terms of total viable plate count (AM) and yeast and mold count (YMC) were evaluated

for all the ALMB samples during storage. The HPP and TP treatments were found to be effective in terms of microbial characteristics as the AM and YMC counts were below the detection limit throughout the storage period (<1 log CFU/mL). However, the untreated samples spoiled within five days of refrigerated storage as indicated by puffed packages. A similar observation of complete inactivation of viable microorganisms during storage was reported earlier for kiwi puree (Fernandez-Sestelo et al. 2013). The results indicated that the natural group of microorganisms present were sensitive to both treatments and the microbial safety of ALMB was well maintained during storage period.

Sensory evaluation

Sensory evaluation of the processed ALMB samples stored at 4 °C was carried out throughout the storage period. The sensory scores were given by trained panellists (N = 10) and are presented in Table 3. Sensory evaluation after immediate processing on the first day indicated that the sensory scores given for HPP treated samples were similar to the untreated ones. Some of the panellists did not like the taste of the thermally treated samples on the first day after processing. The aroma of TP treated samples was also affected as slightly cooked flavour and aroma was noted for these samples. Thermal processing affected the consistency of the ALMB samples, which led to more settling in TP samples than in HPP counterparts.

Table 3 The sensory scores of ALMB samples stored at 4 °C

Sensory attributes	Treatment	Storage (days)										
		0	15	30	45	60	70	80	90	100	110	120
Color	HPP (EVOH)	7.3 ± 1.0	7.2 ± 0.7	6.7 ± 1.1	7.4 ± 1.2	7.3 ± 1.1	7.3 ± 0.6	6.7 ± 1.0	6.0 ± 1.2	5.5 ± 1.1	5.2 ± 0.7	5.2 ± 0.3
	HPP (MetP)	6.0 ± 0.7	7.6 ± 0.9	6.4 ± 1.2	6.8 ± 1.0	5.6 ± 1.2	7.5 ± 1.0	5.4 ± 0.7	6.2 ± 0.7	5.4 ± 0.4	5.3 ± 0.4	5.0 ± 0.6
	TP	5.3 ± 0.8	6.6 ± 0.6	7.2 ± 2.0	5.6 ± 0.5	6.4 ± 0.7	6.2 ± 0.4	6.2 ± 1.0	6.1 ± 1.6	5.7 ± 0.5	5.5 ± 1.0	5.3 ± 0.5
Aroma	HPP (EVOH)	6.8 ± 1.2	7.1 ± 1.1	6.6 ± 1.2	7.3 ± 1.1	7.5 ± 1.0	6.8 ± 0.7	6.8 ± 0.9	6.2 ± 0.9	6.0 ± 1.2	5.6 ± 0.8	5.4 ± 0.2
	HPP (MetP)	6.6 ± 0.7	7.3 ± 0.7	6.2 ± 1.4	7.0 ± 0.8	6.0 ± 0.7	7.2 ± 1.2	6.3 ± 1.7	6.0 ± 1.3	5.7 ± 0.7	5.5 ± 1.1	5.3 ± 0.8
	TP	6.6 ± 0.9	6.8 ± 1.0	5.8 ± 0.8	5.8 ± 1.1	6.3 ± 0.7	6.7 ± 1.2	5.6 ± 1.8	6.5 ± 0.8	5.9 ± 1.0	5.7 ± 0.7	5.5 ± 0.8
Consistency	HPP (EVOH)	6.8 ± 0.7	7.5 ± 0.8	6.8 ± 0.9	7.1 ± 0.7	7.2 ± 0.8	6.6 ± 1.8	6.6 ± 1.3	5.7 ± 1.4	5.6 ± 1.1	5.4 ± 0.6	5.3 ± 1.1
	HPP (MetP)	6.3 ± 1.3	7.3 ± 1.3	6.5 ± 1.0	6.6 ± 1.2	6.1 ± 0.8	7.1 ± 1.4	6.7 ± 0.6	6.1 ± 1.8	5.7 ± 1.1	5.5 ± 0.6	5.2 ± 0.6
	TP	6.0 ± 1.5	6.3 ± 1.5	7.0 ± 0.9	5.7 ± 0.8	5.5 ± 0.9	6.1 ± 1.4	5.4 ± 1.1	5.8 ± 1.4	5.4 ± 1.3	5.4 ± 1.2	5.3 ± 0.6
Taste	HPP (EVOH)	6.9 ± 0.6	7.4 ± 0.7	6.9 ± 1.1	7.6 ± 1.1	7.6 ± 1.3	7.1 ± 1.1	7.0 ± 1.5	6.4 ± 1.5	6.5 ± 0.9	6.3 ± 0.8	5.6 ± 0.6
	HPP (MetP)	7.0 ± 0.8	7.4 ± 0.9	6.7 ± 1.2	7.4 ± 1.1	6.1 ± 1.2	7.2 ± 1.2	5.9 ± 1.0	6.6 ± 0.9	6.1 ± 0.8	5.7 ± 0.7	5.3 ± 0.7
	TP	6.5 ± 0.7	7.0 ± 1.1	6.5 ± 0.7	6.2 ± 1.3	6.6 ± 1.0	6.1 ± 1.5	6.1 ± 0.8	6.2 ± 1.2	6.3 ± 1.1	5.9 ± 1.1	5.6 ± 0.5
Aftertaste	HPP (EVOH)	7.3 ± 0.9	7.5 ± 0.7	6.9 ± 1.0	7.8 ± 0.6	7.3 ± 1.1	6.9 ± 1.1	6.5 ± 1.1	6.3 ± 1.6	6.3 ± 1.5	5.8 ± 0.6	5.2 ± 0.4
	HPP (MetP)	7.1 ± 1.1	7.7 ± 0.9	6.6 ± 0.8	7.6 ± 1.1	6.1 ± 1.2	7.2 ± 1.4	6.5 ± 1.2	6.3 ± 1.5	5.7 ± 1.4	5.6 ± 0.7	5.3 ± 0.5
	TP	6.3 ± 1.0	6.9 ± 1.4	6.6 ± 1.2	6.2 ± 1.4	6.7 ± 0.9	6.4 ± 1.3	6.6 ± 1.4	6.2 ± 1.7	6.4 ± 1.3	6.1 ± 0.6	5.7 ± 1.0

In addition, the aggregation of particles observed, which is not acceptable after immediate processing, whereas, the consistency of the HPP treated sample remained as of fresh ALMB. With the progression of the storage period settling observed in all ALMB samples, however, it was more pronounced in thermally treated samples. After 80 days of storage, the changes in color, aroma and taste were noted, which might be due to the residual POD and PPO activity as discussed earlier. The phase separation was also noticeable after 80 days of storage, possibly due to residual PME activity in ALMB. HPP treated ALMB samples showed pronounced browning after 100 days of storage, which was not preferred by the panellists. Also, taste and aroma of the ALMB reached an unacceptable level along with pronounced phase separation in HPP as well as TP samples. The bitter aftertaste was noted after the 100 days of storage in HPP treated samples.

Shelf life estimation

The shelf life of the ALMB stored at different storage conditions was estimated based on the constraints such as ascorbic acid retention, sensory evaluation, and instrumental color difference of the ALMB samples. Microbial population for all the samples during the storage period was below the detection limit ($< 1 \log \text{CFU/mL}$) ensuring microbial safety throughout the storage period. Hence, it was not considered as a constraint for the shelf life determination. Ascorbic acid degradation can also be considered as indicator for the quality and determination of shelf life. Polydera et al. (2003) reported the 50 % retention as a constraint for shelf life estimation of HPP treated orange juice. A similar approach was considered

in this study and the shelf life obtained for HPP treated ALMB samples stored at 4, 15 and 25 °C was up to 110, 40 and 10 days respectively. Similarly for TP treated samples it was up to 80, 40 and 10 days, respectively. The sensory analysis of ALMB samples stored at 4 °C indicated prominent changes in color after 90 days of storage period, although the other quality attributes were still acceptable. The instrumental color difference (ΔE^*) was more than 5 after 110 days of storage, which was not preferred by the panellists. Hence, the color difference of less than 4 was set as the constraint for shelf life for HPP treated samples. According to this criteria the shelf life of ALMB samples stored at 4, 15 and 25 °C was up to 100, 30 and 5 days, respectively.

Conclusions

The high pressure processing of ALMB samples was found to be effective in retaining the fresh like characteristics of ALMB compared to the thermal processing. The shelf life of HPP treated ALMB samples were extended up to 100 days with refrigerated storage at 4 °C. Temperature during storage played a significant role in restricting the residual enzyme activity and browning reactions in ALMB. Sensory attributes of the ALMB samples stored at 4 °C were unaffected up to 100 days of storage and minimal changes were observed between EVOH and MetP packaged samples. HPP treated ALMB samples were microbiologically safe throughout the storage period. HPP along with mild temperatures up to 60 °C application can be used to enhance the effectiveness of HPP technology to develop shelf stable fruit-based beverages.

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