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High Pressure Processing of Traditional Hardaliye Drink: Effect on Quality and Shelf-Life Extension

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Abstract: Hardaliye, a traditional non-alcoholic beverage, made from red grape pomace from wine production and produced by lactic acid fermentation with addition of different concentrations of whole/ground or heat-treated mustard seeds and of sour cherry leaves. Short shelf life of hardaliye limits its consumption and search in trend to process hardaliye to extend its shelf life. Thus, high hydrostatic pressure treatment of hardaliye drink with determination of changes in physicochemical and sensory properties in addition to microbial inactivation were studied according to Box-Behnken design. Maximum 5.10±0.00, 4.21±0.00, 5.38±0.59 and 5.05±0.22 log reductions were obtained in total mesophilic aerobic bacteria, total mold and yeast, *Brettanomyces bruxellensis* and *Lactobacillus brevis* by HHP. Shelf-life studies of the hardaliye samples were conducted with optimum processing parameters of 490 MPa, 29 ^{o}C for 15 min with the response variables of OD_{520} and inactivation of *L. brevis*. Both control and HHP treated samples were stored at 4 and 22 °C for shelf-life studies. While control samples at 4 and 22 °C were spoiled at the 15th and 3rd days, HHP treated samples were spoiled after 108th and 228th days, respectively.

Keywords: hardaliye drink; high hydrostatic pressure; shelf-life extension; Box-Behnken design; optimization

1. Introduction

Hardaliye is a traditional non-alcoholic beverage produced by a red grape pomace from wine industry. The drink is produced by lactic acid fermentation at room temperature for 7–10 days with addition of with the addition of different concentrations of whole/ground or heat-treated mustard seeds and of sour cherry leaves. If fermentation occurs at lower temperatures, it can be extended up to 20 days. Hardaliye can be consumed either fresh or aged and if it is aged, it may contain alcohol. Its characteristics red/burgundy color reflects the original color of the grapes and has a characteristic very pleasant aroma [1,2]. It is traditionally produced and widely consumed in the Thrace region of Türkiye.

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Grapes and fermentation process deliver the nutritional value of hardaliye; whereas the functional and health promoting properties are attributed to its ingredients of grapes rich with phenolic compounds and mustard seeds containing etheric oils, allyl isothiocyanate and sinigrin which is a cinogenesis-suppressing agent. Moreover, hardaliye also helps to regulate the digestion system and proven to be helpful to prevent coronary heart disease [2]. Even though hardaliye is a very special drink, its production volume is limited due to its shelf life. Current practices to increase shelf life is realized by the addition of sodium benzoate; but it is not accepted by the consumers as concern are raised adverse effects of sodium benzoate on human health. Thus, alternatives to addition of sodium benzoate are in demand. Studies with heat treatment provided shelf-life extension, but physical, health promoting and sensory properties of hardaliye are adversely affected. Except for ultrasonication (US) for processing of hardaliye with respect to determination of changes in physical, bioactive and sensory properties with shelf-life extension [3]; no studies are performed with processing of hardaliye.

High hydrostatic pressure (HHP) is a nonthermal processing technology with application of high isostatic pressures at the range of 100-1000 MPa. Foods are processed by HPP for microbial inactivation, preservation of physicochemical, bioactive and sensory properties with shelf-life extension. Both liquid and solid foods including traditional drinks with perishable nature with and without packaging are successfully processed by HPP [4–8]. Even though different foods were successfully processed by HHP, no studies were conducted for HHP processing of hardaliye drink. Thus, the objectives of the study are (1) to process hardaliye drink by HHP using Box-Benhken design; (2) determine the effects of processing parameters on physical, bioactive and sensory properties of hardaliye; (3) inactivate endogenous and spoilage microflora; (4) optimize and validate the HHP parameters for shelf-life studies, and (5) determine the shelf-life extension of hardaliye at both 4 and 22 ^{o}C.

2. Materials and Method

2.1. Hardaliye Samples

Fresh hardaliye samples produced by cabarnet type grape pomace from wine production were kindly provided by Karlıbağ Hardaliye (Kırklareli, Türkiye). The samples were kept at refrigeration temperature until use.

2.2. Microbial Cultures

Lactobacillus brevis and *Brettanomyces bruxellensis* were isolated from hardaliye and identified by API50 CHB/E and API 20C tests (bioMérieux, Inc., Durham, NC, USA), respectively. Isolated bacteria were subcultured on MRS agar slant and incubated at 30 ± 2 °C for 48 h. The culture was transferred to MRS agar from the saline solution (SS) obtained from the subculture, and the plates were incubated at 30 ± 2 °C for 72 h. The cells from the MRS plates were suspended in SS and collected by centrifugation at 3500 g for 10 min [9]. The cells were inoculated into hardaliye at the final concentration of 10^{5-6} cfu/mL.

Yeast culture -after isolation- was transferred to YPD medium and inoculated at 25 ± 2 °C for 7 days. After subsequent centrifugation at 3250 g for 10 min the collected cells were inoculated into hardaliye at the final concentration of 10^{5-6} cfu/mL [10].

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2.3. High Hydrostatic Pressure

A 2-L pilot scale HHP equipment (Avure, Middletown, OH, USA) using water as pressure medium was utilized to process the samples. 400 mL of the samples vacuum packaged in flexible pouches made from a multilayer polymer/aluminum/polymer film (polyethylene–aluminum–polypropylene) (APACK Packaging Technologies, Istanbul, Turkey) processing. Average temperature increase in addition to average pressure rise and fall rate per 100 MPa pressure increase were 0.5 ± 0.2 °C, 0.5 min and 0.2 min, respectively. Based on preliminary experiments 200-500 MPa pressures, from 3-15 min treatment times (time after achieving the set pressure) and 4-22 °C treatment temperatures were applied according to Box Behnken design (Table 1).

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Table 1. Changes in the physicochemical properties of hardaliye drink by high hydrostatic pressure processing by Box-Behnken design.

Process	Proses code	Pressure (P, MPa)	Treatment time (t, min)	Temperature (T, °C)	pH	Titratable acidity (g/L)	TSS (°Brix)	Conductivity (mS/cm)	Turbidity (NTU)	Reducing sugar (g/L)
Control	Kontrol	–	–	–	3.80±0.00^{ef}	5.80±0.09^{ab}	27.02±0.17^{a}	3.61±0.02^{f}	862.89±3.97^{a}	220.32±2.07^{bcde}
HHP1	YHB1	350	3	40	3.78±0.00^{g}	5.35±0.17^{cdef}	27.00±0.00^{a}	3.67±0.03^{bc}	439.53±2.521^{c}	211.39±7.29^{de}
HHP2	YHB2	200	3	22	3.80±0.01^{ef}	5.55±0.15^{abcde}	27.02±0.04^{a}	3.67±0.02^{bc}	359.41±2.09^{fgh}	242.50±12.54^{abc}
HHP3	YHB3	350	15	40	3.81±0.00^{bcde}	5.65±0.17^{abc}	27.00±0.00^{a}	3.62±0.01^{ef}	340.48±1.27^{i}	227.66±8.58^{abcde}
HHP4	YHB4	350	9	22	3.79±0.01^{f}	5.50±0.179^{abcde}	27.02±0.04^{a}	3.69±0.0^{b}	357.27±1.90^{h}	245.41±17.56^{ab}
HHP5	YHB5	200	15	22	3.80±0.00^{def}	5.65±0.09^{abc}	27.00±0.00^{a}	3.64±0.03^{cdef}	373.92±2.02^{de}	226.79±5.78^{bcde}
HHP6	YHB6	350	3	4	3.80±0.00^{cdef}	5.20±0.09^{def}	27.00±0.00^{a}	3.63±0.03^{cdef}	373.84±2.77^{de}	218.99±3.95^{cde}
HHP7	YHB7	500	3	22	3.80±0.00^{cdef}	5.30±0.09^{cdef}	27.00±0.00^{a}	3.65±0.06^{cdef}	369.98±4.52^{defg}	219.56±3.46^{bcde}
HHP8	YHB8	350	15	4	3.81±0.00^{abcd}	5.35±0.23^{cdef}	26.87±0.12^{a}	3.62±0.01^{ef}	358.90±2.90^{gh}	208.32±1.52^{e}
HHP9	YHB9	500	9	4	3.80±0.00^{cdef}	5.60±0.23^{abcd}	27.00±0.00^{a}	3.67±0.01^{bc}	482.19±10.91^{b}	253.19±5.36^{a}
HHP10	YHB10	350	9	22	3.82±0.00^{ab}	4.95±0.00^{f}	27.00±0.00^{a}	3.70±0.01^{b}	363.84±2.46^{efgh}	220.43±8.72^{bcde}
HHP11	YHB11	500	15	22	3.82±0.00^{a}	5.25±0.15^{cdef}	26.89±0.10^{a}	3.62±0.01^{def}	381.23±1.10^{d}	238.74±11.61^{abc}
HHP12	YHB12	200	9	40	3.81±0.01^{bcdef}	5.15±0.09^{ef}	26.89±0.10^{a}	3.76±0.05^{a}	371.21±3.98^{def}	237.30±5.99^{abcd}
HHP13	YHB13	200	9	4	3.81±0.00^{abc}	5.90±0.17^{a}	26.98±0.04^{a}	3.67±0.02^{bcd}	479.29±5.00^{b}	232.14±3.06^{abcde}
HHP14	YHB14	500	9	40	3.81±0.00^{bcde}	5.45±0.09^{bcde}	27.00±0.00^{a}	3.78±0.01^{a}	370.57±0.97^{defg}	238.21±13.99^{abc}
HHP15	YHB15	350	9	22	3.82±0.00^{a}	5.45±0.17^{bcde}	27.00±0.00^{a}	3.66±0.01^{bcde}	364.69±3.77^{efgh}	228.44±8.17^{abcde}

*Data in the same column with different superscript letter are significantly different ($p \leq 0.05$).

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2.4. Measurement of Physicochemical Properties

pH of the samples was measured by Orion perpHect logR meter (inoLab WTW, Weilheim, Germany). Total soluble solids (TSS, °Brix) measurement was conducted by handheld 507-1 model refractometer (Nippon Optical Works Co. Ltd, Japan). Conductivity of the hardaliye samples were recorded by Sension 5 model (HACH, CO, ABD) handheld conductivity meter, and turbidity (NTU) measurement was performed by (MICRO TPI, Model 20008) turbidity meter. Titratable acidity of hardaliye samples was determined by titrimetric method as lactic acid equivalent. 3,5-dinitrosalicylic acid (DNS) (Sigma Aldrich, Steinheim, Germany) reagent was used to determine the reducing sugar content with glucose (Sigma Aldrich, Steinheim, Germany) used as substrate.

Color parameters of L^*, a^*, and b^* values were recorded by a Hunter Color Flex spectrophotometer (Hunter Associates Laboratory Inc., Reston VA, USA). Chroma (C^*), hue (h^o), and total color difference (ΔE) values were calculated from the measured L^*, a^*, and b^* values. Moreover, color density (IC), color tone (CT) and percent color components of yellow, blue and red indices were calculated by absorbance values from yellow color tone (YCT, OD_{420}), blue color tone (BCT, OD_{520}) and red color tone (RCT, OD_{620}) [11], and color intensity of the samples was recorded by absorbance at 540 nm (PG Instruments T80+UV/VIS model spectrophotometer) [12]). Calculated color parameters were;

$$C^*\sqrt{a^2 + b^2} \quad (1)$$

$$h^0 = \arctan(b/a \quad (2)$$

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (3)$$

$$\text{Color tone} = OD_{420}/OD_{520} \quad (4)$$

$$\%OD_{420} = \frac{OD_{420}}{IC} \times 100 \quad (5)$$

$$\%OD_{520} = \frac{OD_{520}}{IC} \times 100 \quad (6)$$

$$\%OD6_{20} = \frac{OD_{620}}{IC} \times 100 \quad (7)$$

$$\text{Color intensity (IC)} = OD_{420} + OD_{520} + OD_{620} \quad (8)$$

2.5. Measurement of Bioactive Properties

Total antioxidant capacity (TAC, %) of the samples was quantified by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical method [13]. Total phenolic substance content (TPSC, mg/mL) was determined by the Folin-Ciocalteu spectrophotometric method at 720 nm. pH-differential method based on cyanidin-3-glucoside (mg/100 mL) was utilized to determine the total monomeric anthocyanin content (TMAC). Results were expressed as cyanidin 3-glucoside equivalent in mg/L [13].

2.6. Inactivation of Endogenous Microflora

 doi:10.20944/preprints202306.1314.v1

Total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) counts were performed with the samples diluted with 0.1% peptone water. Proper dilutions were surface-plated on plate count agar (PCA, Fluka, Germany) for TMAB and on potato dextrose agar (PDA, Fluka, Germany) acidified with 10% (w/v) tartaric acid (Sigma Chemical Co., Stockholm, Sweden) for TMY, on YPD plates for *B. bruxellensis and on MRS plates for L. brevis*. PCA plates were incubated at 35 ± 2 °C for 24-48 h, PDA plates were incubated at 22 ± 2 °C for 3-5 days, YPD plates were incubated at 28 ± 2 °C for 5 days and MRS plates were incubated at 30 ± 2 °C for 72 h. respectively. The results were expressed as log cfu/mL [4]

2.7. Sensory Analyses

Hardaliye samples at the room temperature were evaluated by 30 trained panelists in three phases by 9-point hedonic scale. First, they were asked to evaluate the samples for appearance (cloudiness-clarity, dullness-shininess, color intensity and particle distribution) and then they were asked smell the samples for the flavor-aroma. Finally, the panelists tasted the samples for juice density, hardaliye taste, bitter taste, sour taste, sweetness and aftertaste [14].

2.8. Shelf-life Studies

Both control and treatment groups (400 mL) under the optimum HHP parameters of 490 MPa, 15 min, and 29 °C were stored at 4 and 22 °C for 228 days for shelf-life studies. pH, conductivity, color (*L**, *a** and *b**), chroma, hue, total color difference, color intensity and inactivations on TMAB and TMY in addition to sensory properties of clarity-cloudiness, shininess-dullness, color intensity, flavor-aroma, bitter taste, sour taste and after taste were measured on 0, 15, 30, 45, 66, 87, 108, 142, 180 and 228 days.

2.9. Experimental Design

The quantities and levels of parameters (pressure, temperature and treatment time) were applied based on preliminary experiments. Effect of processing factors on physical (pH, titratable acidity, total soluble solid, conductivity, turbidity, reducing sugar, color parameters (*L**, *a**, *b**, chroma, hue, total color difference, color intensity, color tone, OD_{420}, OD_{520} and OD_{620}), bioactive (total phenolic substance content, total antioxidant activity and total monomeric anthocyanin content) and sensory properties (cloudiness-clarity, dullness-shininess, color intensity and particle distribution, flavor-aroma, juice density, hardaliye taste, bitter taste, sour taste, sweetness, aftertaste in addition to microbial inactivation (TMAB, TMY, *L. brevis* and *L. bretteromyces*) during high hydrostatic pressure processing of hardaliye drink was evaluated prior to the optimization step.

2.10. Optimization

Thirty-five responses of hardaliye as mention above were developed to model in terms of pressure (X_1, 200 to 500 MPa), temperature (X_2, 4 to 40°C) and treatment time (X_3, 3 to 15 min) using Box-Behnken design (BBD) by Minitab Statistic Software Package (version 17, Minitab Inc. State College, PA, USA). BBD configuration with its (un)coded predictors is summarized in Table 1. For the best-fit

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to the experimental data, the following quadratic regression model was used when all factors and interactions are to be significant:

$$Y_n=b_o+b_{1\times1}+b_2X_2+b_3X_3+\ldots.+b_{25}X_{25}^2$$

where Y_n is the 35 response variables; b_o to b_{25} is the slope coefficients; and X_1, X_2, and X_3 are the predictors of pressure, temperature, and treatment time, respectively. To determine the significant terms of the predictive model, analysis of variance (ANOVA) was performed at a 95% confidence interval ($p<0.05$). Multiple comparisons were made using Tukey's test. The graphical optimization was carried out establish the optimum level of three independent variables, pressure, temperature, and treatment time to achieve desirable responses such as minimum inactivation of *L. brevis* and maximum OD_{520}. The optimum value of multiple responses was determined by using MINITAB optimizer tool.

3. Results and Discussion

3.1. Changes in the Properties of Hardaliye Processed by High Hydrostatic Pressure

The pH values obtained after different HHP conditions applied to the hardaliye drink varied between 3.78 ± 0.00 and 3.82 ± 0.00, while the average pH value of the control group was recorded as 3.80 ± 0.00. Among the HHP treatments, HHP1 (350 MPa, 3 min, 40 °C) had the lowest average pH value, while the highest average pH values were determined for HHP11 (500 MPa, 15 min, 22 °C) and HHP15 (350 MPa, 9 min, 22 °C). According to Tukey's test, the pH of the control group was not significantly different from the pH of the HHP-treated samples of HHP2, HHP3, HHP4, HHP5, HHP6, HHP77, HHP9, HHP12 and HHP14 ($p>0.05$) (Table 1). Only treatment time had a significant effect on pH of the hardaliye.

Titration acidity of the control samples was determined as 5.80 ± 0.09 g/L. Among the HHP applications, HHP10 (350 MPa, 9 min, 22 °C) had the lowest total acidity with 4.95 ± 00 g/L, while the highest value was determined for HHP13 (200 MPa, 9 min, 4 °C) with 5.90 ± 0.17 g/L. The mean initial total acidity of the control group was not significantly different from the total acidity of the HHP2, HHP3, HHP4, HHP5, HHP9, HHP13, HHP14 and HHP15 ($p>0.05$). There also was no significant difference between the total titratable acidity of HHP1, HHP7, HHP8 and HHP11 ($p>0.05$). Titratable acidity value obtained in HHP13 treatment was the closest to the control group (Table 1). Titratable acidity of the samples was only significantly affected by the pressure.

According to the results obtained, the mean TSS values varied between 26.87 ± 0.12 and 27.02 ± 0.17 °Brix. The mean TSS value of the control group was recorded as 27.02 ± 0.17 °Brix. The lowest mean TSS value was determined under HHP8 (350 MPa, 15 min, 4 °C) conditions, while the highest value was determined for the product treated under HHP2 (200 MPa, 3 min, 22 °C) and HHP4 (350 MPa, 9 min, 22 °C), respectively. The average TSS value of the control group without HHP treatment was equal to the average TSS values determined at HHP2 and HHP4. Overall, no significant difference was detected between the control and HHP treated samples for TSS ($p>0.05$) (Table 1).

The average conductivity values varied between 3.61 ± 0.02 and 3.78 ± 0.01 mS/cm. The average conductivity value of the control group was recorded as 3.61 ± 0.02 mS/cm. Among HHP samples, HHP8 (350 MPa, 15 min, 4 °C) had the lowest average conductivity value with 3.61

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± 0.03 mS/cm, while the highest value was determined for HHP14 (500 MPa, 9 min, 40 °C) with 3.78 ± 0.01 mS/cm. The effects of pressure, treatment time and temperature on the conductivity were found significant ($p \leq 0.05$) (Table 1).

Turbidity values of the samples ranged between 340.48 ± 1.27 and 862.89 ± 3.97 NTU. The lowest mean turbidity value was 340.48 ± 1.27 NTU in hardaliye samples treated by HPP3 (350 MPa, 15 min, 40 °C), while the highest value was 862.89 ± 3.97 NTU in the untreated control samples. The effects of pressure, treatment time and temperature alone on the turbidity value of the hardaliye were significant ($p \leq 0.05$). Treatment with HHP resulted in a significant decrease in the turbidity value of the beverage ($p \leq 0.05$) (Table 1). The mean initial reducing sugar content of the control samples was recorded as 220.32 ± 2.07 g/L. HHP8 (350 MPa, 15 min, 4 °C) had the lowest reducing sugar content of 208.32 ± 1.52 g/L, while the highest value was determined for HHP9 (500 MPa, 9 min, 4 °C) with 253.19 ± 5.36 g/L (Table 1). While the effect of pressure and treatment time on the reducing sugar content was found to be significant ($p \leq 0.05$), the effect of temperature variable was found to be insignificant ($p > 0.05$).

The mean initial L^* value was recorded as 3.33 ± 0.23 in the control samples and L^* values of HHP treated samples ranged between 2.05 ± 0.04 and 3.55 ± 0.81. The lowest L^* value was determined in the samples treated by HHP14 (500 MPa, 9 min, 40 °C), while the highest value was determined in the sample treated by HHP9 (500 MPa, 9 min, 4 °C). The control sample was not significantly different from the samples treated by HHP1, HHP5, HHP7, HHP9, HHP11, HHP13 and HHP15 ($p > 0.05$). While the effect of pressure on the L^* value of the hardaliye drink was found to be significant ($p \leq 0.05$), the effect of treatment time and temperature were found to be insignificant ($p > 0.05$) (Table 2). The mean initial a^* value of the control group was recorded as 8.37 ±0.74 and a^* values of the hardaliye samples ranged from 7.38 ± 0.29 to 10.64 ± 1.11. The lowest a^* value was determined for the HHP14 (500 MPa, 9 min, 40 °C) samples, while the highest value was determined for the product treated by HHP5 (200 MPa, 15 min, 22 °C). b^* values of the samples ranged between 0.43 ± 0.08 and 1.74 ± 0.49. The average b^* value of the control samples was recorded as 0.96 ± 0.26. HHP11 (500 MPa, 15 min, 22 °C) samples had the lowest b^* value with 0.43 ± 0.08, while the highest value was determined by HHP5 (200 MPa, 15 min, 22 °C) with 1.746 ± 0.49. No significant difference was detected between the control and the HHP samples for b^* value. While the effect of pressure on the b^* value of hardaliye was found to be significant ($p \leq 0.05$), the effect of processing time and temperature variables was found to be insignificant ($p > 0.05$) (Table 2).

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Table 2. Changes in color properties of hardaliye drink processed by high hydrostatic pressure processing by Box-Behnken design.

Process	L*	a*	b*	C*	h0	ΔE	Color intensity (IC)	Color tone	%OD420	%OD520	%OD620
Control	3.33±0.22^{ab}	8.37±0.76^{b}	0.95±0.26^{abcd}	8.44±0.71^{b}	0.12±0.04^{bcde}	—	4.85±0.01^{ab}	0.43±0.00^{ab}	21.44±0.04^{b}	50.41±0.09^{de}	28.15±0.08^{ab}
HHP1	2.89±0.28^{abcde}	8.74±0.44^{ab}	0.63±0.32^{cd}	8.77±0.47^{ab}	0.07±0.03^{de}	0.75±0.11^{bc}	4.85±0.01^{abc}	0.43±0.00^{ab}	21.60±0.11^{ab}	50.57±0.16^{cde}	27.83±0.06^{bcd}
HHP2	2.22±0.08^{de}	8.14±0.82^{b}	1.67±0.32^{ab}	8.32±0.86^{b}	0.20±0.02^{a}	1.62±0.15^{abc}	4.79±0.02^{d}	0.43±0.00^{ab}	21.61±0.07^{ab}	50.73±0.25^{bcde}	27.65±0.26^{cdef}
HHP3	2.33±0.10^{de}	8.71±1.15^{ab}	1.71±0.52^{a}	8.89±1.23^{ab}	0.19±0.03^{ab}	1.607±0.48^{abc}	4.74±0.01^{e}	0.42±0.01^{ab}	21.48±0.27^{ab}	51.28±0.20^{a}	27.24±0.24^{fg}
HHP4	2.35±0.19^{de}	9.07±1.06^{ab}	1.73±0.41^{a}	9.24±1.12^{ab}	0.19±0.02^{ab}	1.58±0.40^{abc}	4.81±0.01^{bcd}	0.42±0.01^{ab}	21.64±0.29^{ab}	50.91±0.36^{abcd}	27.46±0.20^{defg}
HHP5	3.27±0.09^{abc}	10.64±1.11^{a}	1.74±0.49^{a}	10.78±1.17^{a}	0.16±0.03^{abc}	2.08±1.19^{a}	4.80±0.03^{cd}	0.43±0.01^{ab}	21.76±0.31^{ab}	50.89±0.12^{abcd}	27.35±0.23^{efg}
HHP6	2.29±0.09^{de}	8.08±0.59^{b}	1.47±0.17^{abc}	8.23±0.60^{b}	0.18±0.01^{ab}	1.41±0.17^{abc}	4.79±0.01^{d}	0.43±0.00^{ab}	21.78±0.12^{ab}	50.91±0.11^{abcd}	27.31±0.12^{fg}
HHP7	3.50±0.49^{a}	8.72±0.76^{ab}	0.61±0.44^{cd}	8.75±0.79^{ab}	0.07±0.04^{de}	0.93±0.01^{abc}	4.81±0.01^{cd}	0.43±0.00^{ab}	21.58±0.17^{ab}	51.09±0.07^{ab}	27.32±0.14^{fg}
HHP8	2.31±0.13^{de}	7.95±0.13^{b}	0.70±0.06^{cd}	7.99±0.12^{b}	0.09±0.01^{cde}	1.33±0.12^{abc}	4.80±0.03^{cd}	0.43±0.01^{ab}	21.64±0.17^{ab}	51.03±0.22^{abc}	27.34±0.06^{fg}
HHP9	3.55±0.81^{a}	8.36±0.57^{b}	0.99±0.16^{abcd}	8.43±0.57^{b}	0.12±0.02^{bcde}	0.95±0.318^{abc}	4.81±0.01^{bcd}	0.43±0.00^{ab}	21.53±0.14^{ab}	50.61±0.19^{bcde}	27.86±0.11^{bcd}
HHP10	2.38±0.09^{de}	8.14±0.40^{b}	0.85±0.26^{abcd}	8.19±0.42^{b}	0.10±0.03^{cde}	1.18±0.31^{abc}	4.82±0.01^{bcd}	0.42±0.00^{ab}	21.48±0.04^{ab}	50.84±0.07^{abcd}	27.68±0.10^{cdef}
HHP11	3.46±0.21^{a}	8.84±0.50^{ab}	0.43±0.08^{d}	8.85±0.50^{ab}	0.05±0.01^{e}	0.72±0.18^{bc}	4.80±0.01^{d}	0.42±0.00^{b}	21.39±0.13^{b}	51.09±0.13^{ab}	27.51±0.08^{cdefg}
HHP12	2.45±0.27^{cde}	8.82±0.47^{ab}	0.82±0.24^{bcd}	8.86±0.50^{ab}	0.09±0.03^{cde}	1.02±0.22^{abc}	4.84±0.01^{abcd}	0.42±0.00^{ab}	21.61±0.15^{ab}	50.46±0.06^{de}	27.94±0.16^{bc}
HHP13	2.97±0.21^{abcd}	8.41±0.46^{b}	1.09±0.14^{abcd}	8.48±0.45^{b}	0.13±0.01^{abcd}	0.67±0.33^{c}	4.87±0.01^{a}	0.43±0.00^{ab}	21.36±0.17^{b}	50.21±0.13^{e}	28.43±0.06^{a}
HHP14	2.05±0.04^{e}	7.38±0.29^{b}	1.18±0.11^{abcd}	7.48±0.30^{b}	0.16±0.01^{abc}	1.90±0.23^{ab}	4.84±0.02^{abcd}	0.43±0.00^{ab}	21.53±0.12^{ab}	50.68±0.11^{bcde}	27.79±0.19^{bcde}
HHP15	2.56±0.07^{bcde}	8.41±0.23^{b}	1.25±0.19^{abcd}	8.51±0.21^{b}	0.15±0.02^{abc}	0.94±0.14^{abc}	4.81±0.02^{bcd}	0.43±0.01^{a}	21.97±0.19^{a}	50.83±0.18^{abcd}	27.190.062^{g}

* Data in the same column with different superscript letter are significantly different ($p \leq 0.05$).

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Chroma values of the samples varied between 7.48 ± 0.30 and 10.78 ± 1.17 with the average chroma value of the control group recorded as 8.44 ± 0.71. The lowest chroma value was determined for HHP14 (500 MPa, 9 min, 40 °C) samples, while the highest value was determined for the HHP5 (200 MPa, 15 min, 22 °C), respectively. Effect of HHP processing parameters on chroma value was insignificant ($p > 0.05$) (Table 2).

The hue values of the samples varied between 0.05 ± 0.01 and 0.20 ± 0.02. The hue values of the control samples were recorded as 0.12 ± 0.04. The lowest hue value obtained as a result of HHP treatments was determined at the HHP11 (500 MPa, 15 min, 22 °C), while the highest hue value was determined for the HHP2 (200 MPa, 3 min, 22 °C). Except for HHP2 samples, hue value of the control and HHP samples differed significantly from each other ($p \leq 0.05$) (Table 2). Hue value of the samples were not significantly affected by HPP processing parameters.

The total color difference values of the samples processed with HPP varied between 0.67 ± 0.33 and 2.08 ± 1.19. While HHP13 (200 MPa, 9 min, 4 °C) application had the lowest total color difference value with 0.67 ± 0.33, the highest value was determined for HHP5 (200 MPa, 15 min, 22 °C) with 2.08 ± 1.19 (Table 2). The color intensity (IC) values of hardaliye drinks varied between 4.74 ± 0.01 and 4.87 ± 0.01. The average IC value of the control sample was recorded as 4.85 ± 0.01. The lowest IC value obtained under HHP3 (350 MPa, 15 min, 40 °C), while the highest IC was determined under HHP13 (200 MPa, 9 min, 4 °C) conditions. The mean IC of the control group was significantly different from the mean IC of the YHB2, YHB3, YHB5, YHB6, YHB7, YHB8 and YHB11 ($p \leq 0.05$). While the effect of pressure and temperature on IC was insignificant ($p > 0.05$), the effect of treatment time was found to be significant ($p \leq 0.05$) (Table 2). Color tone of the samples ranged from 0.42 ± 0.01 to 0.43 ± 0.01 with the color tone of 0.43 ± 0.01 for the control samples. The lowest color tone of 0.42 ± 0.01 was observed for the HHP11 (500 MPa, 15 min, 22 °C); whereas the highest color tone value of 0.43 ± 0.01 was measured for HHP15 (350 MPa, 9 min, 22 °C). No significant difference was observed between the control and HHP treated samples for color tone; and HHP processing parameters had no significant effect on color tone of the hardaliye samples (Table 2).

Control hardaliye samples had the mean $\%OY_{420}$ value of 21.44 ± 0.04 and $\%OD_{420}$ values ranged from 21.36 ± 0.17 to 21.97 ± 0.20 among the samples. The lowest $\%OY_{420}$ value was found in the samples treated by HHP13 (200 MPa, 9 min, 4 °C), while the highest value was found in the samples treated by HHP15 (350 MPa, 9 min, 22 °C). In general, no significant difference was observed between the control and HHP treated samples and $\%OY_{420}$ value was not significantly affected by HHP processing parameters (Table 2).

$\%OD_{520}$ values of the hardaliye samples ranged between 50.21 ± 0.13 and 51.28 ± 0.20 with the $\%OD_{520}$ values of 50.41 ± 0.09 for the control samples. HHP13 (200 MPa, 9 min, 4 °C) had the lowest blue color tone of 50.21 ± 0.13%, whereas HHP3 (350 MPa, 15 min, 40 °C) had the highest $\%OD_{520}$ value of 51.28 ± 0.20 (Table 2). Pressure, treatment time and temperature had no effect on $\%OD_{520}$ values of hardaliye ($p > 0.05$). $\%OD_{620}$ values of the samples varied between 27.20 ± 0.06 and 28.43 ± 0.06. The lowest red color tone was determined for the samples treated under HPP15 (350 MPa, 9 min, 22 °C), while the highest value was determined for the samples treated by HHP13 (200 MPa, 9 min, 4

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°C) conditions (Table 2). HHP parameters had significant effect on $\%OD_{620}$ ($p \leq 0.05$).

The mean initial TPSC of the control samples 2310.02 ± 22.91 mg/L changed from 2222.18 ± 36.64 mg/L by HHP1 (350 MPa, 3 min, 40 °C) to 2382.24 ± 17.14 mg/L by HHP9 (500 MPa, 9 min, 4 °C). Although no significant difference was observed between the control and HHP treated samples for TPSC; temperature had significant impact on TPSC (Table 3). The mean initial TAC of the control samples was recorded as 70.20 ± 0.91%. TAC of the HHP-treated samples, on the other hand, varied between 68.91 ± 1.02% and 71.09 ± 0.87%. HHP14 (500 MPa, 9 min, 40 °C) had the lowest TAC of 68.91 ± 1.02%, while the highest value was determined for HHP1 (350 MPa, 3 min, 40 °C) with 71.09 ± 0.87% (Table 3). HHP processing parameters had no significant effect on TAC of the hardaliye samples. The mean average TMAC content of the control samples was 126.91 ± 9.30 mg/L; whereas TMAC contents of the hardaliye samples processed by HHP ranged from 123.25 ± 1.12 mg/L by HHP8 (350 MPa, 15 min, 4 °C) to 150.71 ± 7.34 mg/L by HHP15 (350 MPa, 9 min, 22 °C) (Table 3). While the effect of pressure and treatment time on the TMAC content of YHB-treated hardaliye drink was found to be insignificant ($p > 0.05$), the effect of temperature variable was found to be significant ($p \leq 0.05$).

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Table 3. Changes in bioactive properties of hardaliye drink processed by high hydrostatic pressure processing by Box-Behnken design.

Process	TPSC (mg/L)	TAC (%)	TMAC (mg/L)
Control	2310.02±22.91^{abc}	70.20±0.91^{a}	126.91±9.30^{b}
HHP1	2222.18±36.64^{c}	71.09±0.87^{a}	137.21±8.56^{ab}
HHP2	2312.55±25.87^{abc}	69.80±0.92^{a}	133.03±2.92^{ab}
HHP3	2278.35±14.39^{bc}	70.03±1.34^{a}	136.42±6.06^{ab}
HHP4	2340.01±32.51^{ab}	71.06±1.36^{a}	140.04±4.43^{ab}
HHP5	2236.12±12.31^{c}	70.75±0.99^{a}	140.41±2.10^{ab}
HHP6	2332.83±28.64^{ab}	70.29±0.85^{a}	135.12±2.29^{ab}
HHP7	2348.03±30.49^{ab}	70.79±0.94^{a}	131.23±9.34^{ab}
HHP8	2351.83±33.21^{ab}	68.95±0.26^{a}	123.25±1.12^{b}
HHP9	2382.24±17.14^{a}	69.51±0.77^{a}	130.53±4.26^{ab}
HHP10	2277.93±47.65^{bc}	69.57±0.86^{a}	137.63±8.94^{ab}
HHP11	2346.76±45.35^{ab}	69.81±0.71^{a}	139.25±7.98^{ab}
HHP12	2347.61±27.65^{ab}	69.61±0.87^{a}	133.17±3.57^{ab}
HHP13	2302.84±37.46^{abc}	69.97±0.89^{a}	128.58±15.18^{ab}
HHP14	2236.12±12.04^{c}	68.91±1.02^{a}	130.67±10.85^{ab}
HHP15	2290.38±18.81^{abc}	69.07±0.89^{a}	150.71±7.34^{a}

*Data in the same column with different superscript letter are significantly different ($p \leq 0.05$).

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The mean initial TMAB count of hardaliye was recorded as 5.10 ± 0.02 log cfu/mL. The lowest reduction on TMAB of 0.46 ± 0.04 was provided by HHP2 (200 MPa, 3 min, 22 °C); whereas the highest reduction on TMAB of 5.10±0.00 was provided by HHP3 (350 MPa, 15 min, 40 °C), HHP9 (500 MPa, 9 min, 4 °C) and HHP14 (500 MPa, 9 min, 40 °C) (Table 3). While pressure and temperature significantly affected the TMAB; treatment time was found to be insignificant ($p>0.05$).

The mean initial TMY population of hardaliye was recorded as 4.21±0.04 log cfu/mL. According to the results obtained, the reduction in TMY count ranged from 0.57 ± 0.06 log cfu/mL to 4.21±0.00 log cfu/mL. HHP2 (200 MPa, 3 min, 22 °C) had the lowest decrease value of 0.57±0.06 log cfu/mL; whereas the highest decrease in TMY count was found by HHP3 (350 MPa, 15 min, 40 °C), HHP7 (500 MPa, 3 min, 22 °C), HHP9 (500 MPa, 9 min, 4 °C), HHP11 (500 MPa, 15 min, 22 °C) and HHP14 (500 MPa, 9 min, 40 °C) with 4.21 ± 0.00 log cfu/mL reduction (Table 4). While effect of pressure on TMY inactivation was significant; treatment time and temperature did not have significant effect on TMY inactivation.

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Table 4. Inactivation of microbial flora in hardaliye drink processed by high hydrostatic pressure processing by Box-Behnken design.

Process	TMAB inactivation (log cfu/mL)	TMY inactivation (log cfu/mL)	*Brettanomyces bruxellensis* inactivation (log cfu/mL)	*Lactobacillus brevis* inactivation (log cfu/mL)
Control	–	-	-	-
HHP1	3.06±0.04^{d}	3.21±0.00^{b}	0.56±0.42^{e}	1.57±0.23^{cd}
HHP2	0.46±0.04^{k}	0.57±0.06^{h}	0.50±0.30^{e}	0.16±0.03^{g}
HHP3	5.10±0.00^{a}	4.21±0.00^{a}	4.36±0.43^{a}	3.94±0.48^{b}
HHP4	2.56±0.05^{ef}	2.73±0.03^{c}	0.92±0.45^{cde}	1.77±0.30^{cd}
HHP5	1.10±0.03^{j}	1.17±0.05^{g}	0.75±0.42^{de}	0.75±0.23^{efg}
HHP6	2.36±0.04^{g}	2.51±0.05^{e}	0.53±0.44^{e}	1.13±0.14^{def}
HHP7	4.10±0.00^{b}	4.21±0.00^{a}	4.38±0.40^{a}	5.05±0.22^{a}
HHP8	3.62±0.03^{c}	3.21±0.00^{b}	2.44±0.14^{b}	1.66±0.61^{cd}
HHP9	5.10±0.00^{a}	4.21±0.00^{a}	5.38±0.68^{a}	4.05±0.16^{b}
HHP10	2.56±0.03^{e}	2.73±0.03^{c}	2.04±0.16^{bc}	1.71±0.74^{cd}
HHP11	4.10±0.00^{b}	4.21±0.00^{a}	5.38±0.59^{a}	5.05±0.18^{a}
HHP12	1.76±0.05^{h}	1.77±0.04^{f}	1.07±0.84^{cde}	1.17±0.22^{de}
HHP13	1.56±0.05^{i}	1.69±0.03^{f}	1.54±0.36^{bcde}	0.46±0.16f^{g}
HHP14	5.10±0.00^{a}	4.21±0.00^{a}	1.90±0.15^{a}	5.05±0.38^{a}
HHP15	2.46±0.03^{f}	2.61±0.04^{d}	1.97±0.34^{bcd}	2.23±0.34^{c}

*Data in the same column with different superscript letter are significantly different ($p \leq 0.05$).

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The mean initial *B. bruxellensis* count of 4.91 ± 0.61 log cfu/mL was significantly reduced by all HHP treatments. The lowest decrease of 0.50 ± 0.30 was provided by HHP2 (200 MPa, 15 min, 22 °C); whereas the highest decrease of 5.38 ± 0.59 was provided by HHP11 (500 MPa, 15 min, 40 °C) (Table 4). Both pressure and temperature had significant effect on *B. bruxellensis* inactivation.

The mean initial *L. brevis* population of the control hardaliye samples was recorded as 5.05 ± 0.20 log cfu/mL. After processing the lowest reduction of 0.16 ± 0.03 log cfu/mL was observed by HHP2 (200 MPa, 3 min, 22 °C); whereas the highest reductions of 5.05 ± 0.18 log cfu/mL, 5.05 ± 0.22 and 5.05 ± 0.38 were recorded after HHP11 (500 MPa, 15 min, 22 °C), HHP7 (500 MPa, 3 min, 22 °C) and HHP14 (500 MPa, 9 min, 40 °C), respectively (Table 4). Inactivation of *L. brevis* was significantly affected by pressure, treatment time and temperature.

Applied HHP treatments did not cause significant difference in the sensory properties of cloudiness-clarity, dullness-shininess, color intensity, particle distribution, density of the hardaliye, flavor-aroma, taste, bitter taste, sour taste, sweetness and aftertaste. HHP treated hardaliye samples received higher scores than that of the control samples for all measured properties ($p>0.05$).

3.2. Optimization of High Hydrostatic Pressure Conditions for Hardaliye Drink

After analyzing the effect of physical, chemical, microbiological and sensorial attributes, the selected OD_{520} and inactivation of *L. brevis* were optimized for traditional hardaliye drink after HHP due to consideration of R^2, lack-of-fit value and VIF. The ANOVA results of OD_{520} and inactivation of *L. brevis* values are given in Table 5. According to ANOVA results, the insignificant terms were excluded from the models of the OD_{520} and inactivation of *L. brevis*. According to the polynomial regression model (Table 5), there is a positive correlation between pressure and OD_{520} and also treatment time and OD_{520} value. The quadratic treatment time term increased OD_{520} value at a rate of 0.276% ($p=0.000$), whereas quadratic pressure and treatment time terms decreased OD_{520} value at a rate of 0.182 ($p=0.002$) and 0.187% ($p=0.001$), respectively (Table 5).

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Table 5. Revised ANOVA results and estimated regression coefficients for the coded hardaliye drink by HHP model.

Term	OD_{520}			Inactivation of *Lactobacillus brevis*		
	Coeff.	**VIF**	***p* value**	**Coeff.**	**VIF**	***p* value**
Regression						
Linear						
X_1 (P)	0.148	1.00	0.000	-1.754	1.00	0.000
X_2 (T)				-0.645	1.00	0.012
X_3 (Trt)	0.124	1.00	0.002			
Square						
X_1*X_1	-0.182	1.01	0.002			
X_2*X_2	-0.187	1.01	0.001			
X_3*X_3	0.276	1.01	0.000			
Interaction						
X_1*X_2				0.731	1.00	0.040
X_1*X_3						
X_2*X_3	0.151	1.00	0.006			
Lack-of-fit		0.163			0.316	
Constant	50.86		0.000	1.608		0.000
R^2	0.70			0.61		
$R^2_{(adj)}$	0.65			0.58		
$R^2_{(pred)}$	0.58			0.54		

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The degree of effective operational conditions on the responses such as OD_{520} and inactivation of *L. brevis* can be inferred from comparing the magnitudes of the coefficients of regression models. Pressure was the most important factor with the highest rate increase in pressure for OD_{520} (0.148) and inactivation of *L. brevis* (1.754) (Table 5). The R^2 indicates that 70 and 61% of the variation in the OD_{520} and inactivation of *L. brevis* value can be attributed to the HHP processing conditions analyzed by this model while the remaining 30 and 39% variation is the error, respectively. The goodness-of –fit (R^2_{adj}) of the models showed that 0.65 and 0.58 of variations in OD_{520} and inactivation of *L.brevis*, respectively. The insignificant lack of fit values for these two models also showed that the model fitted the experimental data well (Table 5). The operational settings were optimized to maximize the OD_{520} value and minimize the inactivation of *L. brevis*. The best solution for multi response optimization which based on the composite desirability function was represented in Figure 1. Ideal composite desirability function was close to 1. The maximum OD_{520} (51.27) and maximum inactivation of *L. brevis* (0.0061) were obtained with the optimum operational conditions (490 MPa, 29 °C for 15 min) (Figure 2).

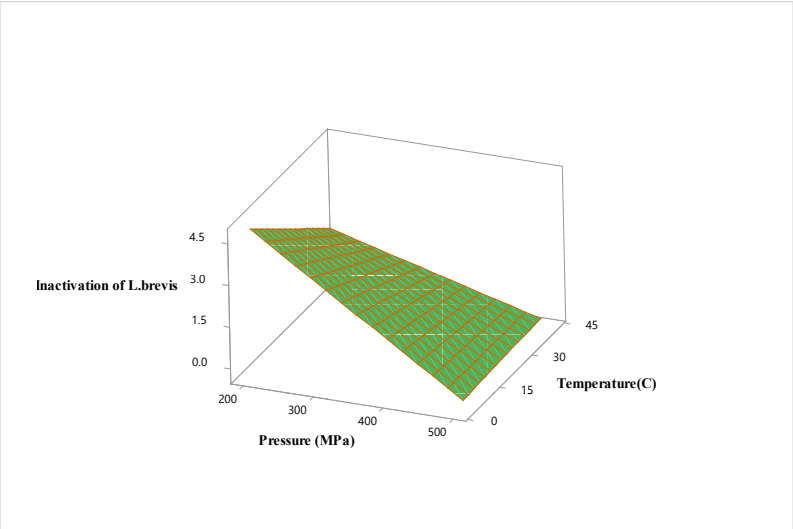
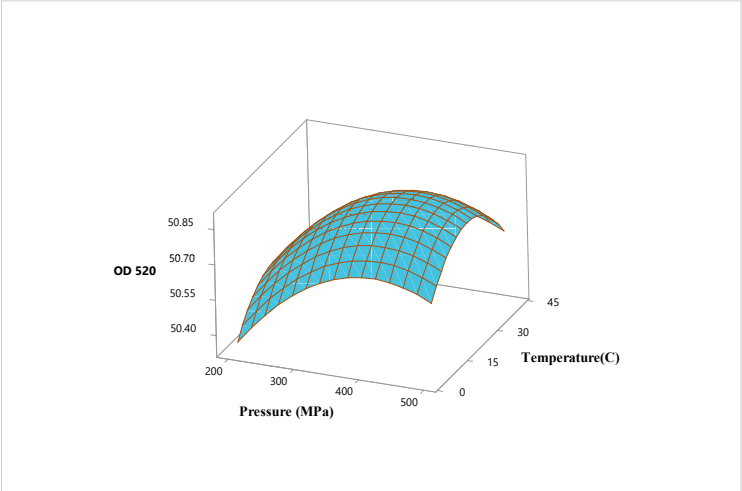


Figure 1. Response surface plot of HHP condition optimization for inactivation of *Lactobacillus brevis* in hardaliye drink.



(**a**)

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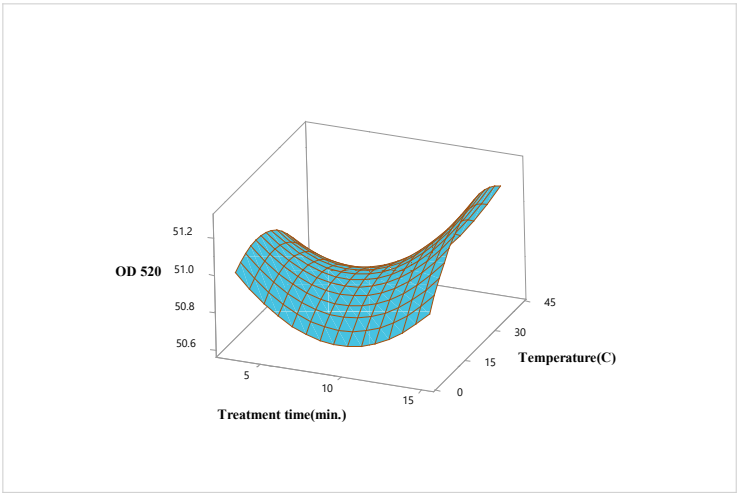
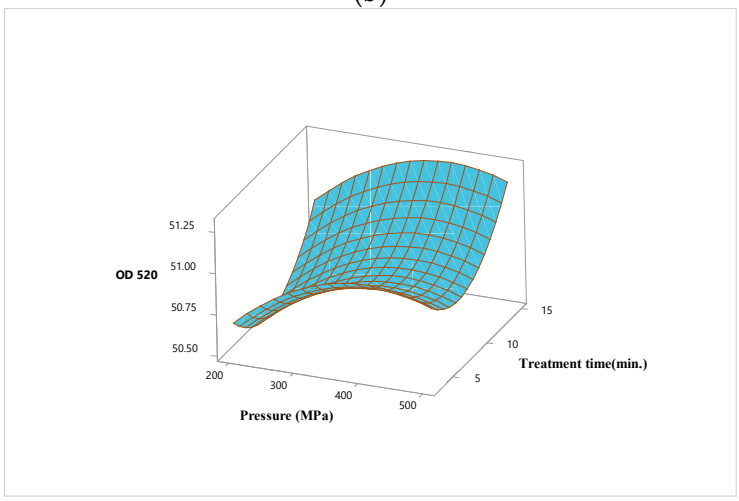


(**b**)



(**c**)

Figure 2. Response surface plots showing interaction effects of pressure and temperature (**a**), temperature and treatment time (**b**), pressure and treatment time (**c**) on the OD_{520} for hardaliye drinkReferences.

The effects of the HHP process conditions on the multiple responses (inactivation of *L. brevis* and OD_{520}) were represented using the 3D surface plots. Both pressure and temperature have a linear positive effect on inactivation of *L.brevis* (Figure 1). Inactivation of *L. brevis* was affected by both pressure and temperature and fell with the increased pressure and temperature at 9 min. Inactivation of *L.brevis* decreased with the increased pressure under the lowest temperature (4°C) (Figure 1). The highest OD520 value was obtained at 400 MPa and 22°C. The pressure enhances OD_{520} value justifying the significant square of pressure and temperature terms in model (Figure 2a). The OD_{520} value with increased temperature under the longest treatment time at an increasing rate (Figure 2b). The longest treatment time maximized OD $_{520}$ value at the highest pressure (500 MPa) (Figure 2c).

3.3. Shelf-life Studies of Hardaliye Drink

Control hardaliye samples stored at 4°C spoiled after 15 days, whereas the control samples stored at 22°C spoiled after 3 days. HHP treated samples stored at 4 and 22°C, on the other hand, spoiled after 228 and 108 days, respectively. Although storage temperature did not cause significant difference on HHP treated samples, pH of the all

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samples significantly decreased by increased storage time ($p \leq 0.05$). While pH of the control samples at 4 °C (3.69 ± 0.03) at the beginning of the shelf-life studies changed to 3.52 ± 0.04; pH of the HHP treated samples at 4 and 22 °C (3.83 ± 0.02 and 3.77 ± 0.03) changed to 3.69 ± 0.04 and 3.69 ± 0.09 at the end of the shelf-life studies, respectively (Table 6). Conductivity of the HHP treated samples did not significantly change neither by storage temperature nor by storage time ($p > 0.05$) (Table 6).

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Table 6. Changes in the physical properties of hardaliye during shelf-life studies.

		Storage temperature			
		4°C		22°C	
	Days	Control	HHP treated	Control	HHP treated
pH	0	3.69±0.03^{Ba}	3.83±0.02^{Aa}	3.78±0.03^{A}	3.77±0.03^{Aa}
	15	3.52±0.04^{Bb}	3.85±0.04^{Aa}		3.76±0.04^{Ab}
	30		3.72±0.03^{Abc}		3.71±0.02^{Ab}
	45		3.74±0.04 ^{Ab}		3.75±0.03^{Aab}
	66		3.80±0.03 ^{Aab}		3.74±0.03 ^{Aab}
	87		3.66±0.09^{Ac}		3.72±0.07^{Ab}
	108		3.69±0.02^{Abc}		3.66±0.09^{Ab}
	142		3.68±0.05^{Abc}		
	180		3.68±0.04^{Abc}		
	228		3.69±0.04^{Abc}		
	Days	Control	HHP treated	Control	HHP treated
Conductivity (µS/cm)	0	3.42±0.03^{Aa}	3.42±0.03^{A}	3.43±0.03^{A}	3.42±0.03^{Aa}
	15	3.41±0.03^{Ba}	3.41±0.03^{Ba}		3.48±0.05^{Aa}
	30		3.50±0.04^{A}		3.50±0.04^{Aa}
	45		3.47±0.04^{Aa}		3.52±0.04^{Aa}
	66		3.51±0.03^{Aa}		3.52±0.03^{Aa}
	87		3.44±0.03^{Aa}		3.48±0.04^{Aa}
	108		3.45±0.04^{Ba}		3.51±0.03^{Aa}
	142		3.42±0.04^{Aa}		
	180		3.42±0.04^{Aa}		
	228		3.47±0.04^{Aa}		
	Days	Control	HHP treated	Control	HHP treated
Color *L**	0	11.81±1.64^{Aa}	11.59±0.93^{Aa}	9.48±0.84^{B}	11.81±1.64^{Aa}
	15	2.98±0.02^{Bb}	3.98±0.12^{Ab}		3.60±0.36^{Ab}
	30		3.79±0.35^{Ab}		3.67±0.45^{Ab}
	45		3.68±0.33^{Ab}		3.40±0.43^{Ab}
	66		3.52±0.24^{Ab}		3.33±0.35^{Ab}
	87		3.52±0.36^{Ab}		3.10±0.58^{Ab}
	108		3.53±0.25^{Ab}		3.13±0.45^{Ab}

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	142		3.42±0.34^{Ab}		
	180		3.42±0.34^{Ab}		
	228		3.47±0.34^{Ab}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
	0	32.96±0.64^{Aa}	32.75±3.24^{A}	32.84±0.99^{A}	32.82±1.99^{Aa}
	15	14.04±0.12^{Bb}	13.46±2.22^{Ab}		12.49±1.12^{b}
	30		12.68±1.15^{Ab}		8.78±1.10^{Ac}
	45		12.42±1.17^{Ab}		8.51±0.60^{Ac}
	66		12.49±1.15^{Ab}		5.95±0.19^{Ad}
Color *a**	87		12.14±1.14^{Ab}		5.10±0.58^{Ad}
	108		12.16±1.25^{Ab}		5.11±0.45^{Ad}
	142		11.42±1.34^{Ab}		
	180		9.76±0.34^{Ac}		
	228		8.47±0.34^{Ad}		

	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
	0	12.87±1.58^{Aa}	12.19±1.42^{Aa}	12.26±1.01^{A}	13.80±2.58^{Aa}
	15	3.26±0.11^{Ab}	3.27±0.18^{Ab}		3.10±0.14^{Ab}
	30		3.25±0.06^{Ab}		2.49±0.10^{Bc}
	45		3.27±0.16 ^{Ab}		2.44±0.20^{Bc}
	66		3.24±0.13 ^{Ab}		2.47±0.30 ^{Bc}
	87		3.12±0.36^{Ab}		2.31±0.31^{Bc}
Color *b**	108		3.09±0.68^{Ab}		2.36±0.17^{Bc}
	142		2.57±0.64^{Ac}		
	180		2.08±0.34^{Ac}		
	228		3.03±0.08^{Ac}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
	0	15.47±2.78^{Aa}	17.24±1.44^{Aa}	15.41±1.44^{A}	13.89±2.58^{Aa}
	15	12.41±0.41^{Ab}	13.81±0.52^{Ab}		13.86±0.43^{Aa}
	30		13.04±0.14^{Ab}		9.07±0.80^{Bb}
	45		12.85±0.16 ^{Abc}		6.87±0.55^{Bc}
	66		12.86±0.12 ^{Abc}		6.57±0.49 ^{Bc}
Chroma	87		12.52±0.10^{Abc}		6.50±0.38^{Bc}
	108		12.16±1.49^{Abc}		5.62±0.89^{Bd}

	142		11.99±1.31^{Abc}		
	180		10.36±0.76^{Abc}		
	228		9.98±0.20^{Ad}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
Hue	0	0.36±0.03^{Aa}	0.34±0.08^{Aa}	0.33±0.22^{A}	0.38±0.03^{Aa}
	15	0.23±0.01^{Ab}	0.22±0.01^{Ab}		0.24±0.01^{Ab}
	30		0.22±0.01^{Ab}		0.25±0.01^{Bb}
	45		0.23±0.03 ^{Ab}		0.23±0.03^{Ab}
	66		0.25±0.02 ^{Ab}		0.23±0.03^{Ab}
	87		0.24±0.02^{Ab}		0.22±0.02^{Ab}
	108		0.25±0.03^{Ab}		0.17±0.02^{Bd}
	142		0.24±0.03^{Ab}		
	180		0.23±0.02^{Ab}		
	228		0.21±0.04^{Ab}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
Total color difference	0	0.00±0.00^{Ba}	1.32±0.12^{Aa}	0.00±0.00^{B}	1.38±0.13^{Aa}
	15	0.21±0.02^{Ab}	1.22±0.11^{Aa}		1.24±0.11^{Aa}
	30		1.22±0.10^{Aa}		1.25±0.10^{Aa}
	45		1.20±0.11 ^{Aa}		1.20±0.11^{Aa}
	66		1.25±0.12 ^{Aa}		1.21±0.11^{Aa}
	87		1.24±0.22^{Aa}		1.22±0.10^{Aa}
	108		1.25±0.13^{Aa}		1.24±0.12^{Aa}
	142		1.24±0.12^{Aa}		
	180		1.23±0.12^{Aa}		
	228		1.21±0.14^{A}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
Color intensity	0	4.90±0.37^{Aa}	4.90±0.36^{Aa}	4.59±0.33^{A}	4.29±0.40^{Aa}
	15	3.87±0.04^{Ab}	3.97±0.12^{Ab}		4.05±0.14^{Aa}
	30		4.16±0.23^{Ab}		4.39±0.24^{a}
	45		4.07±0.14 ^{Ab}		4.11±0.17^{Aa}
	66		4.00±0.21 ^{Ab}		4.29±0.15^{Aa}
	87		2.65±0.32^{Ac}		3.20±0.13^{Ab}
	108		2.28±0.16^{Ac}		3.24±0.21^{Ab}
	142		2.24±0.10^{Ac}		

180	2.23±0.12^{Ac}
228	2.22±0.13^{Ac}

*Data in the same column with different lowercase superscript letter and data in the same row with uppercase superscript letter are significantly different (p≤0.05).

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Color *L** value of the hardaliye samples were significantly decreased by storage time and temperature. While color *L** value control samples at 4 °C (11.81 ± 1.64) and HHP treated samples at 4 and 22 °C (11.59±0.93 and 11.81±1.64) at the first day of shelf-life studies decreased to 2.98 ± 0.02 after 15 day of the storage; *L** value of HHP treated samples at 4 and 22°C decreased to 3.47 ± 0.34 and 3.13 ± 0.45 after 228 and 108th day of the storage, respectively ($p \leq 0.05$) (Table 6). Color *a** value of control and HHP treated samples at 4°C and HHP treated samples at 22°C, 32.96 ± 0.64, 32.75 ± 3.24 and 32.82 ± 1.99; significantly reduced to 14.04 ± 0.12, 8.47 ± 0.34 and 5.11 ± 0.45, consequently (Table 6). Color *b** values of 12.87 ± 1.58, 12.19 ± 1.42 and 13.80 ± 2.58 for control and HHP treated samples at 4°C and HHP treated samples at 22 °C at the first day of shelf-life studies decreased to 3.26 ± 0.11, 3.03 ± 0.08 and 2.36 ± 0.17 by the end of the 15th, 228th and 108th day ($p \leq 0.05$) (Table 6).

In parallel to color values significant decrease in chroma values of the samples were observed. Chroma values of 15.47 ± 2.78, 17.24 ± 1.44 and 13.89 ± 2.58 for control and HHP treated samples at 4 °C and HHP treated samples at 22 °C at the beginning of the shelf-life studies decreased to 12.41 ± 0.41, 9.98 ± 0.20 and 5.62 ± 0.89, respectively after 15th, 228 and 108th day of the shelf-life study ($p \leq 0.05$) (Table 6). Hue values of the control samples at 4 °C (0.36 ± 0.03), and HHP treated samples at 4 °C (0.34 ± 0.08) and 22 °C (0.38 ± 0.03) decreased to 0.23 ± 0.01, 0.21 ± 0.04 and 0.17 ± 0.02 at the end of the 15th, 228 and 108th day of the storage, consequently ($p \leq 0.05$) (Table 6). Except for the control samples at 4°C no significant change was observed for the total color difference of the HHP treated samples at both 4 and 22 °C ($p > 0.05$). Color intensity of the control samples at 4 °C and HHP treated samples at 4 and 22°C (4.90 ± 0.37, 4.90 ± 0.36 and 4.29 ± 0.40) significantly decreased to 3.87 ± 0.04, 2.22 ± 0.13 and 3.24 ± 0.21 by the storage time. Both storage time and temperature had significant effect on *L**, *a** *b** values and color intensity of the samples ($p \leq 0.05$) (Table 6).

A significant increase was observed in both TMAB and TMY counts of the all samples with storage time. While HPP treated samples at 4 and 22 °C increased from 0.00 ± 0.00 and 0.03 ± 0.02 log cfu/mL to 2.62 ± 0.13 and 3.56 ± 0.30 log cfu/mL; TMAB of the control samples increased from 4.00 ± 0.49 log cfu/mL to 6.56 ± 0.24 log cfu/mL ($p \leq 0.05$) (Table 7). TMY count of the control samples at 4 °C and HHP treated samples at both 4 and 22 °C increased from 3.33 ± 0.26, 0.00 ± 0.00 and 0.00 ± 0.02 log cfu/mL to 4.37 ± 0.22, 2.78 ± 0.14 and 2.84 ± 0.28 log cfu/mL, respectively. Increase in both TMAC and TMY have significantly affected by both storage time and temperature as samples at 22 °C had higher microbial count than that of the samples at 4°C (Table 7).

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Table 7. Changes in the endogenous microflora of hardaliye during shelf-life studies.

	Storage temperature				
		4°C		22°C	
	Days	Control	HHP treated	Control	HHP treated
TMAB	0	4.00±0.49^{Aa}	0.00±0.00^{Bd}	4.00±0.33^{A}	0.3±0.02^{Be}
	15	6.56±0.24^{Ab}	0.47±0.10^{Cc}		1.02±0.0^{Bd}
	30		0.49±0.13^{Bc}		1.12±0.24^{Ad}
	45		0.58±0.11 ^{Ac}		1.31±0.16^{Ad}
	66		1.38±0.21 ^{Ab}		2.06±0.15^{Ac}
	87		1.40±0.18^{Ab}		2.61±0.16^{Ab}
	108		2.62±0.20^{Aa}		3.56±0.30^{Aa}
	142		2.24±0.10^{Aa}		
	180		2.31±0.12^{Aa}		
	228		2.62±0.13^{Aa}		
	Days	Control	HHP treated	Control	HHP treated
TMY	0	3.33±0.26^{Aa}	0.00±0.00^{Be}	3.67±0.44^{A}	0.00±0.02g
	15	4.37±0.22^{Ab}	0.00±0.00^{Ce}		0.56±0.0^{Bf}
	30		0.00±0.00e		0.84±0.16^{Ae}
	45		0.38±0.10 ^{Ad}		1.04±0.06^{Ad}
	66		1.55±0.20^{Ac}		1.46±0.10^{Ac}
	87		1.86±0.18^{Ac}		2.02±0.47^{Ab}
	108		1.98±0.16^{Ac}		2.84±0.28^{Aa}
	142		2.12±0.14^{Ab}		
	180		2.48±0.18^{Ab}		
	228		2.78±0.14^{Aa}		

*Data in the same column with different lowercase superscript letter and data in the same row with uppercase superscript letter are significantly different (p≤0.05).

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A significant decrease in the clarity and increase in cloudiness of the hardaliye drink was observed both by increase in time and in storage temperature. Clarity-cloudiness of the control samples at 4 °C and HHP treated samples at both 4 and 22 °C (9.33 ± 0.89, 9.60 ± 0.44 and 9.88 ± 0.43 reduced to 6.77 ± 0.22, 6.56 ± 0.24 and 5.94 ± 0.20B, respectively ($p \leq 0.05$) (Table 8). Similarly, shininess of the samples decreased by the storage temperature revealing increase in dullness. While shininess of the control samples at 4 °C decreased from 9.22 ± 0.38 to 5.40 ± 0.23, shininess of the HHP treated samples at 4 and 22 °C decreased from 9.66 ± 0.41 and 9.33 ± 0.28 to 7.05 ± 0.28 and 7.02 ± 0.32, respectively ($p \leq 0.05$) (Table 8). Color intensity of all samples were significantly decreased by storage time and temperature as well. The mean initial color intensity of the control samples at 4 °C and HHP treated samples at both 4 and 22 °C (7.33 ± 1.56, 8.46 ± 0.38 and 8.22 ± 0.38) reduced to 6.44 ± 0.49, 6.00 ± 0.24 and 6.14 ± 0.38, consequently ($p \leq 0.05$) (Table 8). Flavor-aroma of the both control and HHP treated samples significantly decreased by the storage time. While flavor-aroma of the control samples at 4 °C reduced from 7.03 ±1.44 to 6.44±0.49, flavor-aroma of the HHP treated samples of 8.46 ± 0.38 and 8.22 ± 0.37 at 4 and 22 °C decreased to 6.00 ± 0.24 and 6.14 ± 0.35, consequently ($p \leq 0.05$) (Table 8). Both bitter and sour taste of hardaliye drinks were significantly increased by storage time and temperature. The mean bitter taste of control samples at 4 °C (4.44 ± 0.72) increased to 6.41 ± 0.59; whereas HHP treated samples at 4 and 22 °C (3.66 ± 0.66 and 4.02 ± 0.42 increased to 4.02 ± 0.28 and 4.34 ± 0.30, respectively ($p \leq 0.05$) (Table 8). The mean sour taste of 4.42 ± 0.50, 3.44 ± 0.40 and 4.02 ± 0.42 for the control and HPP treated samples at 4 °C and HHP treated samples at 22 °C increased to 6.98 ± 0.58, 4.02 ± 0.28 and 4.34 ±0.30, consequently ($p \leq 0.05$) (Table 8). Aftertaste of control samples at 4 °C (6.78 ± 0.22) and HHP treated samples at 22 °C (7.04 ± 0.39 were significantly reduced to 3.48 ± 0.58 and 5.67 ± 0.50; whereas HHP treated samples at 4 °C (7.84 ± 0.40) reduced to 7.84 ± 0.40 with an insignificant difference ($p > 0.05$) (Table 8).

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Table 8. Changes in the sensory properties of hardaliye during shelf-life studies.

	Storage temperature				
		4°C		22°C	
	Days	Control	HHP treated	Control	HHP treated
Clarity-Cloudiness	0	9.33±0.89^{Aa}	9.60±0.44^{Aa}	9.00±0.50^{A}	9.88±0.43^{Aa}
	15	6.77±0.22^{Bb}	9.00±0.66^{Aa}		8.44±0.72^{Aa}
	30		8.60±0.80^{Aa}		8.33±0.20^{Aa}
	45		7.80±0.10 ^{Aab}		7.04±0.26^{Ab}
	66		7.68±0.32^{Ab}		6.32±0.14^{Bc}
	87		7.22±0.18^{Ab}		6.05±0.22^{Bc}
	108		6.98±0.26^{Ac}		5.94±0.20B^{c}
	142		6.88±0.34^{Ac}		
	180		6.58±0.28^{Ac}		
	228		6.56±0.24^{Ac}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
Shininess-Dullness	0	9.22±0.38^{Aa}	9.66±0.41^{Aa}	9.21±0.50^{A}	9.33±0.28^{Aa}
	15	5.40±0.23^{Cb}	9.10±0.60^{Aa}		8.12±0.60^{Bab}
	30		8.96±0.46^{Aa}		8.00±0.23^{Ab}
	45		8.14±0.13^{Aab}		7.65±0.32^{Bb}
	66		8.08±0.30^{b}		7.50±0.22^{Bb}
	87		7.69±0.26^{Ab}		7.42±0.26^{Ab}
	108		7.62±0.24^{Ac}		7.02±0.32^{Ab}
	142		7.22±0.39^{Ac}		
	180		7.11±0.42^{Ac}		
	228		7.05±0.28^{Ac}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
Color intensity	0	7.33±1.56^{Aa}	8.46±0.38^{Aa}	7.33±0.44^{A}	8.22±0.38^{Aa}
	15	6.44±0.49^{Cb}	8.23±0.46^{Aa}		8.01±0.35^{Bb}
	30		8.11±0.32^{Aa}		7.89±0.26^{Ab}
	45		7.98±0.39^{Aab}		7.33±0.43^{bc}
	66		7.65±0.42^{b}		7.22±0.38^{Bc}
	87		7.03±0.36^{Ab}		7.02±0.16^{Ac}
	108		6.82±0.28^{Ab}		6.14±0.38^{Ad}

	142		6.18±0.42^{Abc}		
	180		6.01±0.32^{Ac}		
	228		6.00±0.24^{Ac}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
	0	7.03±1.44^{Aa}	8.46±0.38^{Aa}	7.00±0.44^{A}	8.22±0.37^{Aa}
	15	6.44±0.49^{Bb}	8.23±0.46^{Aa}		8.01±0.35^{Ab}
	30		8.11±0.32^{Aa}		7.89±0.26^{Ab}
	45		7.98±0.39^{Aab}		7.33±0.43^{Ac}
	66		7.65±0.42^{Ab}		7.22±0.38^{Ac}
Flavor-aroma	87		7.03±0.36^{Ab}		7.02±0.16^{Ac}
	108		6.82±0.28^{Ab}		6.14±0.25^{Ad}
	142		6.18±0.42^{Abc}		
	180		6.01±0.32^{Ac}		
	228		6.00±0.24^{Ac}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
	0	4.44±0.72^{Aa}	3.66±0.66^{Aa}	4.33±0.524^{A}	4.02±0.42^{Aa}
	15	6.41±0.59^{Cb}	3.67±0.40^{Aa}		4.08±0.28^{Bb}
	30		3.65±0.36^{Aa}		4.09±0.20^{Ab}
	45		3.76±0.30^{Aab}		4.03±0.23^{bc}
	66		3.78±0.40^{b}		4.12±0.30^{Bc}
Bitter taste	87		3.80±0.32^{Ab}		4.22±0.26^{Ac}
	108		3.96±0.20^{Ab}		4.34±0.30^{Ad}
	142		3.98±0.40^{Abc}		
	180		4.04±0.30^{Ac}		
	228		4.02±0.28^{Ac}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
	0	4.42±0.50^{Aa}	3.44±0.40^{Aa}	4.33±0.52^{A}	4.02±0.42^{Aa}
	15	6.98±0.58^{Cb}	3.40±0.43^{Aa}		4.08±0.28^{Bb}
	30		4.01±0.42^{Aa}		4.09±0.20^{Ab}
	45		4.02±0.38^{Aab}		4.03±0.23^{bc}
	66		3.78±0.40^{Ab}		4.12±0.30^{Bc}
Sour taste	87		3.80±0.32^{Ab}		4.22±0.26^{Ac}
	108		3.96±0.20^{Ab}		4.34±0.30^{Ad}
	142		3.98±0.40^{Abc}		

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	180		4.04±0.30^{Ac}		
	228		4.02±0.28^{Ac}		
	Days	**Control**	**HHP treated**	**Control**	**HHP treated**
After taste	0	6.78±0.22^{Aa}	7.84±0.40^{Aa}	6.32±0.55^{A}	7.04±0.39^{Aa}
	15	3.48±0.58^{Cb}	7.64±0.60^{Aa}		6.68±0.28^{Bb}
	30		7.33±0.39^{Aa}		6.29±0.35^{Ab}
	45		7.34±0.44^{Aa}		6.20±0.42^{bc}
	66		7.67±0.36^{Aa}		6.18±0.36^{Bb}
	87		7.56±0.44^{Aa}		6.08±0.42^{Bb}
	108		7.49±0.38^{Aa}		5.67±0.50^{Bb}
	142		7.41±0.39^{Aa}		
	180		7.38±0.49^{Aa}		
	228		7.26±0.51^{Aa}		

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*Data in the same column with different lowercase superscript letter and data in the same row with uppercase superscript letter are significantly different ($p \leq 0.05$)

Although hardaliye is very unique with its production method and sensory properties, its consumption is very limited due to short shelf-life. Studies related to determination of some properties of hardaliye produced from Müşküle grapes with addition of mustard seeds (1.5%) and potassium benzoate (0.1%) fermented at 30 °C revealed that resulted drink had 17.5% TSS, 3.9 g/L total acidity (tartaric acid), 0.37% (v/v) alcohol content, 0.147 g/L volatile acid content and pH of 4.09 with the TPSC of 272.53 mg GAE/L [15]. Properties of hardaliye may change depending on the minor differences in production method and raw materials thus, differences may be seen among different hardaliye samples. Especially grape variety plays a major role in color, flavor and chemical properties of hardaliye.

Hardaliye produced from papazkarası blue-black grapes stored at both 4 and 20 °C for 60 days revealed the highest proportion of red color at the beginning of the shelf-life studies; however, 60 and 78% losses in anthocyanin content were reported by the end of the shelf-life studies at 4 and 20 °C, respectively. TPSC and TAC value of the samples were measured as 1743 ± 8.67 mg GAE/L and 8.53 mM Trolox/mL in the fresh beverage, consequently. Higher storage temperature revealed higher amount of anthocyanin loss during storage accompanied by increased polymeric color values and other color parameters revealing polymerization of the anthocyanins [16]. Similar to current study color properties of the hardaliye significantly reduced by both storage time and temperature.

Shelf-life extension of traditional products is of great concern for food industry. In fact, utilization of novel processing technologies such as ultraviolet (UV), pulsed electric fields (PEF), as well as HHP were tested to process different products. For example, PEF processing of traditional licrorice root sherbet (LRS) by various processing parameters of electric field strength, treatment time and processing temperatures did reveal no significant changes in most of the measured properties with significant amount of endogenous microflora. PEF treated LRS samples had shelf life of 40 days; whereas control samples had a 5 day of shelf life at 4 °C [13].

HHP processing of traditional fermented turnip juice (shalgam) by 3-15 min treatment time, 4-40 °C treatment temperature and 200-500 MPa pressure revealed optimum processing parameters of 34.23 °C, 15 min, and 500 MPa. HHP treatment provided over 90 days of extended shelf life at both 4 and 22 °C under optimum processing parameters [4]. HHP processing of LRS by 200–500 MPa pressure, 3-15 min treatment time and 4-40°C treatment temperature revealed optimum operational conditions of 500 MPa pressure, 9.90 min treatment time and 18.5°C treatment temperature. Shelf-life studies conducted with the optimum HHP operating conditions resulted with 25-day of storage compare to 2- and 7-days shelf life of the control samples at both 4°C and 22°C [14].

In general, HHP processing of juices resulted in no or slight changes in physicochemical properties. For example, HHP treatment of grapefruit juice by 600 MPa for 5 min preserved antioxidants and antioxidant capacity of the juice samples with ensuring microbiological safety at 4°C for 21 days [17]. HHP processing of white grape juice concentrate (GJC) by 200, 300 and 400 MPa for 2 and 4 min at room temperature (20 ± 2°C) provided significant reduction on *Botyritis cinerea*. TAC and total flavonoid content of HHP-treated samples were

significant decreased during storage at 4°C for 35 days [18]. HHP processing of cloudy ginger juice resulted in no significant change pH, TSS, TA, TAC, and color with 3 log cfu/mL reduction in microbial load. Color darkening with increase in TPSC were reported during storage at 4 and 22°C [19].

4. Conclusions

Increasing consumer demand for additive-free, high-quality and fresh-like fruit and vegetable products especially fermented traditional products such as hardaliye attracts attention to novel processing technologies in addition to concept of minimal processing. HHP, as a prime example, is one of the best alternatives to thermal processing to preserve physicochemical, bioactive and sensory properties of juices and drinks. As one of the traditional fermented drink, hardaliye has unique physical properties of flavor, aroma and taste in addition to color and aftertaste. However, current practices to extend its shelf-life involve addition of antimicrobial agents such as sodium benzoate causing unpleasant aroma formation in hardaliye. Addition of sodium benzoate is not preferred by the consumers but it is the only feasible approach to provide shelf-life extension of hardaliye. It is shown in this study that HHP provides a possible alternative to extend shelf life of hardaliye without addition of any antimicrobial agent. Thus, future studies need to focus on feasibility of HHP on hardaliye processing.

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