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High pressure processing of licorice drink with respect to quality characteristics, microbial inactivation, and shelf-life extension

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Abstract

High hydrostatic pressure (HHP) processing of traditional licorice drink with 200–500 MPa, 3–15 min, and 4°C–40°C revealed optimum operational conditions of 500-MPa pressure, 9.90 min and 18.5°C. Shelf-life studies with the optimum HHP settings were conducted for a 25-day storage at 4°C and 22°C. Control samples at 22°C and 4°C were deteriorated on Days 2 and 7, whereas the HHP-processed samples had a shelf life of 25 days. Initial pH, L^*, a^*, b^*, and C^* values and glabridin concentration significantly decreased, but conductivity and turbidity values, total mesophilic aerobic count and total mold and yeast count significantly increased, and no significant change was observed for the initial h°, titratable acidity (TA), glycyrrhizin concentration, and sensory properties for the HHP-processed samples during shelf-life studies. Except for vanillic, caffeic, and acetic acids and miristein, no significant change was observed on phenolic compounds and organic acids.

Practical applications

Due to perishable nature, licorice drink has very short shelf life, and this situation restricts large-scale production and marketing. Heat processing causes degradation on physical and sensory properties of licorice drink. HHP can achieve shelf life extension of licorice drink up to 1 month without a significant adverse impact on most of the quality parameters enabling large-scale production.

1 | INTRODUCTION

Liquorice (*Glycyrrhiza glabra*) is one of the oldest and well known plants in ancient China, Egypt, and Rome and have been used in medical, food, and cosmetic industries owing to its saponins, flavanones, flavonoids, amines, glucose, sucrose, amino acids, gums, essential oils, and starch present in its roots (Liu, Chen, et al., 2016). Glycyrrhizic acid up to 50 times as much sweet as sucrose consisting of one molecule of glycyrrhetinic acid, and two molecules of glucuronic acid is the major compound in licorice root (You et al., 2016).

Licorice drink (sherbet) is one of the popular traditional drinks consumed especially in summer in Turkey and some Middle Eastern countries. Licorice drink is prepared via the water extraction of the dried shredded licorice roots for at least 12 hr at room temperature, the removal of roots from liquid part, and its cooling for several hours. It has refreshing sweet taste and gold yellowish color. Although licorice drink is very popular, its consumption is very limited due to the microbial spoilage (Al-Balawnih et al., 2017). Because efforts to extend its shelf life through the heat processing have deteriorated its physical and sensory properties, alternative technologies are being sought to process licorice drink with the purpose of preserving its physical and sensory properties. Such previous studies involved pulsed electric field (PEF) treatment, combination of mild heat and PEF (Uzuner & Evrendilek, 2017) and ultrasonication (Bakay, 2019).

High hydrostatic pressure (HHP) is nonthermal processing technology that successfully applied to different food samples. Mango

juice (Liu et al., 2014), *Lonicera caerulea* berries (Liu, Xu, et al., 2016), *Granny Smith* (GS) apple purée (Landl et al., 2010), clear and cloudy Se-enriched kiwifruit juices (Xu et al., 2018) and kimchi (Park et al., 2017) are some of the food samples processed by HHP for the preservation of physicochemical and sensory properties, retention of bioactive compounds, microbial inactivation, and shelf life extension. There exist a few studies such as Aday et al. (2018) and Genis (2016) about the HHP processing of licorice drink with inactivation of *Escherichia coli*, *Salmonella typhimurium*, and *Geobacillus stearothermophilus*. Even though HHP processing of licorice drink is reported in the literature, effect of processing parameters on quality attributes with process optimization and shelf life extension are not reported. Thus, the objective of this study was to quantify changes in HHP-processed licorice drink in terms of the physicochemical, bioactive and sensory properties, microbial inactivation and to determine the optimum HHP processing conditions based on the Box–Bhenken design for shelf-life studies conducted at 4°C and 22°C.

2 | MATERIALS AND METHODS

2.1 | Licorice root samples

Dried shredded licorice roots were purchased from a local supplier (Ankara, Turkey). The licorice drink was prepared by immersing 10 g of root in 1-L water at room temperature and leaving at 4°C for 16 hr. The drink samples were filtered to remove the particles, and a clear drink with goldish-yellow color was obtained. The samples were kept at refrigeration temperature and processed at the same day of preparation.

2.2 | High hydrostatic pressure

Aliquots of licorice root (450 ml) were packed in flexible pouches made of a multilayer polymer/aluminum/polymer film (polyethylene- aluminum–polypropylene) (APACK Packaging Technologies, Istanbul, Turkey), sealed in a vacuum sealer, and placed into a hydrostatic pressure vessel (2-L capacity pilot-scale HHP unit, Avure, Middletown, OH, USA). The samples were pressurized from 200 to 500 MPa for 2 to 15 min at 4°C to 22°C according to the Box–Behnken design (BBD) (Table 1).

2.3 | Physical properties

pH was measured at room temperature, using a pH meter (pH-2005 model, JP Selecta SA, Barcelona, Spain). Conductivity (mS cm^{-1}) was measured using a handheld conductivity meter (Sension 5 model, HACH, CO, ABD). Turbidity (NTU) was analyzed using a turbidimeter MICRO TPI, Model 20008), whereas titratable acidity (TA, g 100^{-1} ml^{-1}) was determined as glycyrrhizic acid equivalent, using the titrimetric method (AOAC, 1990).

TABLE 1 (Un)coded variables of Box–Behnken design for physical, bioactive, sensory properties, and microbial inactivation of licorice drink processed by high hydrostatic pressure

Process number	Temperature (°C) X_1	Treatment time (min) X_1	Pressure (MPa) X_1
Control	–	–	–
1	40 (+1)	3 (−1)	350 (0)
2	22 (0)	3 (−1)	200 (−1)
3	40 (+1)	15 (+1)	350 (0)
4[a]	22 (0)	9 (0)	350 (0)
5	22 (0)	15 (+1)	200 (−1)
6	4 (−1)	3 (−1)	350 (0)
7	22 (0)	3 (−1)	500 (+1)
8	4 (−1)	15 (+1)	350 (0)
9	4 (−1)	9 (0)	500 (+1)
10[a]	22 (0)	9 (0)	350 (0)
11	22 (0)	15 (+1)	500 (+1)
12	40 (+1)	9 (0)	200 (−1)
13	4 (−1)	9 (0)	200 (−1)
14	40 (+1)	9 (0)	500 (+1)
15[a]	22 (0)	9 (0)	350 (0)

[a]Mid-point.

Color L^*, a^*, and b^* values were measured using a Hunter Color Flex spectrophotometer (Hunter Associates Laboratory Inc., Reston VA, USA). Chroma (C^*), hue (h°), and total color difference (ΔE) were calculated from the L^*, a^*, and b^* values. Color density (IC); color tone; percent color components of yellow, blue, and red as yellow color tone (YCT, OD_{420}); blue color tone (BCT, OD_{520}); and red color tone (RCT, OD_{620}) were measured. Spectrophotometric measurements were performed by PG Instruments T80 + UV/VIS model spectrophotometer against distilled water (Ribereau-Gayon et al., 2006).

3,5-Dinitrosalicylic acid (DNS) reagent contained 1% DNS, 2% NaOH, and 20% sodium potassium tartrate (wt/vol) was used to calculate reducing sugar content (RSC). Glucose solutions at the concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 g/L were used as substrate, and a calibration curve was prepared using color intensity measured at 540 nm (Sengupta et al., 2000). All chemicals and standards were obtained from Sigma Aldrich (Steinheim, Germany) and Sigma Chemical Co, (Stockholm, Sweden), respectively.

2.4 | Total antioxidant capacity and total content of phenolic substances

Total antioxidant capacity (TAC) and total phenolic substance content (TPSC) were determined using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH, Sigma Chemical Co., Stockholm, Sweden) free radical method described by Moon and Terao (1998) and the Folin-Ciocalteu method at 720 nm by Abdullakasim et al. (2007), respectively.

2.5 | Organic acids

Acetic, formic, and fumaric acid concentrations was determined with a high-performance liquid chromatography (HPLC) (Sturm et al., 2003). Five milliliters of the samples were mixed with 20-ml, 5-mM H_2SO_4, and the mixture was centrifuged at 4°C for 10 min at 6,500 ×*g*. The supernatant was filtered through a 0.45-μm PTFE filter (Micron Separations Inc., Westboro, MA, USA), and 20 μl of the filtrate were injected to HPLC system coupled with the XTerra column (5 μm particle size, 4.6 mm diameter, and 250 mm length, Waters, Ireland) at 50°C. Isocratic elution was performed with 5-mM H_2SO_4 (0.5 ml/min) for 30 min with the identification of the peaks at 210 nm for lactic acid, 230 nm for fumaric acid, and 244 nm for acetic acid. Concentrations of organic acids were quantified comparing their peak areas against the standard curves obtained specifically for the reference solutions (Sigma Chemical Co., Stockholm, Sweden) in concentrations ranging from 5 to 200 mg/L for acetic and lactic acid and from 0.05 to 50 mg/L for fumaric acid, respectively (Sturm et al., 2003).

2.6 | Phenolic compounds

Mixture of 5 ml of samples and 10 ml of 80% methanol was centrifuged at 6,500 ×*g* at 4°C for 10 min and degassed in the ultrasound water bath for 10 min at room temperature (Justesen et al., 1998). After filtration of the supernatant by using a 0.45-μm PTFE filter, 20 μl of the filtrates were injected to the HPLC equipped with XTerra column fitted with C18 guard column (ACE, 4.6 × 250 mm, UK). Mobile phase formed by 2% formic acid (A) and 100% acetonitrile (B) at a rate of 0.8 ml/min at gradient flow were used at the column temperature of 30°C. Photodiode array (PhDA) detector was used at 280 nm for gallic acid, 320 nm for *p*-coumaric acid, chlorogenic acid and myricetin, 290 nm for vanillic acid, and 320 nm for caffeic acid, respectively. Concentration of each phenolic compound was quantified comparing its peak area against the standard curve obtained specifically for the reference solutions in concentrations ranging from 0.25 to 200 mg/L. All chemicals and standards were obtained from Sigma Aldrich (Steinheim, Germany) and Sigma Chemical Co (Stockholm, Sweden), respectively.

2.7 | Glycyrrhizin and glabridin contents

Sample extraction and HPLC analysis with some modifications were performed according to the methods described by Tian et al. (2008). Five milliliters of the samples were mixed with 50-ml ethanol : water mixture (30:70 vol/vol), and extraction was completed in ultrasonic water for 5 min. The samples were filtered through a 0.45-μm filter, and 25 μl of the samples were injected to HPLC with C18 column (150 × 4.6 mm). Mobile phase at a flow rate of 1 ml/min was formed with ethanol : water (70:30 vol/vol) mixture containing 1% acetic acid. PhDA detector at 252 nm was used. Concentration of each compound was quantified comparing its peak area against the standard curve in concentrations ranging from 0.025 to 5 mg/L for glabridin and 0.5 to 800 mg^{-1} for glycyrrhizin, respectively. Standards were obtained from Sigma Chemical Co, (Stockholm, Sweden).

2.8 | Microbial cultures

Bacillus circulans and *Candida tropicalis* cultures were isolated from licorice drink by API50 CHB/E and API 20C tests (bioMérieux, Inc., Durham, NC, USA), respectively. Isolated culture of *B. circulans* were grown on nutrient agar, and the plates were incubated at 25°C for 48 hr. *C. tropicalis* were grown on yeast extract agar (YEA, Fluka, Seelze, Germany) and the plates were incubated at 37°C for 72 hr. Both cultures were inoculated into licorice drink at the level of 10^5–10^6 cfu/ml.

2.9 | Microbial enumeration

Inactivations of total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) were performed by surface plating of the appropriate dilutions in 0.1% (wt/vol) peptone onto PCA for TMAB and PDA acidified with 10% (wt/vol) tartaric acid for TMY. For the count of *B. circulans*, appropriate dilutions were surface plated on nutrient agar, whereas dilutions for *C. tropicalis* were plated on YEA. PCA and YEA plates were incubated at 35 ± 2°C for 24–48 hr, whereas PDA and nutrient agar plates were incubated at 22 ± 2°C for 3–5 days, respectively. Results were reported as log cfu/ml. Agars and tartaric acid were obtained from Fluka (Seelze, Germany).

2.10 | Concentrations of metal ions

An inductively coupled plasma mass spectroscopy (ICP-MS) (XSERIES 2, Thermo Scientific, Schwerte, Germany) was used to determine the metal ion concentration. Analytical masses were ^{11}B, ^{23}Na, ^{24}Mg, ^{39}K, ^{40}Ca, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{111}Cd, ^{137}Ba, ^{202}Hg, and ^{208}Pb (Kurtoglu et al., 2014). A calibration curve for each element was prepared with the concentrations from 2.5 to 1,000 mg/kg. Limits of detection (LOD) and quantification (LOQ) values were calculated, and recovery values were reported as 70%–120%.

2.11 | Sensory analyses

Quantitative descriptive analysis (QDA) was used for the sensory analyses of the licorice drink samples. The samples were evaluated for the sensory properties of cloudiness-clarity, dullness-shininess, color intensity, particle distribution, flavor, juice density, licorice

taste, bitter taste, sour taste, sweetness, and aftertaste. Nine-point hedonic scale test was performed with 30 trained panelists (Evrendilek, 2017).

2.12 | Shelf-life studies

Shelf life of the licorice drink samples was performed with the best HHP processing conditions found from the optimization studies. Five hundred milliliters of the samples in flexible pouches were stored at both 4°C and 22°C for shelf-life studies. The sampling was made on 0th, 15th, 20th, and 25th day of the storage. pH, conductivity, turbidity, L^*, a^*, b^*, C^*, h^o, ΔE, TMAB, TMY, phenolic compounds, organic acids, glycyrrhizin and glabridin contents, as well as metal ion concentration and sensory analyses were performed during the shelf-life studies. Licorice drink is usually consumed in summer during which its temperature rises above 20°C; thus, the samples were also stored at 22°C to determine its shelf life at the higher temperatures.

2.13 | Data analyses

Thirty-three responses of licorice drink as pH, conductivity, turbidity, TA, color parameters, reducing sugar, TAC, TPSC, inactivation of TMAB, TMY, *B. circulans* and *C. tropicalis*, as well as sensory analyses were used for the optimization studies as a function of pressure (200 to 500 MPa), treatment time (3 to 15 min), and treatment temperature (4°C to 22°C) using the BBD with a quadratic model. The overall BBD configuration with its (un)coded predictors is presented in Table 1. All statistical analyses were performed with MINITAB 17.0 (Minitab Inc. State College, PA, USA). The following quadratic regression model was used in order to best fit the experimental data:

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \cdots + b_{35}X_{35}^2 \quad (1)$$

where Y_n is the response variable for 33 responses; b_0 to b_{35} are slope coefficients; and X_1, X_2, and X_3 are the magnitude of pressure, treatment time, and treatment temperature, respectively. Additional experiments in triplicate were carried out under the optimal conditions determined to validate the models. Analysis of variance (ANOVA) and regression models were performed at 95% confidence interval in order to define the significant terms of the predictive model, ($p \leq .05$). The multiple comparisons were made using Tukey's test. To verify the predicted model, the coefficient of variation (CV, %) was computed as follows:

$$CV = \frac{\sigma}{\overset{!!}{X}} 100 \quad (2)$$

where σ is sample standard deviation and $\overset{!!}{X}$ is sample mean.

3 | RESULTS AND DISCUSSIONS

3.1 | Effect of HHP on licorice drink properties

The mean initial pH, conductivity, turbidity, and RSC of the licorice drink samples were measured as 6.92 ± 0.00, 383.3 ± 0.58 mS cm^{-1}, 28.94 ± 0.24 NTU, and 0.35 ± 0.00 g/L. Depending on the applied HHP processing parameters, significant differences were observed among the control and the processed samples for pH, conductivity, turbidity, and RSC, whereas no significant difference was observed for the TA of the samples as it did not change with HHP processing (Table 2).

Significant changes on the mean initial L^*, a^*, b^*, C^*, h°, CI, color tone, YCT, RCT, and BCT color values of 52.32 ± 0.00, 6.15 ± 0.01, 53.24 ± 0.11, 53.60 ± 0.10, 1.45 ± 0.00, 1.05 ± 0.00, 4.41 ± 0.04, 73.29 ± 0.14, 17.06 ± 0.12, and 7.64 ± 0.01 of the control samples were observed depending on the applied parameters (Table 3). HHP, on the other hand, provided either significant increase or no significant change in the mean initial TAC and TPSC of 18.33 ± 0.06% and 0.12 ± 0.00 GAE ml^{-1} of the control samples (Table 4).

The mean initial TMAB and TMY counts of 5.61 ± 0.29 and 5.48 ± 0.20 log cfu/ml changed from 2.28 ± 0.15 to 3.98 ± 0.45 and from 1.83 ± 0.01 to 3.55 ± 0.15 log cfu/ml, respectively. Whereas the mean initial *B. circulans* of 5.39 ± 0.42 log cfu/ml ranged from 2.26 ± 0.03 to 4.23 ± 0.33 log cfu/ml, the mean initial *C. tropicalis* of 5.45 ± 0.26 log cfu/ml ranged from 2.37 ± 0.09 to 4.11 ± 0.20 log cfu/ml with HHP treatments. Maximum 3.33 ± 0.16, 3.65 ± 0.01, 3.13 ± 0.04, and 3.08 ± 0.10 log inactivation were obtained in the mean initial TMAB, TMY, *B. circulans*, and *C. tropicalis* by HHP11 treatments, respectively (Table 5).

Cloudiness-clarity, dullness-shininess, color intensity, particle distribution, flavor, juice density, licorice taste, bitter taste, sour taste, sweetness, and aftertaste of the control samples estimated did not significantly changed with applied HHP parameters (Table 6). Due to specific phenolics including glabridin, glabrene, and glabrol as well as more than 300 flavonoids including flavanones and chalcones such as liquiritin, liquiritigenin, glabrolide, and licoflavonol, licorice root extract is used as active ingredient in different medicines and foods (Zhang & Ye, 2009). Thus, it is important to preserve phenolics and flavonoids because they provide health promoting properties. It is reported that HHP causes a disruption in the large molecules such as protein and lipids; however, small molecules like saponins and flavonoids remain unaffected (Linton & Patterson, 2000). Our results supported the idea of HHP being ineffective on small molecules because no significant decrease was observed on TPSC and TAC. In parallel to our results, Aday et al. (2018) reported that HHP of licorice drink at 450 MPa for 5 min revealed no significant differences between the control and the treated samples in pH, TSS, TPSC, total flavonoids, TAC, and glycyrrhizic acid content. Moreover, sensory analyses for flavor and appearance revealed no significant difference between the

TABLE 2 Changes in the physical properties of licorice drink processed by high hydrostatic pressure

Process	pH	Conductivity (mS cm^{-1})	Turbidity (NTU)	TA (g 100^{-1} ml^{-1})	Reducing sugar (g/L)
Control	6.92 ± 0.002^{a}	383.3 ± 0.577^{a}	28.94 ± 0.235^{a}	0.0204 ± 0.000^{a}	0.351 ± 0.0007^{a}
350 MPa/40°C/3 min	7.04 ± 0.000^{bc}	376.3 ± 1.528^{b}	40.67 ± 0.521^{b}	0.0204 ± 0.000^{a}	0.378 ± 0.0013^{bcd}
200 MPa/22°C/3 min	7.03 ± 0.005^{cd}	376.3 ± 0.577^{b}	44.89 ± 0.448^{cd}	0.0204 ± 0.000^{a}	0.374 ± 0.0012^{ef}
350 MPa/40°C/15 min	7.05 ± 0.000^{b}	378.3 ± 0.577^{cdef}	40.59 ± 0.546^{b}	0.0204 ± 0.000^{a}	0.378 ± 0.0007^{bc}
350 MPa/22°C/9 min	7.03 ± 0.005^{cd}	376.6 ± 0.577^{bf}	40.57 ± 0.495^{b}	0.0204 ± 0.000^{a}	0.376 ± 0.0010^{bcde}
200 MPa/22°C/15 min	7.02 ± 0.000^{ef}	379.3 ± 0.577^{cdg}	42.82 ± 0.500^{ef}	0.0204 ± 0.000^{a}	0.379 ± 0.0010^{b}
350 MPa/4°C/3 min	7.03 ± 0.005^{cd}	377.6 ± 0.577^{bdef}	44.04 ± 1.017^{cde}	0.0204 ± 0.000^{a}	0.376 ± 0.0008^{cdef}
500 MPa/22°C/3 min	7.01 ± 0.000^{f}	379.0 ± 0.000^{cdg}	40.50 ± 0.396^{b}	0.0204 ± 0.000^{a}	0.379 ± 0.0010^{b}
350 MPa/4°C/15 min	7.01 ± 0.000^{f}	378.6 ± 0.577^{cde}	41.12 ± 0.731^{bf}	0.0204 ± 0.000^{a}	0.379 ± 0.0013^{b}
500 MPa/4°C/9 min	7.03 ± 0.000^{cde}	378.0 ± 0.000^{bdef}	43.61 ± 1.003^{de}	0.0204 ± 0.000^{a}	0.374 ± 0.0010^{ef}
350 MPa/22°C/9 min	7.01 ± 0.000^{f}	379.0 ± 0.000^{cdg}	40.65 ± 0.387^{b}	0.0204 ± 0.000^{a}	0.376 ± 0.0010^{bcde}
500 MPa/22°C/15 min	7.02 ± 0.005^{de}	378.6 ± 0.577^{cde}	45.87 ± 0.641^{c}	0.0204 ± 0.000^{a}	0.375 ± 0.0002^{def}
200 MPa/40°C/9 min	7.04 ± 0.000^{bc}	380.0 ± 0.000^{cg}	41.18 ± 0.083^{bf}	0.0204 ± 0.000^{a}	0.376 ± 0.0007^{bcde}
200 MPa/4°C/9 min	7.04 ± 0.000^{bc}	377.0 ± 1.000^{bef}	40.92 ± 0.065^{b}	0.0204 ± 0.000^{a}	0.373 ± 0.0005^{f}
500 MPa/40°C/9 min	7.03 ± 0.005^{cd}	380.0 ± 0.000^{cg}	44.07 ± 1.180^{cde}	0.0204 ± 0.000^{a}	0.375 ± 0.0007^{f}
350 MPa/22°C/9 min	7.01 ± 0.000^{f}	380.6 ± 0.577^{g}	42.31 ± 0.482^{bef}	0.0204 ± 0.000^{a}	0.376 ± 0.0010^{cdef}

Note: Data in the same column with different superscript letter are significantly different ($p \leq .05$).

control and treatment samples under the same processing conditions (Aday et al., 2018).

Changes in color properties were also observed in wine samples. HHP treatment at 650 MPa at ambient temperature (around 18°C) for 2 hr resulted in the decrease of color intensity and simultaneous increase of tint. Both the absorbance of wine samples at 420 nm (yellow color) and 520 nm (red color) decreased after HHP. Although an increase in $L^*$and b^* was observed after HHP, a decrease was detected both in a^* and C^* (Tao et al., 2012). It was concluded that HHP (at low and moderate temperatures), in general, has been reported to have a limited effect on the compounds responsible for the color of fruits and vegetables (e.g., chlorophyll, carotenoids, and anthocyanins), but due to incomplete inactivation of enzymes and microorganisms, which can result in undesired chemical reactions (both enzymatic and nonenzymatic) in the food matrix, the color compounds of HHP-processed fruits and vegetables may change during storage (Oey et al., 2008).

It was observed that magnitude of pressure, processing time, and temperature affected properties of licorice drink with applied pressure and processing time having bigger impact on measured properties and especially on microbial inactivation. It was also reported by the previous studies that the effectiveness of the HHP greatly depend on technological parameters, such as pressure, holding time and temperature and, to lesser extent, the type and the physiological state of microorganisms (Gao et al., 2006; Hugas et al., 2002). Both increase in pressure and treatment holding time result in an increase of microbial inactivation (Ritz et al., 2000). It is also reported that food intrinsic factors can also affect the microbial resistance to high pressure (Ritz et al., 2000). In fact, the highest amount of reduction on microbial counts was observed with 500 MPa/22°C/15 min when pressure was the highest and treatment time was the longest.

Level of inactivation on both endogenous microflora as well as inoculated bacteria in licorice drink processed by HHP in previous studies, on the other hand, were higher than that of the present study. Processing of licorice drink fully inactivated TMY and total coliforms with 250, 355, and 450 MPa for 1 and 5 min, and achieved more than five log reductions in the inoculated *E. coli* and *S. typhimurium* with 450 MPa for 5 min (Aday et al., 2018). A complete inactivation on the mean initial total mesophilic aerobic spore (TMAS) of 2.53 ± 0.64 log cfu/ml in licorice drink was obtained with mid-range temperatures (20°C–30°C) under 450 MPa for 5-, 15-, and 30-min treatment time. Also, inoculated *G. stearothermophilus* spores at 1.8 ± 0.14 log cfu/ml level was reduced by 1 log at 450 MPa for 30 min (Genis, 2016).

Processing of licorice drink by 17, 23, and 30 kV/cm and heat treatments by 70°C for 3 min, 80°C for 3 min, and 90°C for 3 min, and combination of mild heat (40°C for 3 min) + PEF (23 kV/cm) revealed that PEF and mild heat + PEF treatments did not cause significant difference on pH, TA, conductivity, TSS, color values, TAC, and TPSC, whereas all heat treatments revealed significant difference on measured properties compared with control samples. Inactivation of TAMB, TMY, *E. coli* and *Salmonella enteritidis* increased with an increase in electric field strength and temperature. Combination of mild heat + PEF was more effective for microbial inactivation than that of the other treatments. Although increase in temperatures caused a decrease on sensory scores, samples treated by PEF and mild heat + PEF treatments were not significantly different from the control samples and from the each other. Thus, it was concluded that heat treatments caused degradation of physicochemical properties

TABLE 3 Changes on the color properties of licorice drink processed by high hydrostatic pressure

Process	L^*	a^*	b^*	Chroma	Hue	Total color difference	Color intensity (abs)	Color tone	Yellow color tone (%)	Red color tone (%)	Blue color tone (%)
Control	52.32 ± 0.007^{a}	6.15 ± 0.010^{a}	53.24 ± 0.111^{a}	53.60 ± 0.109^{a}	1.455 ± 0.0003^{a}	–	1.056 ± 0.001^{a}	4.413 ± 0.042^{a}	73.29 ± 0.144^{a}	17.06 ± 0.128^{a}	7.64 ± 0.016^{a}
350 MPa/40°C/3 min	52.42 ± 0.010^{a}	7.74 ± 0.030^{b}	54.67 ± 0.163^{bc}	55.21 ± 0.158^{b}	1.430 ± 0.0009^{b}	2.133 ± 0.086^{a}	1.100 ± 0.017^{bc}	3.847 ± 0.145^{b}	73.70 ± 0.257^{abc}	18.32 ± 0.142^{bcde}	7.96 ± 0.156^{abc}
200 MPa/22°C/3 min	52.61 ± 0.005^{b}	6.48 ± 0.005^{c}	53.56 ± 0.158^{d}	53.95 ± 0.156^{c}	1.450 ± 0.0004^{cde}	0.547 ± 0.092^{b}	1.091 ± 0.007^{bcd}	3.947 ± 0.086^{bc}	73.62 ± 0.297^{ac}	18.65 ± 0.329^{bc}	7.72 ± 0.047^{ac}
350 MPa/40°C/15 min	53.88 ± 0.122^{c}	5.92 ± 0.085^{d}	53.68 ± 0.035^{d}	54.01 ± 0.033^{c}	1.450 ± 0.0015^{cde}	1.632 ± 0.105^{cd}	1.103 ± 0.011^{b}	3.864 ± 0.130^{b}	73.04 ± 0.550^{a}	18.91 ± 0.492^{b}	8.03 ± 0.061^{abc}
350 MPa/22°C/9 min	52.75 ± 0.025^{b}	6.77 ± 0.026^{e}	54.04 ± 0.087^{e}	54.46 ± 0.086^{d}	1.446 ± 0.0005^{f}	1.092 ± 0.055^{e}	1.083 ± 0.008^{bcde}	4.073 ± 0.057^{bcd}	73.96 ± 0.382^{abcd}	18.15 ± 0.163^{cdef}	7.87 ± 0.223^{abc}
200 MPa/22°C/15 min	51.04 ± 0.010^{d}	7.14 ± 0.028^{f}	53.62 ± 0.085^{d}	54.10 ± 0.081^{c}	1.438 ± 0.0007^{g}	1.666 ± 0.016^{c}	1.089 ± 0.011^{bcd}	3.940 ± 0.126^{bc}	73.38 ± 0.558^{a}	18.63 ± 0.455^{bcd}	7.98 ± 0.103^{abc}
350 MPa/4°C/3 min	51.89 ± 0.015^{e}	6.79 ± 0.037^{e}	54.48 ± 0.055^{c}	54.90 ± 0.055^{e}	1.446 ± 0.0006^{fh}	1.457 ± 0.047^{dfg}	1.074 ± 0.008^{ade}	4.189 ± 0.050^{ade}	74.24 ± 0.255^{abcde}	17.72 ± 0.156^{aefgh}	8.03 ± 0.132^{abc}
500 MPa/22°C/3 min	51.45 ± 0.005^{f}	6.75 ± 0.010^{eg}	54.11 ± 0.078^{e}	54.52 ± 0.076^{d}	1.447 ± 0.0003^{fh}	1.363 ± 0.042^{gh}	1.061 ± 0.002^{ae}	4.246 ± 0.026^{ade}	74.70 ± 0.048^{bdefg}	17.59 ± 0.100^{afgh}	7.69 ± 0.068^{ac}
350 MPa/4°C/15 min	51.41 ± 0.015^{f}	6.83 ± 0.028^{e}	54.12 ± 0.020^{e}	54.55 ± 0.017^{d}	1.445 ± 0.0005^{f}	1.434 ± 0.012^{fg}	1.057 ± 0.005^{a}	4.388 ± 0.017^{a}	75.15 ± 0.186^{efg}	17.12 ± 0.025^{a}	7.72 ± 0.161^{ac}
500 MPa/4°C/9 min	53.28 ± 0.141^{g}	6.36 ± 0.062^{h}	53.52 ± 0.075^{ad}	53.90 ± 0.067^{c}	1.450 ± 0.0013^{cde}	1.224 ± 0.060^{eh}	1.058 ± 0.005^{ae}	4.408 ± 0.045^{a}	75.29 ± 0.226^{fg}	17.08 ± 0.147^{a}	7.69 ± 0.112^{ac}
350 MPa/22°C/9 min	51.71 ± 0.025^{h}	6.56 ± 0.030^{ci}	54.14 ± 0.070^{e}	54.54 ± 0.072^{d}	1.450 ± 0.0004^{cde}	1.160 ± 0.075^{e}	1.058 ± 0.015^{ae}	4.368 ± 0.090^{ae}	74.94 ± 0.581^{defg}	17.15 ± 0.223^{ah}	7.90 ± 0.358^{abc}
500 MPa/22°C/15 min	51.65 ± 0.010^{h}	6.63 ± 0.005^{i}	54.08 ± 0.078^{e}	54.48 ± 0.077^{d}	1.448 ± 0.0001^{eh}	1.174 ± 0.056^{eh}	1.063 ± 0.001^{ae}	4.334 ± 0.025^{ae}	75.63 ± 0.228^{f}	17.92 ± 0.074^{defg}	7.73 ± 0.067^{abc}
200 MPa/40°C/9 min	52.62 ± 0.005^{b}	7.60 ± 0.034^{j}	54.90 ± 0.188^{b}	55.42 ± 0.182^{b}	1.433 ± 0.0010^{i}	2.219 ± 0.120^{a}	1.061 ± 0.007^{ae}	4.331 ± 0.036^{ae}	74.71 ± 0.280^{bdefg}	17.24 ± 0.092^{agh}	8.04 ± 0.221^{abc}
200 MPa/4°C/9 min	53.84 ± 0.010^{c}	6.35 ± 0.020^{h}	53.13 ± 0.020^{a}	53.50 ± 0.021^{a}	1.451 ± 0.0003^{c}	1.717 ± 0.012^{c}	1.091 ± 0.001^{bcd}	4.076 ± 0.013^{bcd}	73.67 ± 0.025^{ac}	18.07 ± 0.064^{cdef}	8.24 ± 0.084^{b}
500 MPa/40°C/9 min	53.20 ± 0.047^{g}	6.65 ± 0.040^{gi}	54.45 ± 0.047^{c}	54.86 ± 0.042^{e}	1.449 ± 0.0008^{de}	1.575 ± 0.050^{cdf}	1.062 ± 0.007^{ae}	4.234 ± 0.093^{ade}	74.54 ± 0.434^{bcdeg}	17.60 ± 0.319^{afgh}	7.84 ± 0.340^{abc}
350 MPa/22°C/9 min	51.65 ± 0.011^{h}	6.49 ± 0.011^{c}	54.11 ± 0.015^{e}	54.50 ± 0.014^{d}	1.451 ± 0.0002^{cd}	1.145 ± 0.002^{e}	1.077 ± 0.006^{acde}	4.142 ± 0.050^{cde}	73.97 ± 0.203^{abcd}	17.85 ± 0.169^{efgh}	8.17 ± 0.049^{bc}

Note: Data in the same column with different superscript letter are significantly different ($p \leq .05$).

and nonthermal treatments alone or combination with mild temperatures can successfully be applied for licorice drink processing (Uzuner & Evrendilek, 2017). Thermosonication treatment of licorice drink at 60°C for 20 min resulted in 7.08 log cfu/ml reduction in the *E. coli* K-12, did not significantly change the color values, pH, TA, turbidity, TAC, total flavonoid, and TPSC and exhibited no negative effect on the physicochemical properties (Ozturk, 2019). Previous studies with HHP lacked the quantification of its effect on bioactive properties and shelf life, the main issues with the licorice drink. Thus, not only did the present study explored its effect on the physicochemical, bioactive, and sensory properties of licorice drink but also on the shelf-life extension.

TABLE 4 Changes on the bioactive properties of licorice drink processed by high hydrostatic pressure

Process	Total antioxidant capacity (%)	Total phenolic substance content (GAE ml^{-1})
Control	18.33 ± 0.066^{a}	0.128 ± 0.0002^{a}
350 MPa/40°C/3 min	18.83 ± 0.115^{bcde}	0.128 ± 0.0011^{a}
200 MPa/22°C/3 min	18.68 ± 0.100^{defg}	0.128 ± 0.0005^{a}
350 MPa/40°C/15 min	18.57 ± 0.043^{afg}	0.130 ± 0.0007^{ab}
350 MPa/22°C/9 min	18.91 ± 0.109^{bcd}	0.130 ± 0.0002^{ab}
200 MPa/22°C/15 min	18.53 ± 0.075^{ag}	0.130 ± 0.0000^{ab}
350 MPa/4°C/3 min	19.02 ± 0.090^{b}	0.130 ± 0.0008^{ab}
500 MPa/22°C/3 min	18.66 ± 0.043^{defg}	0.127 ± 0.0007^{a}
350 MPa/4°C/15 min	18.98 ± 0.025^{bc}	0.127 ± 0.0009^{a}
500 MPa/4°C/9 min	18.50 ± 0.090^{ag}	0.127 ± 0.0005^{a}
350 MPa/22°C/9 min	18.68 ± 0.050^{defg}	0.127 ± 0.0002^{a}
500 MPa/22°C/15 min	19.00 ± 0.090^{b}	0.128 ± 0.0005^{a}
200 MPa/40°C/9 min	18.79 ± 0.087^{bcdef}	0.127 ± 0.0002^{a}
200 MPa/4°C/9 min	18.62 ± 0.075^{efg}	0.128 ± 0.0002^{a}
500 MPa/40°C/9 min	18.73 ± 0.090^{cdefg}	0.127 ± 0.0002^{a}
350 MPa/22°C/9 min	18.69 ± 0.133^{defg}	0.127 ± 0.0009^{a}

Note: Data in the same column with different superscript letter are significantly different ($p \leq .05$).

3.2 | Modeling and optimization

Based on ANOVA results, the insignificant terms were excluded from the models of the YCT, RSC, and inactivation of *B. circulans* and *C. tropicalis* (Table 7). The significant factors ($p \leq .05$) were pressure, treatment time, and temperature; and interaction of treatment time and temperature and interaction of pressure and treatment time for YCT, RSC, and inactivation of *B. circulans* and *C. tropicalis* (Table 7). Pressure and treatment time had a negative effect on *B. circulans* and *C. tropicalis* but a positive effect on YCT. Temperature had a negative effect on YCT, whereas both treatment time and temperature had a positive effect on RSC. The significant quadratic terms were found for the pressure ($p \leq .05$) with a positive effect on YCT and a negative effect on RSC, *B. circulans* and *C. tropicalis*. Also, a quadratic term of treatment time had a positive effect on YCT and a negative effect on RSC, *B. circulans* and *C. tropicalis*. While a quadratic term of treatment time had a positive effect on RSC and *B. circulans*, a quadratic term of temperature had a positive significant effect on *B. circulans*. There was a significant interaction between pressure and treatment time with a positive effect on YCT but with a negative effect on RSC, *B. circulans*, and *C. tropicalis*. The pressure-by-temperature and treatment

TABLE 5 Log reductions of total mesophilic aerobic bacteria, total mold and yeast, *Bacillus circulans*, and *Candida tropicalis* on licorice drink processed by high hydrostatic pressure

Process	Total mesophilic aerobic bacteria (log cfu/ml)	Total mold and yeast (log cfu/ml)	*Bacillus circulans* (log kob ml^{-1})	*Candida tropicalis* (log cfu/ml)
350 MPa/40°C/3 min	2.34 ± 0.112^{bcde}	2.50 ± 0.115^{bcd}	1.29 ± 0.240^{bc}	1.50 ± 0.098^{bcd}
200 MPa/22°C/3 min	1.63 ± 0.457^{f}	1.56 ± 0.399^{e}	1.16 ± 0.338^{b}	1.34 ± 0.203^{bc}
350 MPa/40°C/15 min	2.26 ± 0.161^{bcde}	2.46 ± 0.115^{bcd}	1.80 ± 0.223^{cde}	2.02 ± 0.116^{efg}
350 MPa/22°C/9 min	2.52 ± 0.137^{bcde}	2.45 ± 0.234^{bcd}	1.89 ± 0.090^{cde}	1.85 ± 0.084^{def}
200 MPa/22°C/15 min	2.06 ± 0.208^{bcf}	2.16 ± 0.258^{bce}	1.42 ± 0.225^{bcd}	1.35 ± 0.318^{bc}
350 MPa/4°C/3 min	2.49 ± 0.113^{bcde}	2.27 ± 0.341^{bc}	1.32 ± 0.223^{bc}	1.37 ± 0.224^{bc}
500 MPa/22°C/3 min	2.78 ± 0.066^{eg}	2.51 ± 0.211^{bcd}	1.86 ± 0.051^{cde}	1.91 ± 0.055^{defg}
350 MPa/4°C/15 min	2.55 ± 0.211^{bcde}	2.56 ± 0.332^{bcd}	1.81 ± 0.176^{cde}	1.95 ± 0.087^{defg}
500 MPa/4°C/9 min	2.43 ± 0.224^{bcde}	2.36 ± 0.262^{bcd}	2.38 ± 0.025^{e}	2.38 ± 0.073^{g}
350 MPa/22°C/9 min	2.68 ± 0.041^{cde}	2.69 ± 0.011^{cd}	1.99 ± 0.091^{de}	1.90 ± 0.040^{def}
500 MPa/22°C/15 min	3.33 ± 0.158^{g}	3.65 ± 0.012^{f}	3.13 ± 0.035^{f}	3.08 ± 0.099^{h}
200 MPa/40°C/9 min	2.02 ± 0.250^{bf}	1.97 ± 0.122^{be}	1.38 ± 0.260^{bcd}	1.54 ± 0.134^{bcd}
200 MPa/4°C/9 min	2.09 ± 0.314^{bcdf}	1.93 ± 0.159^{be}	1.42 ± 0.221^{bcd}	1.50 ± 0.152^{bcd}
500 MPa/40°C/9 min	2.79 ± 0.029^{eg}	2.96 ± 0.112^{d}	2.39 ± 0.041^{e}	2.29 ± 0.128^{fg}
350 MPa/22°C/9 min	2.73 ± 0.100^{deg}	2.76 ± 0.040^{cd}	1.86 ± 0.091^{cde}	1.81 ± 0.138^{cde}

Note: Data in the same column with different superscript letter are significantly different ($p \leq .05$).

TABLE 6 Changes on the sensory properties of licorice drink processed by high hydrostatic pressure

Process	Cloudiness-clarity	Dullness-shininess	Color intensity	Particle distribution	Flavor	Juice density	Licorice taste	Bitter taste	Sour taste	Sweetness	After taste
Control	8.50 ± 1.09^{a}	7.95 ± 0.50^{b}	8.77 ± 0.25^{c}	8.38 ± 0.34^{d}	8.67 ± 0.16^{e}	8.45 ± 0.09^{f}	8.83 ± 0.16^{g}	8.61 ± 0.34^{h}	8.83 ± 0.60^{i}	9.55 ± 0.25^{j}	8.95 ± 0.10^{k}
350 MPa/40°C/3 min	8.33 ± 0.57^{a}	8.00 ± 1.33^{b}	8.22 ± 0.84^{c}	8.33 ± 0.87^{d}	8.22 ± 1.07^{e}	8.55 ± 0.96^{f}	9.00 ± 0.57^{g}	8.00 ± 0.33^{h}	8.33 ± 0.33^{i}	9.00 ± 0.33^{j}	8.11 ± 0.51^{k}
200 MPa/22°C/3 min	8.55 ± 0.51^{a}	8.00 ± 0.57^{b}	8.55 ± 0.19^{c}	8.44 ± 0.76^{d}	8.55 ± 0.69^{e}	8.33 ± 0.57^{f}	7.77 ± 0.69^{g}	8.77 ± 0.50^{h}	8.66 ± 0.33^{i}	9.11 ± 0.38^{j}	8.44 ± 0.19^{k}
350 MPa/40°C/15 min	7.90 ± 0.69^{a}	8.44 ± 0.69^{b}	8.22 ± 0.38^{c}	8.22 ± 0.19^{d}	8.44 ± 0.19^{e}	8.33 ± 0.57^{f}	8.22 ± 0.38^{g}	7.77 ± 0.69^{h}	8.55 ± 0.51^{i}	8.67 ± 0.33^{j}	8.89 ± 0.19^{k}
350 MPa/22°C/9 min	9.11 ± 0.69^{a}	8.44 ± 0.69^{b}	8.22 ± 0.50^{c}	8.44 ± 0.38^{d}	8.33 ± 0.33^{e}	8.22 ± 0.38^{f}	8.22 ± 0.19^{g}	8.67 ± 0.57^{h}	8.89 ± 0.38^{i}	9.00 ± 0.33^{j}	8.44 ± 0.38^{k}
200 MPa/22°C/15 min	8.55 ± 0.38^{a}	8.66 ± 0.57^{b}	8.55 ± 0.51^{c}	7.78 ± 0.84^{d}	8.44 ± 0.19^{e}	8.22 ± 0.50^{f}	8.33 ± 0.33^{g}	8.22 ± 0.50^{h}	8.55 ± 1.01^{i}	9.22 ± 0.50^{j}	8.55 ± 0.19^{k}
350 MPa/4°C/3 min	8.55 ± 0.51^{a}	8.33 ± 0.50^{b}	8.00 ± 0.33^{c}	8.22 ± 0.50^{d}	8.11 ± 0.51^{e}	8.77 ± 0.50^{f}	8.11 ± 0.38^{g}	8.55 ± 0.38^{h}	8.89 ± 0.38^{i}	8.55 ± 0.19^{j}	8.44 ± 0.38^{k}
500 MPa/22°C/3 min	9.00 ± 0.33^{a}	8.55 ± 0.19^{b}	8.66 ± 0.57^{c}	8.11 ± 0.69^{d}	8.00 ± 0.33^{e}	8.33 ± 0.33^{f}	8.55 ± 0.38^{g}	8.55 ± 0.69^{h}	8.44 ± 0.38^{i}	8.89 ± 0.19^{j}	8.67 ± 0.66^{k}
350 MPa/4°C/15 min	8.44 ± 0.19^{a}	8.33 ± 0.33^{b}	8.22 ± 0.19^{c}	9.11 ± 0.69^{d}	8.55 ± 1.17^{e}	8.77 ± 0.33^{f}	8.33 ± 0.33^{g}	8.55 ± 0.69^{h}	8.55 ± 0.51^{i}	8.78 ± 0.19^{j}	8.00 ± 0.67^{k}
500 MPa/4°C/9 min	8.33 ± 0.66^{a}	8.55 ± 0.19^{b}	8.78 ± 0.84^{c}	8.78 ± 0.19^{d}	8.55 ± 1.17^{e}	8.67 ± 0.33^{f}	8.11 ± 0.96^{g}	8.67 ± 0.33^{h}	8.44 ± 0.38^{i}	9.00 ± 0.33^{j}	8.89 ± 0.38^{k}
350 MPa/22°C/9 min	8.66 ± 0.33^{a}	8.55 ± 0.96^{b}	8.77 ± 0.69^{c}	8.44 ± 0.51^{d}	8.66 ± 0.33^{e}	8.55 ± 0.19^{f}	7.89 ± 0.19^{g}	8.33 ± 0.57^{h}	8.55 ± 0.19^{i}	8.78 ± 0.84^{j}	8.77 ± 0.69^{k}
500 MPa/22°C/15 min	8.11 ± 0.69^{a}	8.78 ± 0.84^{b}	7.99 ± 0.57^{c}	8.11 ± 0.51^{d}	8.22 ± 1.01^{e}	8.44 ± 0.51^{f}	8.11 ± 0.38^{g}	8.44 ± 0.69^{h}	8.89 ± 0.69^{i}	9.00 ± 0.88^{j}	8.89 ± 0.38^{k}
200 MPa/40°C/9 min	8.77 ± 0.38^{a}	8.55 ± 0.51^{b}	8.11 ± 1.38^{c}	8.44 ± 0.19^{d}	7.77 ± 0.38^{e}	8.11 ± 0.38^{f}	8.33 ± 0.66^{g}	8.00 ± 0.33^{h}	9.00 ± 0.33^{i}	8.55 ± 0.19^{j}	8.11 ± 0.38^{k}
200 MPa/4°C/9 min	8.33 ± 0.33^{a}	7.89 ± 0.19^{b}	7.78 ± 0.38^{c}	8.55 ± 0.69^{d}	8.44 ± 0.38^{e}	9.22 ± 0.50^{f}	8.67 ± 0.50^{g}	8.33 ± 0.33^{h}	8.55 ± 1.01^{i}	9.00 ± 0.57^{j}	8.77 ± 0.50^{k}
500 MPa/40°C/9 min	7.77 ± 0.50^{a}	8.22 ± 0.50^{b}	8.00 ± 0.33^{c}	8.67 ± 0.19^{d}	8.22 ± 0.19^{e}	8.22 ± 0.19^{f}	8.33 ± 0.33^{g}	8.33 ± 0.33^{h}	8.55 ± 0.19^{i}	9.22 ± 0.50^{j}	8.44 ± 0.38^{k}
350 MPa/22°C/9 min	8.77 ± 0.50^{a}	8.44 ± 0.19^{b}	8.22 ± 0.50^{c}	8.55 ± 0.19^{d}	8.44 ± 0.19^{e}	8.55 ± 0.38^{f}	8.55 ± 0.19^{g}	8.67 ± 0.33^{h}	8.33 ± 0.57^{i}	9.00 ± 0.57^{j}	8.44 ± 0.19^{k}

Note: Data in the same column with different superscript letter are significantly different ($p \leq .05$).

TABLE 7 Revised analysis of variance (ANOVA) results and estimated regression coefficients for the transformed coded yellow color tone, reducing sugar content, and numbers of *Bacillus circulans* and *Candida tropicalis* model by high hydrostatic pressure

	Yellow color tone		Reducing sugar content		*Bacillus circulans*		*Candida tropicalis*	
Terms	Coeff	*p* value	Coeff	*p* value	Coeff	*p* value	Coeff	*p* value
Linear								
X_1 (*P*)	0.678	0.000	–	–	−0.546	0.000	−0.494	0.000
X_2 (*t*)	0.255	0.000	0.0006	0.002	−0.315	0.000	−0.281	0.000
X_3 (*T*)	−0.369	0.000	0.0004	0.041	–	–	–	–
Square								
$X_1 \times X_1$	0.288	0.000	−0.0015	0.000	−0.160	0.005	−01.49	0.003
$X_2 \times X_2$	–	–	0.0019	0.000	0.178	0.002	–	–
$X_3 \times X_3$	–	–	–	–	0.177	0.002	–	–
Interaction								
$X_1 \times X_2$	0.207	0.003	−0.0020	0.000	−0.251	0.000	−0.296	0.003
$X_1 \times X_3$	−0.369	0.000	−0.0011	0.000	–	–	–	–
$X_2 \times X_3$	−0.283	0.000	−0.0007	0.010	–	–	–	–
Lack-of-fit	–	0.137	–	0.092	–	0.492		0.086
Constant	74.11	0.000	0.3762	0.000	3.481	0.000	3.680	0.000
R^2	0.91	0.81	0.90	0.90				
R^2 (adj)	0.90		0.78		0.88		0.89	
R^2 (pred)	0.88		0.72		0.85		0.86	

time-by-temperature interactions had a negative effect on both YCT and RSC. The insignificant lack-of-fit values for the four models also indicated that the model fitted the experimental data well (Table 7). The degree of influence of the operational conditions on the YCT, RSC, *B. circulans*, and *C. tropicalis* can be inferred from comparing the magnitudes of the coefficients of the quadratic regression models. According to Table 7, the pressure was the most important factor for YCT (0.678), *B. circulans* (0.546), and *C. tropicalis* (0.494), whereas the treatment time was the most important factor for *B. circulans* (0.315), and the treatment temperature was the most important factor for YCT (0.369) (Table 7). Table 8 shows the regression equations for YCT, RSC, *B. circulans*, and *C. tropicalis*. The goodness-of-fit (R^2) values of the models were 0.91%, 0.81%, 0.90%, and 0.90% for YCT, RSC, *B. circulans*, and *C. tropicalis*, respectively (Table 8).

TABLE 8 Regression equations for the coded yellow color tone, reducing sugar content, and numbers of *Bacillus circulans* and *Candida tropicalis* models by high hydrostatic pressure

Response	Models	Equation	R^2	VIF	CV (%)
Yellow color tone	Quadratic	$Y_1=74.11+0.678\times X_1+0.255\times X_2-0.369\times X_3+0.288\times X_2^1+0.207\times X_1\times X_2-0.369\times X_1\times X_3-0.283\times X_2\times X_3$	0.91	1.00	0.139
Reducing sugar content	Quadratic	$Y_2=0.376+0.0006\times X_2+0.0004\times X_3-0.0015\times X_1^2+0.0019\times X_2^2-0.0020\times X_1\times X_2-0.0011\times X_1\times X_3-0.0007\times X_2\times X_3$	0.81	1.00	0.897
Bacillus circulans	Quadratic	$Y_3=3.481-0.546\times X_1-0.315\times X_2-0.160\times X_1^2+0.178\times X_2^2+0.177\times X_3^2-0.251\times X_1\times X_2$	0.90	1.00	1.363
Candida tropicalis	Quadratic	$Y_4 = 3.680 - 0.494 \times X_1 - 0.281 \times X_2 - 0.149 \times X_1^2 - 0.296 \times X_1 \times X_2$	0.90	1.00	0.097

Abbreviations: CV, coefficient of variation; VIF, variance inflation factor.

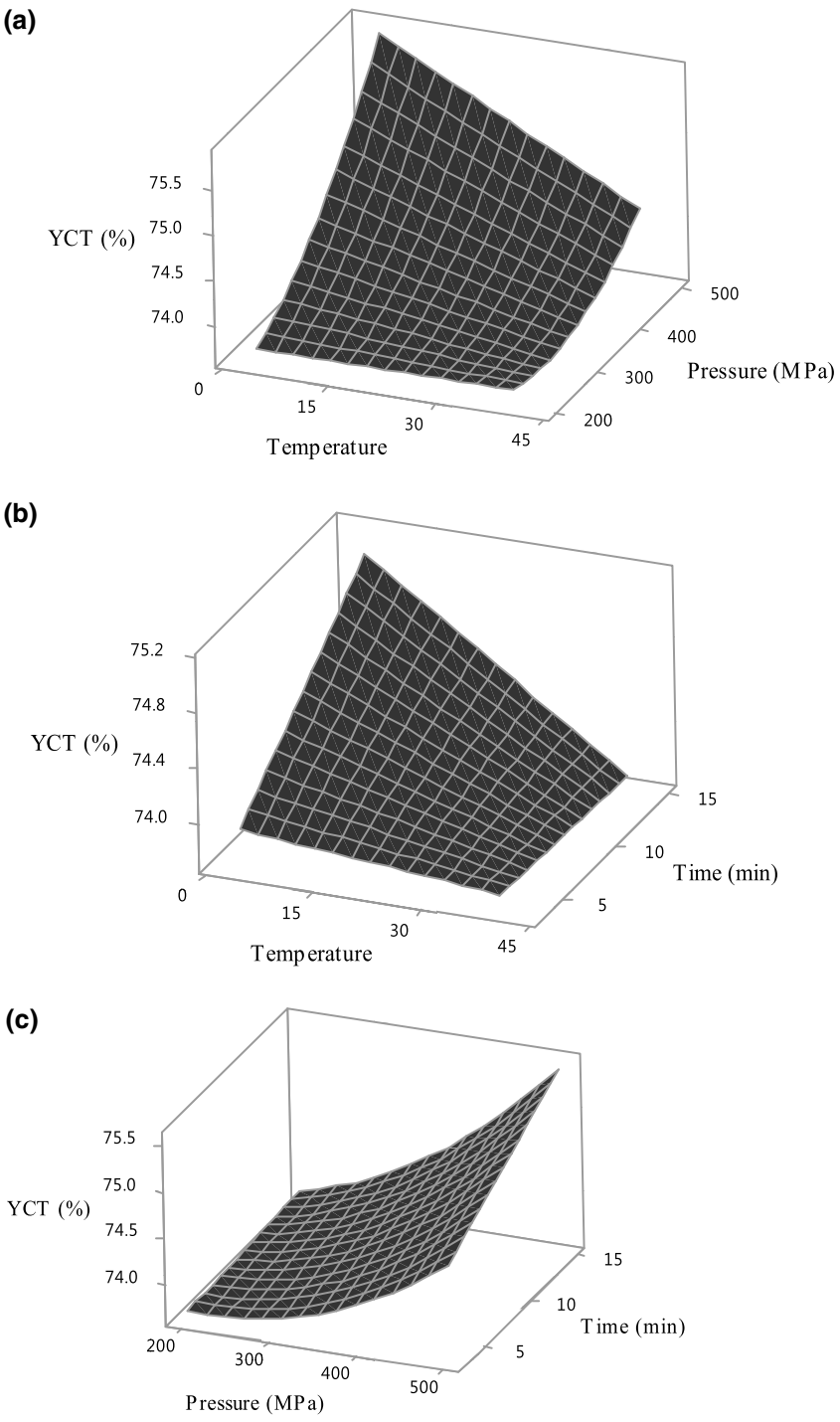

FIGURE 1 Effect of (a) temperature versus pressure, (b) temperature versus treatment time, and (c) pressure versus treatment time on yellow color tone of licorice drink processed by high hydrostatic pressure

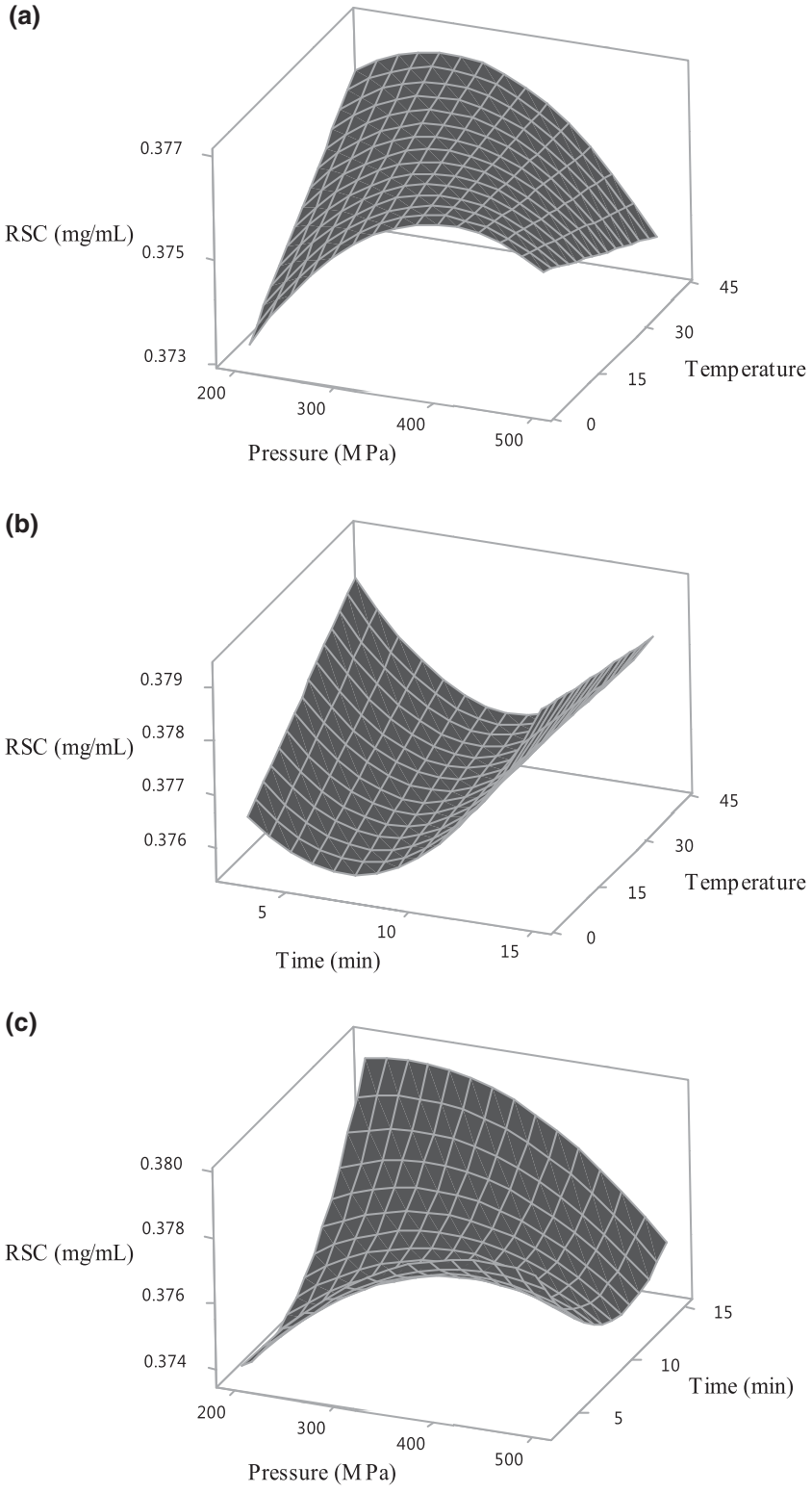

FIGURE 2 Effect of (a) pressure versus temperature, (b) treatment time versus temperature, and (c) pressure versus treatment time on reducing sugar content of licorice drink processed by high hydrostatic pressure

The surface plots were used to visualize how the operational settings simultaneously influenced the multiple responses of the licorice drink processed with HHP (Figures 1–4). The highest pressure maximized YCT value at the lowest temperature (15°C) (Figure 1a), and YCT value increased with the increased treatment time above 5 min (Figure 1b). The highest pressure maximized YCT value at the highest time (15 min) (Figure 1c). RSC increased with the increased pressure under the lowest temperature at an increasing rate (Figure 2a). The lowest treatment time maximized RSC values at the highest temperature (45°C) (Figure 2b). RSC increased with the increased treatment time under the lowest pressure (200 MPa) at an increasing rate (Figure 2c). *B. circulans* decreased with the increased treatment time under the lowest temperature at an increasing rate (Figure 3a). The highest pressure minimized *B. circulans* at 15°C (Figure 3b). The

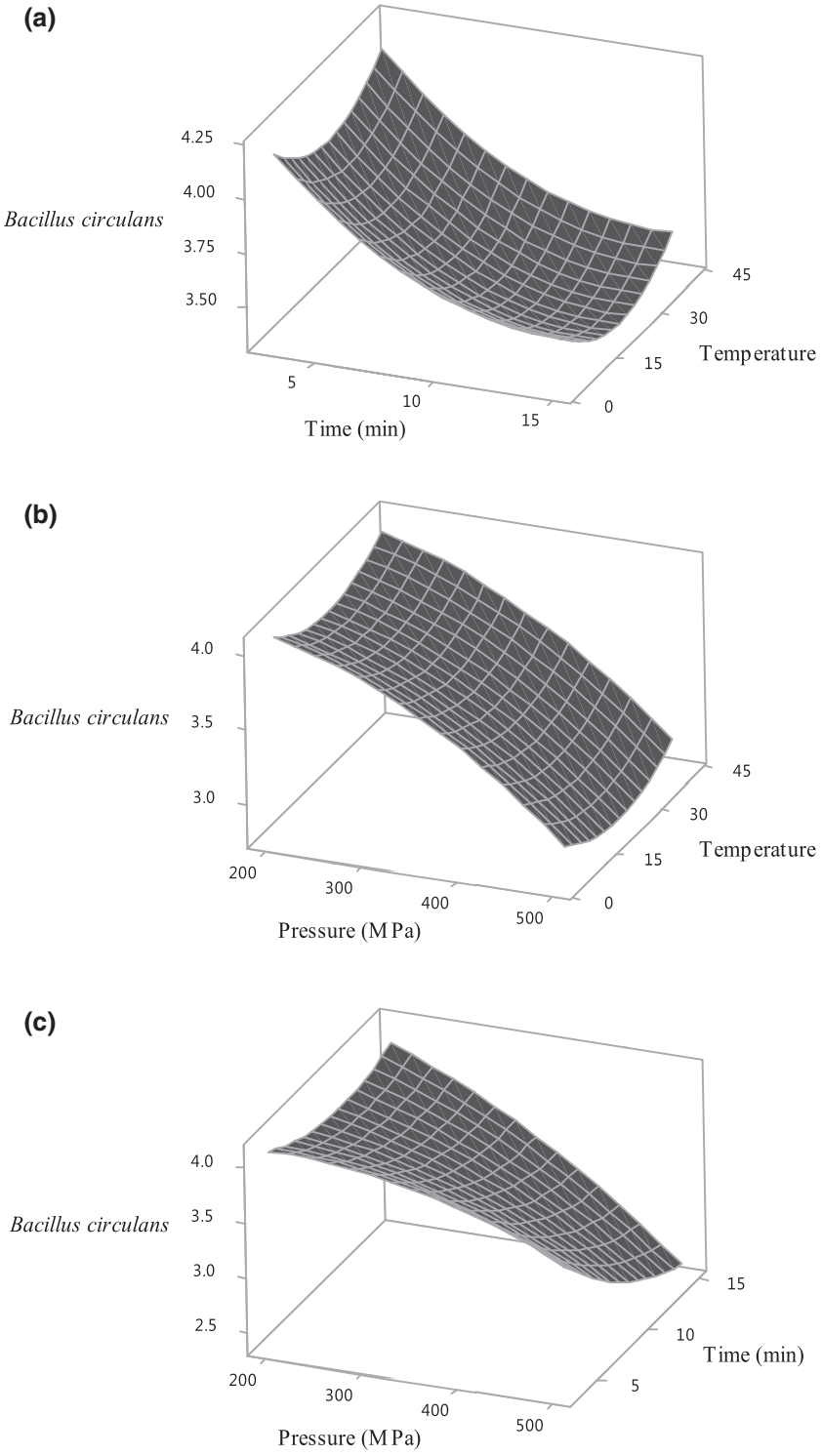

FIGURE 3 Effect of (a) treatment time versus temperature, (b) pressure versus temperature, and (c) pressure versus treatment time on *Bacillus circulans* inactivation on licorice drink processed by high hydrostatic pressure

highest pressure minimized *B. circulans* at the highest treatment time (15 min) (Figure 3c). *C. tropicalis* decreased with the increased treatment time and pressure linearly (Figure 4).

The operational settings were optimized to minimize the mean initial numbers of *B. circulans* and *C. tropicalis* and to target YCT and RSC of the licorice drink quality properties. The multiple-response optimization was based on the composite desirability (*D*) of zero to one (ideal), a geometric mean of individual desirabilities (*d*). The optimum operational conditions were achieved with 500 MPa, 9.90 min and 18.5°C. The minimum numbers of *B. circulans* (2.70 log cfu/ml), *C. tropicalis* (2.95 log cfu/ml), and target YCT (75.3) and RSC (0.375) were obtained with the optimum operational conditions. These conditions were experimentally tested to validate the predictive power of the models. The resultant YCT, RSC, *B. circulans*, and *C. tropicalis* values of 75.15 ± 0.313, 0.379 ± 0.001, 2.59 ± 0.080, and 2.96 ± 0.040 log cfu/ml, respectively, indicated no

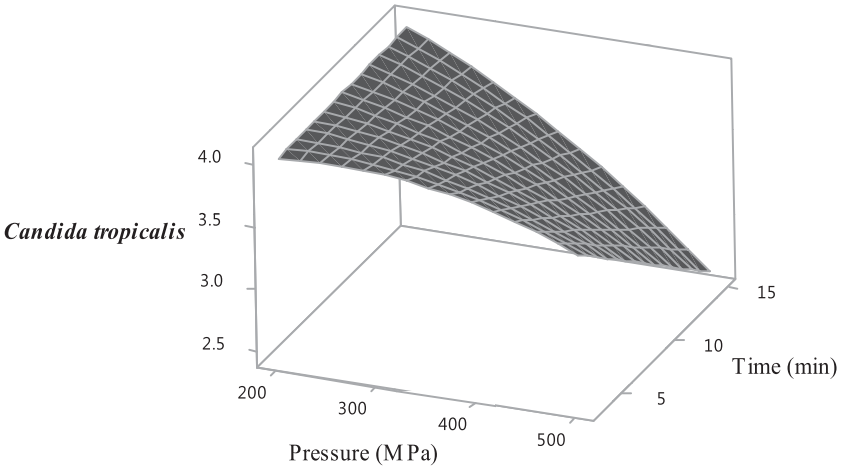

FIGURE 4 Effect of pressure versus treatment time on *Candida tropicalis* inactivation on licorice drink processed by high hydrostatic pressure

significant difference between the measured and predicted values. The CV values for YCT, RSC, *B. circulans*, and *C. tropicalis* were determined to be 0.139%, 0.897%, 1.363%, and 0.097% (Table 8). The smaller CV values showed the better reproducibility of the model.

3.3 | Shelf-life studies

Shelf-life studies were performed between the control and the treated samples using the optimum operational conditions of 500 MPa, 9.90 min, and 18.5°C. The control samples at 22°C and 4°C were spoiled at the 2nd and 7th days of the shelf-life studies. HHP-treated samples had the shelf life of over 25 days at both 4°C and 22°C. A significant decrease was observed in the mean initial pH of the control and treated samples over the storage time. HHP-treated samples stored at 4°C had significantly higher pH than did the treated samples at 22°C. In contrast to pH, conductivity increased during shelf-life studies ($p \leq .05$), and treated samples at 22°C had significantly higher conductivity than did treated samples at 4°C. Although color L^*, a^*, b^*, and C^* values decreased, turbidity values significantly increased over the storage time ($p \leq .05$). Both h° and TA values did not significantly change over the storage time ($p \leq .05$). Treated samples at 4°C had significantly higher L^*, a^*, b^*, and C^* values than the treated samples at 22°C ($p \leq .05$). No significant difference was observed in h° value between the treated samples stored at 4°C and 22°C ($p > .05$) (Table 9). Glycyrrhizin concentrations did not significantly change with storage time and storage temperature ($p > .05$). Glabridin concentration, on the other hand, significantly decreased with storage time ($p \leq .05$) but did not change with storage temperature ($p > .05$) (Table 10).

HHP-treated samples had significantly lower initial gallic, *p*-cumaric, and chlorogenic acid and miristein concentrations than that of the control samples at the beginning of the shelf-life studies. But their initial concentrations in control samples were significantly decreased by storage time, whereas mean initial gallic, *p*-cumaric, and chlorogenic acid concentrations of the treated samples did not significantly change with the storage time and storage temperature ($p > .05$). No significant difference was observed for vanillic and caffeic acids at the beginning of the shelf-life studies, but their initial concentrations and the initial concentration of miristein significantly fell with the storage time ($p \leq .05$). Also, treated samples stored at 4°C had significantly higher miristein, vanillic, and caffeic acid concentrations than treated samples stored at 22°C (Table 11).

No significant difference was observed between the control and treated samples at both 4°C and 22°C at the beginning of the shelf-life studies for organic acids. The mean initial concentration of the acetic acid significantly dropped with storage time ($p \leq .05$) with no significant decrease for both formic and fumaric acids. The difference between the treated samples at 4°C and 22°C for acetic, formic, and fumaric acids was not significant ($p > .05$) (Table 12).

The mean initial TMAB count of 5.52 ± 0.68 log cfu/ml significantly reduced to 2.36 ± 0.30 log cfu/ml, whereas the initial TMY count of 5.50 ± 0.69 log cfu/ml significantly reduced to 2.29 ± 0.32 log cfu/ml with HHP treatments. Both TMAB and TMY counts significantly increased with the storage temperature and time. The treated samples stored at 22°C had higher TMAB count than the treated samples at 4°C (Table 13).

The mean initial Na (10.34 ± 0.77 ppm), Mg (18.96 ± 0.93 ppm), K (50.04 ± 2.50 ppm), Ca (25.47 ± 2.14 ppm), B (29.23 ± 4.10 ppm), Cr (1.30 ± 0.51 ppm), Mn (143.68 ± 6.35 ppm), Fe (240.00 ± 8.42 ppm), Co (2.70 ± 0.55 ppm), Ni (22.63 ± 3.29 ppm), Cu (27.30 ± 4.73 ppm), Zn (147.27 ± 8.84 ppm), As (0.36 ± 0.10 ppm), and Ba (32.29 ± 2.20 ppm) were not significantly affected by processing, storage time, and storage temperature ($p > .05$). Hg and Pb were not detected in the samples.

The cloudiness-clarity (9.80 ± 0.44), dullness-shininess (9.60 ± 0.54), color intensity (9.40 ± 0.54), particle distribution (9.40 ± 0.54), flavor (9.60 ± 0.40), juice density (9.40 ± 0.46), licorice taste (9.80 ± 0.44), bitter taste (9.60 ± 0.44), sour taste (9.60 ± 0.24), sweetness (10.00 ± 0.00), and aftertaste (9.80 ± 0.44) did not significantly change with storage time and temperature.

Licorice drink is usually consumed at room temperature but its shelf life is only 1 day at this temperature. Thus, refrigerated storage is applied to extend its shelf life for several days. The samples were stored at 4°C and 22°C to observe shelf life extension and compare

TABLE 9 Changes on the measured properties of licorice drink during storage at both 4°C and 22°C

		Temperature			
		4°C		22°C	
	Days of storage	Control	HHP processed	Control	HHP processed
pH	0	7.00°±°0.01^{Aa}	7.03°±°0.04^{Ba}	7.00 ± 0.01^{Aa}	7.03 ± 0.05^{Aa}
	7	6.62°±°0.02^{Cb}	7.01°±°0.05^{Aa}		6.93 ± 0.04^{Bb}
	15		6.92°±°0.05^{Ab}		6.49 ± 0.04^{Bc}
	25		6.88 ± 0.06^{Ac}		6.29 ± 0.04^{Dd}
Conductivity (μS cm^{-1})	0	361.2 ± 10.31^{Aa}	357.6 ± 0.77^{Ba}	361.2 ± 1.309^{Aa}	357.6 ± 0.77^{Ba}
	7	492.7 ± 1.92^{Ab}	374.3 ± 1.94^{Cb}		488.6 ± 2.60^{Db}
	15		392.4 ± 1.67^{Cc}		514.0 ± 1.00^{Dc}
	25		442.4 ± 1.81^{Cd}		523.6 ± 1.12^{Dd}
*L**	0	52.02 ± 0.58^{Aa}	52.10 ± 0.31^{Aa}	52.02 ± 0.58^{Aa}	52.10 ± 0.31^{Aa}
	7	49.05 ± 0.34^{Bb}	51.75 ± 0.39^{Aa}		46.82 ± 0.24^{Cb}
	15		51.17 ± 0.38^{Ab}		46.39 ± 0.31^{Bc}
	25		48.14 ± 0.41^{Ac}		44.86 ± 0.28^{Bd}
*a**	0	6.76 ± 0.22^{Aa}	6.70 ± 0.35^{Aa}	6.76 ± 0.22^{Aa}	6.70 ± 0.35^{Aa}
	7	5.96 ± 0.20^{Bb}	6.53 ± 0.07^{Aa}		6.17 ± 0.13^{Bb}
	15		6.11 ± 0.09^{Ab}		5.76 ± 0.12^{Bc}
	25		5.72 ± 0.16^{Ac}		4.81 ± 0.26^{Bd}
*b**	0	52.95 ± 0.32^{Aa}	54.21 ± 0.20^{Ba}	52.95 ± 0.32^{Aa}	54.21 ± 0.20^{Ba}
	7	46.33 ± 0.47^{Bb}	52.79 ± 0.32^{Ab}		46.92 ± 0.23^{Bb}
	15		52.13 ± 0.24^{Ac}		45.01 ± 0.40^{Ac}
	25		48.90 ± 0.57^{Ad}		43.28 ± 0.55^{Bd}
*C**	0	53.38 ± 0.31^{Ba}	54.62 ± 0.18^{Aa}	53.38 ± 0.31^{Ba}	54.62 ± 0.18^{Aa}
	7	46.71 ± 0.45^{Bb}	53.19 ± 0.32^{Ab}		47.33 ± 0.23^{Bb}
	15		52.48 ± 0.25^{Ac}		45.38 ± 0.41^{Bc}
	25		49.23 ± 0.55^{Ad}		43.54 ± 0.54^{Bd}
h°	0	1.443 ± 0.04^{Aa}	1.447 ± 0.04^{Aa}	1.443 ± 0.04^{Aa}	1.447 ± 0.03^{Aa}
	7	1.442 ± 0.04^{Aa}	1.447 ± 0.04^{Aa}		1.440 ± 0.04^{Aa}
	15		1.454 ± 0.03^{Aa}		1.443 ± 0.05^{Aa}
	25		1.454 ± 0.04^{Aa}		1.459 ± 0.04^{Aa}
Turbidity (NTU)	0	26.80 ± 0.46^{Ba}	43.85 ± 0.48^{Aa}	26.80 ± 0.46^{Ba}	43.85 ± 0.48^{Aa}
	7	58.7 ± 1.45^{Bb}	52.51 ± 0.80^{Cb}		114.9 ± 1.218^{Ab}
	15		63.89 ± 0.35^{Bc}		142.1 ± 0.458^{Ac}
	25		93.41 ± 0.84^{Bd}		162.8 ± 1.20^{Ad}
TA (g 100^{-1} ml^{-1})	0	0.0204 ± 0.00^{Aa}	0.0204 ± 0.00^{Aa}	0.0204 ± 0.00^{Aa}	0.0204 ± 0.00^{Aa}
	7	0.0204 ± 0.00^{Aa}	0.0204 ± 0.00^{Aa}		0.0204 ± 0.00^{Aa}
	15		0.0204 ± 0.00^{Aa}		0.0204 ± 0.00^{Aa}
	25		0.0204 ± 0.00^{Aa}		0.0204 ± 0.00^{Aa}

Note: Data in the same column with different lowercase superscript letter and data in the same row with different uppercase letter are significantly different ($p \leq .05$).

Abbreviation: HHP, high hydrostatic pressure.

the effect of the storage temperatures on the quality properties. Previous studies conducted by HHP also reported increase in shelf life at both 4°C and 22°C. Most of the physiochemical properties of licorice drink were not significantly affected by pressure (450 MPa for 5 min at 10°C) and storage temperature (4°C and 20°C) for 4-week storage. Microbial growth of the treated samples was significantly

TABLE 10 Changes on the glycyrrhizin and glabridin concentrations (mg/L) of licorice drink during storage at both 4°C and 22°C

		Temperature			
		4°C		22°C	
	Days of storage	Control	HHP processed	Control	HHP processed
Glycyrrhizin	0	503.4 ± 25.64^{Aa}	499.0 ± 16.17^{a}	503.4 ± 25.64^{Aa}	499.0 ± 16.17^{Aa}
	7	498.5 ± 23.50^{Aa}	486.0 ± 14.28^{Aa}		479.6 ± 29.12^{Aa}
	15		471.8 ± 16.10^{Aa}		447.7 ± 28.71^{Aa}
	25		466.8 ± 17.52^{Aa}		435.2 ± 22.12^{Aa}
Glabridin	0	0.331 ± 0.05^{aA}	0.366 ± 0.19^{Aa}	0.331 ± 0.05^{Aa}	0.366 ± 0.19^{Aa}
	7	0.291 ± 0.14^{Aa}	0.318 ± 0.31^{Ab}		0.304 ± 0.24^{Ab}
	15		0.289 ± 0.24^{Ab}		0.245 ± 0.21^{Ac}
	25		0.276 ± 0.31^{Ab}		0.237 ± 0.22^{Ac}

Note: Data in the same column with different lowercase superscript letter and data in the same row with different uppercase letter are significantly different ($p \leq .05$).

Abbreviation: HHP, high hydrostatic pressure.

TABLE 11 Changes on the phenolic compounds (mg/L) of licorice drink during storage at both 4°C and 22°C

		Temperature			
		4°C		22°C	
	Days of storage	Control	HHP processed	Control	HHP processed
Gallic acid	0	8.35 ± 0.30^{Aa}	6.99 ± 0.36^{Ba}	8.35 ± 0.30^{Aa}	6.99 ± 0.36^{Ba}
	7	6.54 ± 0.17^{Ab}	6.84 ± 0.15^{Aa}		6.82 ± 0.52^{Aa}
	15		6.76 ± 0.71^{Aa}		6.68 ± 0.97^{Aa}
	25		6.70 ± 0.09^{Aa}		6.56 ± 0.24^{Aa}
p-cumaric acid	0	1.44 ± 0.11^{Aa}	0.91 ± 0.09^{Ba}	1.44 ± 0.11^{Aa}	0.91 ± 0.09^{Ba}
	7	1.03 ± 0.10^{Ab}	0.89 ± 0.11^{Aa}		0.79 ± 0.29^{Aa}
	15		0.88 ± 0.12^{Aa}		0.69 ± 0.11^{Aa}
	25		0.80 ± 0.07^{Aa}		0.73 ± 0.09^{Aa}
Chlorogenic acid	0	2.01 ± 0.22^{Aa}	1.38 ± 0.16^{Ba}	2.01 ± 0.22^{Aab}	1.38 ± 0.16^{Ba}
	7	1.23 ± 0.27^{Ab}	1.21 ± 0.18^{Aa}		1.18 ± 0.09^{Aa}
	15		1.10 ± 0.13^{Aa}		1.06 ± 0.27^{Aa}
	25		1.10 ± 0.21^{Aa}		0.95 ± 0.36^{Aa}
Vanillic acid	0	20.97 ± 2.55^{Aa}	19.31 ± 1.82^{Aa}	20.97 ± 2.55^{Aa}	19.31 ± 1.82^{Aa}
	7	16.08 ± 1.05^{Bb}	19.15 ± 1.47^{Aa}		14.90 ± 3.90^{Bab}
	15		17.28 ± 1.61^{Aab}		11.80 ± 1.03^{Bb}
	25		15.13 ± 1.67^{Ab}		9.68 ± 1.78^{Bb}
Caffeic acid	0	2.77 ± 0.80^{Aa}	2.97 ± 0.28^{Aa}	2.77 ± 0.80^{Aa}	2.97 ± 0.28^{Aa}
	7	1.72 ± 0.13^{Ab}	1.65 ± 0.06^{Ab}		1.58 ± 0.50^{Ab}
	15		1.37 ± 0.11^{Ac}		0.81 ± 0.11^{Bc}
	25		1.11 ± 0.09^{Ac}		0.53 ± 0.16^{Bd}
Miristein	0	0.663 ± 0.05^{Aa}	0.382 ± 0.10^{Ba}	0.663 ± 0.05^{Aa}	0.382 ± 0.10^{Ba}
	7	0.401 ± 0.11^{Ab}	0.397 ± 0.13^{Aa}		0.356 ± 0.17^{Ba}
	15		0.365 ± 0.16^{Ab}		0.311 ± 0.14^{Bb}
	25		0.355 ± 0.12^{Ab}		0.293 ± 0.10^{Bb}

Note.: Data in the same column with different lowercase superscript letter and data in the same row with different uppercase letter are significantly different ($p \leq .05$).

Abbreviation: HHP, high hydrostatic pressure.

TABLE 12 Changes on the organic acid concentrations (mg/L) of licorice drink during storage at both 4°C and 22°C

		Temperature			
		4°C		22°C	
	Days of storage	Control	HHP processed	Control	HHP processed
Acetic acid	0	80.40 ± 8.33^{Aa}	80.00 ± 5.70^{Aa}	80.40 ± 8.33^{Aa}	80.00 ± 5.70^{Aa}
	7	71.13 ± 5.02^{Aa}	77.03 ± 8.66^{Aab}		73.30 ± 7.30^{Aa}
	15		70.99 ± 2.83^{Aab}		69.80 ± 10.66^{Aab}
	25		67.75 ± 5.76^{Ab}		62.30 ± 8.70^{Ab}
Formic acid	0	42.23 ± 4.42^{Aa}	43.92 ± 8.20^{Aa}	42.23 ± 4.42^{Aa}	43.92 ± 8.20^{Aa}
	7	40.30 ± 4.90^{Aa}	42.74 ± 3.56^{Aa}		41.65 ± 5.39^{Aa}
	15		42.99 ± 1.62^{Aa}		39.81 ± 5.12^{Aa}
	25		42.75 ± 4.20^{Aa}		38.47 ± 6.60^{Aa}
Fumaric acid	0	0.784 ± 0.46^{Aa}	0.744 ± 0.11^{Aa}	0.784 ± 0.46^{Aa}	0.744 ± 0.11^{Aa}
	7	0.738 ± 0.16^{Aa}	0.733 ± 0.10^{Aa}		0.715 ± 0.18^{Aa}
	15		0.719 ± 0.12^{Aa}		0.712 ± 0.47^{Aa}
	25		0.703 ± 0.18^{Aa}		0.700 ± 0.33^{Aa}

Note: Data in the same column with different lowercase superscript letter and data in the same row with different uppercase letter are significantly different ($p \leq .05$).

Abbreviation: HHP, high hydrostatic pressure.

TABLE 13 Changes on the total mesophilic aerobic bacteria and total mold and yeast (log cfu/ml) of licorice drink during storage at both 4°C and 22°C

		Temperature			
		4°C		22°C	
	Days of storage	Control	HHP processed	Control	HHP processed
Total mesophilic aerobic bacteria	0	5.52 ± 0.68^{Aa}	2.36 ± 0.30^{Ba}	5.52 ± 0.68^{Aa}	2.36 ± 0.30^{Ba}
	7	7.32 ± 0.57^{Ab}	3.26 ± 0.23^{Cb}		4.06 ± 0.49^{Bb}
	15		3.43 ± 0.49^{Bb}		4.53 ± 0.23^{Ab}
	25		4.46 ± 0.17^{Bc}		5.12 ± 0.36^{Ac}
Total mold and yeast	0	5.50 ± 0.69^{Aa}	2.29 ± 0.32^{Ba}	5.50 ± 0.69^{Aa}	2.29 ± 0.32^{Ba}
	7	7.29 ± 0.62^{Ab}	3.21 ± 0.21^{Cb}		4.04 ± 0.49^{Bb}
	15		4.02 ± 0.46^{Bc}		5.51 ± 0.21^{Ac}
	25		5.42 ± 0.17^{Bd}		6.12 ± 0.26^{Ad}

Note: Data in the same column with different lowercase superscript letter and data in the same row with different uppercase letter are significantly different ($p \leq .05$).

Abbreviation: HHP, high hydrostatic pressure.

lower than that of the control samples (Aday et al., 2018). However, previous studies lacked of sensory analyses during shelf-life studies, thus the consumer acceptance of the licorice drink during shelf-life studies was not known. Present study indicated that HHP treated samples were acceptable up to 25 days at both 4°C and 22°C.

4 | CONCLUSIONS

Licorice drink, even though it is very popular and well known, has very short shelf life due to its high pH value. Thus, efforts have made to increase its shelf life by HHP processing. Changes on physicochemical and sensorial properties as well as inactivation of endogenous microflora and spoilage bacteria was performed by 200–500 MPa pressure, 3- to 15-min processing time, and 4°C–40°C processing temperature determined by BBD. Optimization results showed that 500-MPa pressure, 9.90 min, and 18.5°C were the optimum operational conditions to process licorice drink samples; thus, shelf-life studies performed at both 4°C and 22°C with the samples treated by optimum HHP processing parameters. Control samples at 22°C and 4°C were deteriorated on Days 2 and 7, whereas the HHP-processed samples had a shelf life of 25 days. Initial pH, L^*, a^*, b^*, and

C^* values and glabridin concentration significantly decreased, but conductivity and turbidity values, total mesophilic aerobic count, and TMY count significantly increased, and no significant change was observed for the initial h° and TA values, glycyrrhizin concentrations, sensory properties, and metal ion concentrations for the HHP-processed samples during shelf life at both 4°C and 22°C. Except for miristein and vanillic, caffeic, and acetic acids, no significant change was observed on phenolic compounds and organic acids.

Applied HHP parameters provided microbial inactivation and shelf life extension of licorice drink without adversely affecting most of the physicochemical properties. The highest microbial reductions observed after 500 MPa/22°C/15 min were 3.33 ± 0.158 log for TMAB, 3.65 ± 0.012 log for TMY, 3.13 ± 0.035 log for *B. circulans*, and 3.08 ± 0.099 log for *C. tropicalis*. Even though the highest inactivation was below the USDA requirements for fruit juices (5-log inactivation); still HHP was effective to extend shelf life of the licorice drink. Further studies need to be conducted with the determination of HHP processing parameters providing 5-log reduction of *E. coli* O157:H7, shelf life extension, and changes on physicochemical properties as well as aroma active compounds during shelf-life studies.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Conceptualization; Data curation; Funding acquisition; Methodology; Project administration; Supervision; Validation; Writing-original draft; Writing-review & editing: Gulsun Akdemir Evrendilek. *Formal analysis; Methodology; Visualization*: Sahin Bakay. *Methodology; Software; Validation; Visualization*: Sibel Uzuner.

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