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Research Article

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Combined effects of acidification and high-pressure processing on microbial inactivation, bioactive compounds and antioxidant activity of liquorice root sherbet

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Abstract

High Hydrostatic Pressure Processing (HPP) has gained more attention in the fruit and vegetable industry in recent years. In this study, the optimal acidification parameters (citric acid alone and combination with ascorbic acid at the pH range 3.0 to 4.5) were determined and the effect of HPP conditions (pressures 250- 450 MPa and exposure times 1-5 min) on acidified liquorice root sherbet (ALRS) were investigated. Results showed that acidification of LRS by only citric acid had higher aroma and flavor scores. HP treatments were effective to reduce the yeast and mold (YM) count, total coliforms (TC), and inoculated pathogens (*Escherichia coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028) in ALRS. Although acidification of LRS achieved a significant reduction in glycyrrhizic acid (GA) content, further treatment by HPP did not affect pH, the contents of total phenolic, total soluble solids, flavonoid, and GA or the antioxidant capacity of ALRS. Our results suggest that acidification and HPP treatments could be used to increase consumer acceptability and extend the shelf life of LRS.

Keywords: Liquorice root sherbet, Acidification, High pressure, Quality characteristics

Introduction

Liquorice root has been utilized in many traditional medical formulations to combat diseases in Asian and Mediterranean countries (Fung and Linn, 2017). Triterpenoid saponins and flavonoids are the main compounds in liquorice and major bioactive components are glycyrrhizin and its derivatives (Zhang and Ye, 2009). Glycyrrhizic acid (GA) in the liquorice contains one molecule of glycyrrthetinic acid and two molecules of glucuronic acid. GA is 50 times sweeter than sucrose and is generally consumed in herbal tea products and infusions (Obolentseva et al., 1999).

In the Middle East countries such as Turkey, liquorice roots are used to make a traditional drink known as ‘‘Liquorice Root Sherbet’’ which is served cold by street vendors (Aday et al., 2018; Maskan, 1999). The roots are shredded and extracted by water to prepare sherbet (Arino et al., 2007; Roden, 2008). A traditional method for producing sherbet in Turkey includes keeping the licorice roots in tap water (30-50°C) for 3 days to extract color, but the process is uncontrolled (Cınar, 2012). However, the shelf-life of sherbet is only a few days after production because of the low-acidity, high water activity and absence of pasteurization (Aday et al., 2018).

As heat treatment adversely affects the sensorial properties of LRS, there have been some attempts on utilizing non-

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thermal techniques for cold pasteurization of LRS. Uzuner and Evrendilek (2017) applied pulsed electric field (PEF) and mild heat (40°C) on LRS and obtained a considerable reduction in microbial load and *E. coli* O157: H7 in liquorice sherbet. Additionally, PEF treatment maintained physical and sensorial properties better than those of heat treatment (90°C for 3 min). Çam et al. (2014) showed that the UV-C process did not change the physical and chemical properties of LRS. However, there was a limited decrease both total aerobic microbial count and total yeast and mold count in the same study. In our previous study, we have shown the effectiveness of HP treatment on the inactivation of microorganisms in LRS (Aday et al., 2018).

As is the case with many food preservation technologies, non-thermal technologies give the best results in extending the shelf life of foods, when they are combined with other hurdles. Based on this approach, HPP and acidification of LRS could be used to increase storage life while preserving its quality. Increasing the acidity of food can be thought of as one 'hurdle' to prolong stability and shelf life. Acidified foods are classified as foods with a pH value lower than 4.6 due to acidic food compounds or the addition of acid according to the U.S. Code of Federal Regulations (Theron and Lues, 2010). Vegetative forms of bacteria are less resistant to pressure and have low pH in foods and also acidity enhances of microorganisms during HPP (Smelt, 1998). Non-thermal techniques such as high hydrostatic pressure can be used in foods as another 'hurdle' to inactivate spoilage and pathogenic microorganisms and enzymes while preserving the colour and nutritional components of commodities (Chaikham et al., 2017). The effectiveness of the process is related to the type and count of microorganisms, duration, and level of pressure, temperature, acidity, and composition of the food. Acidity is one of the critical factors in pressure treatment to inactivate microorganisms in foods because, in acidic foods, pressure-damaged cells fail to repair themselves (Linton et al., 1999).

Our previous work has shown the effectiveness of HP treatment on liquorice sherbet and for the inactivation of microorganisms (Aday et al., 2018). In this study, the optimal acidification parameters for LRS were determined by sensory test and the impact of HPP on the inactivation of microorganisms in acidified LRS was investigated. The effects of combined acidification and HPP on nutritional and bioactive components of LRS were also determined.

Materials and Methods

Chemicals and reagents

Acetonitrile, acetic acid, ABTS, glycyrrhizic acid, ammonium salt, quercetin, trolox, and gallic acid were obtained from Sigma-Aldrich (Darmstadt, Germany). Plate count agar (PCA), Folin-Ciocalteu reagent (2 N), eosin methylene blue (EMB) agar, dichloran-rose bengal chloramphenicol agar (DRBC), xylose lysine deoxycholate (XLD) agar and violet red bile agar with mug (VRB-MUG) were supplied by Merck (Darmstadt, Germany).

Preparation of sherbet

Liquorice roots were obtained from the company located in Diyarbakır, TURKEY. Roots were gently washed with water to eliminate soil residues. Then, the length of the roots was reduced to approximately 20 cm and allowed to dry for one day at room temperature. Dried roots were ground using a mallet to obtain liquorice fibres (Aday et al., 2018). Liquorice roots were extracted according to the procedure of Cınar (2012) by using water in the ratio of 1:20 (w/w) for an hour at room temperature. The prepared solution was sealed with paraffin wax to prevent evaporation. A filter paper was used to remove impurities after the extraction process.

Optimization of the acidification process

Freshly prepared LRS was acidified by citric acid (2 M) alone or in combination with ascorbic acid (100 mg ascorbic acid/100 mL LRS) by adjusting the pH of LRS to 3.0, 3.5, 4.0 and 4.5. After the addition of acids, LRS passed through a filter paper for preventing unacceptable haze formation.

High pressure treatment

Sterile bags (4 × 10 cm) (InterscienceBagFilter®) were filled with 5ml sherbet samples and carefully sealed by a heat sealer without leaving any air bubbles inside the bags. The sealed bags were then packed for the second time in plastic pouches and sealed under vacuum using a vacuum packing machine (Model MV-20, Lipovak, Turkey). Model MSE-CIP-WB-5500 high-pressure equipment (MSE Technology, Turkey) was used for the treatment and further details of the system were previously presented by Bulut (2014) and Aday et al. (2018). The parameters used in pressure treatments were selected as 250 MPa, 355 MPa, 450 MPa for the pressure levels and exposure times of 1 and 5 min according to the earlier study that we carried out high-pressure parameters between 249 and 450 MPa and exposure times between 3 and 17 min by performing central composite design (Aday et al., 2018).

Microbiological analysis

The pour plate method was used to determine the total viable count computed from duplicate inoculated plates by using plate count agar (PCA) at 37 °C for 24-48 h. Inoculated DRBC plates were incubated for 5 days at 25 °C for the enumeration of yeasts and molds (AOAC, 2000). Violet Red Bile (VRB) plates with 4-methylumbelliferone glucuronide (*MUG*) were used to enumerate total coliforms and *Eschericihia* coli at 37 °C for 24-48 h (Halkman and Sağdaş, 2011). Salmonella spp. and Listeria spp. counts of freshly prepared ALRS analyzed according to ISO 6579-2:2012 and ISO 11290-2:2017, respectively. Inoculations of *E. coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028 were performed by using the procedure of Bulut (2014). Eosin Methylene Blue (EMB) and Xylose Lysine Deoxycholate (XLD) agar were used to the enumeration of inoculated *E. coli* and *S.* Typhimurium according to the spread plate technique, respectively, and the plates were incubated for 24-48 h at 37 °C.

Total soluble solids and pH analyses

Total soluble solids content (TSS) and pH of the sherbet were determined using a refractometer (Atago PAL1, Japan) and pH meter (Ohaus Starter 3100, USA), respectively. Measurements were conducted at room temperature.

Colour

Determination of sherbet colour was carried out using a colourimeter (Minolta CR 400, Japan). CIELAB color space was used and readings were recorded as L^*, a^* and b^* values

(Colgecen and Aday, 2015).

Total phenolic content (TPC)

The total phenolic content was determined according to the procedure of Singleton and Rossi (1965). The absorbance of each solution (samples treated with Folin reagent) was recorded at 765 nm against reagent blank solution (Water+Folin+$NaCO_3$) and results were stated as mg gallic acid/L.

Total flavonoids content (TFC)

The procedure described by Tohma and Gulçin (2010) was used to measure total flavonoid content. The absorbance of sample solution was measured at 415 nm against ethanol (96 %) after waiting 40 min at room temperature. Results were expressed as milligrams of quercetin equivalents/L.

Trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacities of the samples were analyzed under Re et al. (1999). Phosphate buffer solution (pH 7.4) was used to dilute ABTS radical to 0.7 (±0.2) at 734 nm. Samples in different quantities (10, 20 and 30 µL) were added to this solution and absorbance readings were recorded after 6 min. TEAC values were expressed as µmol Trolox/mL.

Glycyrrhizic acid content (GA)

The glycyrrhizic acid content of the sherbets was determined using high-pressure liquid chromatography (Shimadzu, Japan) based on a method as described by Helmy et al. (2013). Phenomenex Gemini C18 (250 mm, 4.6 ID, 5µ) column in isocratic flow was used in the separation process. The mobile phase contains 40% acetonitrile, 60% ultrapure water and 1% acetic acid. The injection volume was 20 µL and the flow rate was selected as 1.0 ml/min. The column temperature was set at 30°C (Aday et al., 2018). Results were reported as mg glycyrrhizic acid/L.

Sensory analysis

The consumer acceptability test was performed by using a 7-point hedonic scale to choose the best acidification condition for LRS. Overall preference, appearance, aroma and flavor properties of the samples, served in 100 ml cups at room temperature, determined by participants (12 male and 3 female) who were untrained but regularly consumed this traditional beverage as part of their daily diet, using a mean liking score of 7 point scale. On the scale, 7 represent 'like extremely' and 1 represent 'dislike extremely' (Meilgaard et al., 2006).

Statistical analysis

Statistical significance of the acidification conditions and the factors (pressure levels and holding time) were determined using the ANOVA procedure. Tukey *post-hoc* test was performed to compare the means ($p<0.05$). Data analyses were carried out with SAS V8.2 software.

Results and Discussion

Selection of acidification conditions for LRS

Sensorial scores of LRS for the different acidification processes were shown in Table 1. Acidification of LRS by the combination of citric acid and ascorbic acid to different pH values did not cause any difference regarding the overall acceptability, appearance, aroma and flavor attributes. Similar trends were obtained in the LRS acidified by only citric acid, except aroma and flavor scores. Acidification with citric acid alone at pH 4.5 had significantly higher values for aroma and flavor. However, pH 4.5 is a critical point for choosing the pasteurization or sterilization operation for food products. Therefore, in order to study the effect of the HP treatments on the acidified LRS, acidification with citric acid alone at pH 4.2 was selected as the optimum treatment.

Table 1. Sensorial scores of liquorice root sherbet (LRS) for the different acidification processes (Values are expressed as the mean ± SD).

pH Values	Acidification of LRS by citric acid alone		
	Overall acceptability	Colour	Aroma and flavor
3.0	3.06^{a}±2.12	3.40^{a}±2.09	2.86^{b}±2.44
3.5	3.20^{a}±1.97	2.73^{a}±1.62	2.73^{b}±2.15
4.0	3.26^{a}±1.53	2.80^{a}±1.26	2.66^{b}±1.71
4.5	4.40^{a}±1.72	3.00^{a}±1.46	4.53^{a}±2.09
pH Values	Acidification of LRS by a combination of citric acid and ascorbic acid		
	Overall acceptability	Colour	Aroma and flavor
3.0	3.13^{a}±2.16	3.06^{a}±2.37	3.00^{a}±2.10
3.5	2.26^{a}±1.27	2.20^{a}±1.26	2.40^{a}±1.35
4.0	2.66^{a}±1.49	2.86^{a}±1.40	2.60^{a}±1.76
4.5	3.40^{a}±1.80	2.80^{a}±1.56	3.53^{a}±2.03

$^{a-c}$ Mean in the same column with different letters are significantly different ($p \leq 0.05$).

Physicochemical properties and microbiological quality of acidified LRS

The physicochemical characteristics and microbial quality of freshly made acidified LRS are reported in Table 2. With a pH of 4.21±0.09, ALRS can be classified as acidic food according to the U.S. Code of Federal Regulations (Theron and Lues, 2010). In contrast to our previous study (Aday et al., 2018) related to microbial quality, it might be explained that the acidification process resulted in higher rates of microbial inactivation. This finding corroborates the ideas of International

et al. (2009) reported that the low pH of the product causes denaturation of intracellular proteins of microorganisms. Food pathogens such as *Escherichia coli, Salmonella* spp. and *Listeria* spp. were not detected in our samples.

Table 2. Physicochemical properties and microbial loads of the acidified liquorice sherbet (Values are expressed as the mean ± SD)

Physicochemical properties and microbial loads	Acidified Liquorice Sherbet
pH	4.21±0.09
TSS (°Bx)	0.50±0.00
L*	72,30±4,42
a*	-0,76±1,66
b*	37,46±2,77
Glycyrrhyzic acid content (mg/L)	174.57±38.17
Total Phenolic Content (µg GA/mL)	308.28±42.71
Total Flavonoid (µg QE/mL)	6.56±4.29
Antioxidant Capacity (TEAC)(µmolTrolox /mL)	3,49±1,10
Total Aerobic Count (Log CFU/mL)	3,915±0.58
Yeast and Mold (Log CFU/mL)	2,038±0.47
Total Coliform (Log CFU/mL)	2,151±0.37
Escherichia coli	<10
Salmonella spp.	0/25 mL
Listeria spp.	0/25 mL

The acidification process caused an approximately seven-fold reduction in the glycyrrhizic acid content of ALRS (174.57 mg/L) compared to non-acidified LRS (1172.44 mg/L) that we previously published (Aday et al., 2018). Glycyrrhizic acid is the main compound in liquorice sherbet which gives an unpleasant, persistent aftertaste and characteristic aroma (Komes et al., 2016). Therefore, the acidification process can be a realistic option to increase the consumer acceptability of liquorice sherbets by lowering the glycyrrhizic acid content due to the breakage of bonds between glycone and aglycone in an acidic medium (Visht, 2014).

The other bioactive compounds of ALRS such as total phenolics (308.28 mg GA/L) and total flavonoids (6.56 mg QE/L) showed lower values than non-acidified LRS reported as 379.72 mg GA/L and 25.18 mg QE/L, respectively (Aday et al., 2018).

Effectiveness of high pressure on the bacterial flora of acidified LRS

Figure 1 shows the inactivation data of total aerobic microorganisms in ALRS treated by different high-pressure levels and holding times. High-pressure treatments at all levels reduced the TAC significantly by about 1.75 log CFU/mL ($p<0.05$). Obtained results are in agreement with the works of (Manas and Mackey, 2004; Tewari and Juneja, 2008) who reported that inactivation is influenced by pressure level, treatment time, and microbial types. The fact that the reduction in TAC in ALRS after a 1 m pressure treatment at 250 MPa (1.7 log CFU/mL) was slightly lower than the TAC obtained in ALRS treated at 450 MPa for 5 min (1.8 log CFU/mL), implies that the remaining microbial population (~2,5 log CFU/mL) were more likely to be aerobic spores (Aday et al., 2018). This statement could be supported by the findings of Genis et al. (2016), where they reported that the average aerobic mesophilic spore counts of LRS were 2,53 log CFU/mL and the growth of anaerobic mesophilic spores were not observed.

The initial population of yeast and mold (YM) was about 2.03 log CFU/mL. Changes in the YM counts after HPP treatments were shown in Figure 2. HPP treatments above the 355MPa were effective to achieve a complete elimination of the YM populations (min detection level 1 CFU/mL). Our results are in accordance with the results of Mukhopadhyay and Panja (2008) and Huang et al. (2013) who reported that pressures severe than 300 MPa, reduced YM counts below the limit of detection (1 log CFU/mL) in cantaloupe puree and strawberry puree, respectively.

With regard to the effects of the HPP treatments on total coliforms (TC), HPP treatments at all pressure-time combinations completely eliminated the TC in ALRS (Figure 3), which translate into a min of 2.15 log CFU/ mL reduction even after a 1 min treatment at 250 MPa. It is possible that HPP affected the morphology and disrupted the non-covalent bonds and cell membrane of the microorganisms (Casadei et al., 2002; Patterson, 2005; Tewari and Juneja, 2008). In addition, our results corroborate the results of Sreedevi et al. (2017) who showed that HPP levels above the 300 MPa resulted in a complete reduction of TC in sugarcane juice.

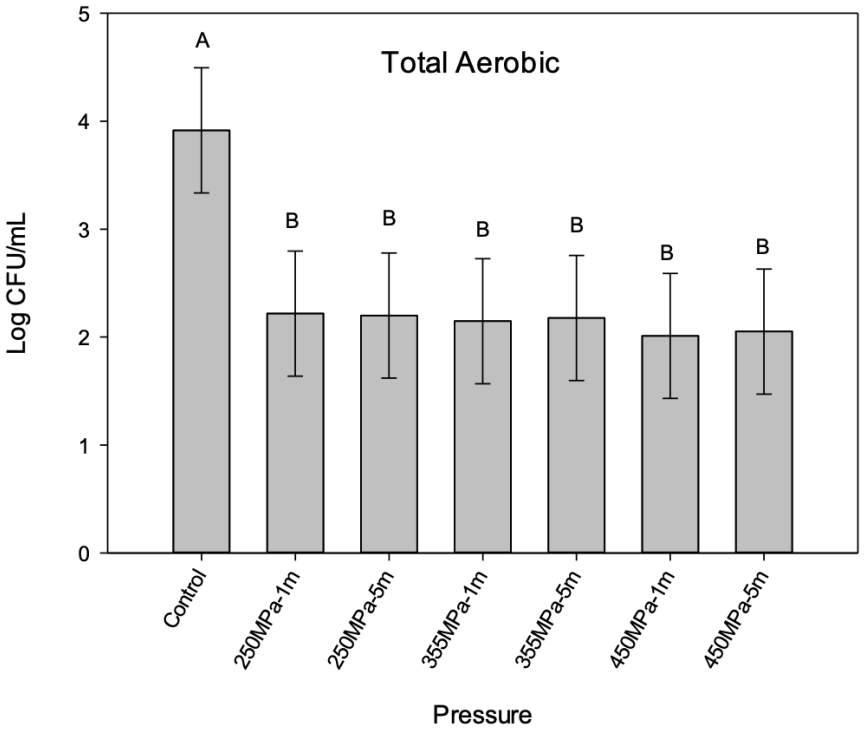

Figure 1. Changes in the counts of total aerobic microorganisms in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

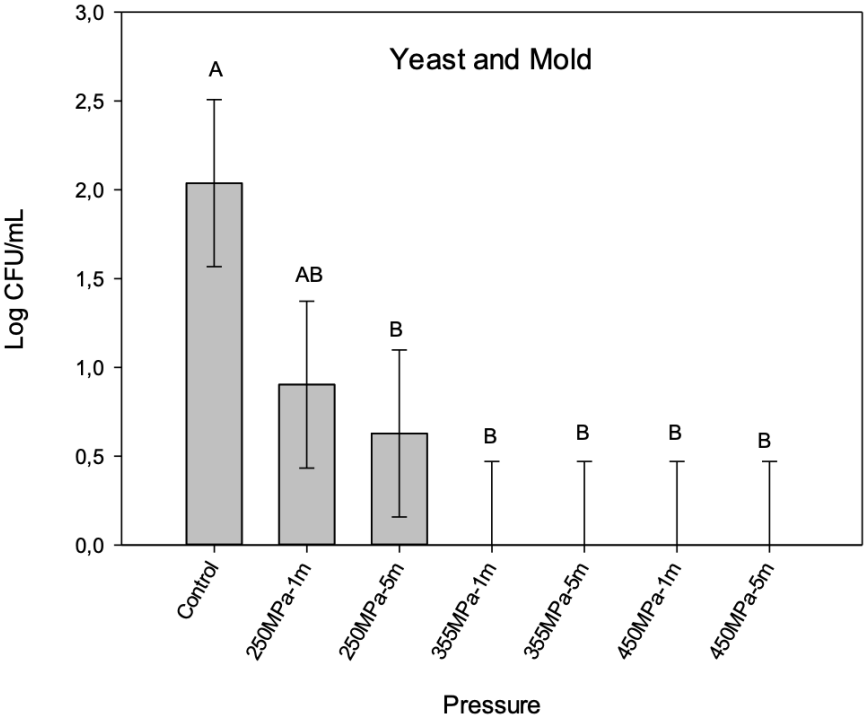

Figure 2. Changes in the counts of yeast and mold in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

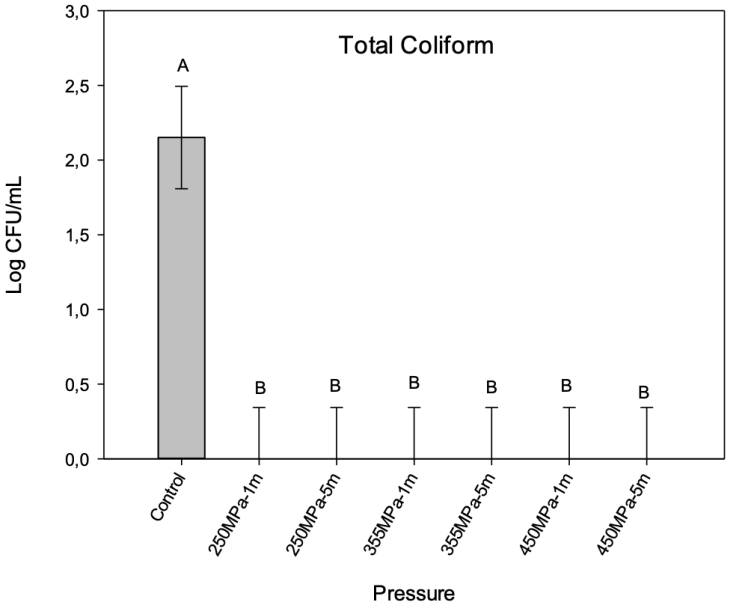

Figure 3. Changes in the counts of total coliform in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

Effectiveness of high-pressure treatment on pathogens

Survival counts of *E. coli* ATCC 25922 as a function of holding time and pressure are presented in Figure 4. Pressures above 355 MPa and 250 MPa-5min treatments had a more pronounced effect on the inactivation of *E.coli* whereas the effect of 250MPa-1min was limited. Pressures at 355 MPa and 450 MPa reduced the count of *E.coli* by 6 and 7 log CFU/mL cycles, respectively. Obtained results are in accordance with the report of (Bayındırlı et al., 2006) who showed that around 7 log reduction was observed in apple/apricot juices by using 350MPa-5m treatment. In our study, only 1 log reduction was observed after a 1 min treatment at 250 MPa, which is consistent with the results of Duong et al. (2015) who demonstrated about 1 log inactivation of E. coli NZRM 916 in feijoa puree after a 2 min treatment at 200 MPa.

Complete reduction of the *Salmonella* Typhimurium ATCC 1428, was achieved for the pressures severe than 355 MPa (Figure 5). These findings corroborate the results of Alpas and Bozoglu (2000) reported that HPP levels at 300 MPa for 5 min resulted in 8 log decrease in S. Typhi in orange juice. In our study, greater than 5 log reduction was noticed for 250MPa-5m treatment, while the reduction was about 3 log for the 250MPa-1m treatment.

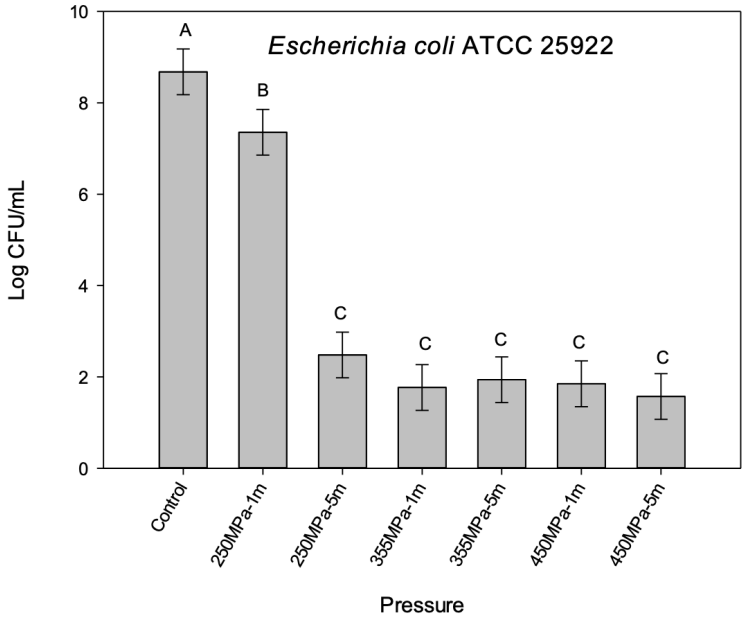

Figure 4. Changes in the counts of *E. coli* ATCC 25922in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

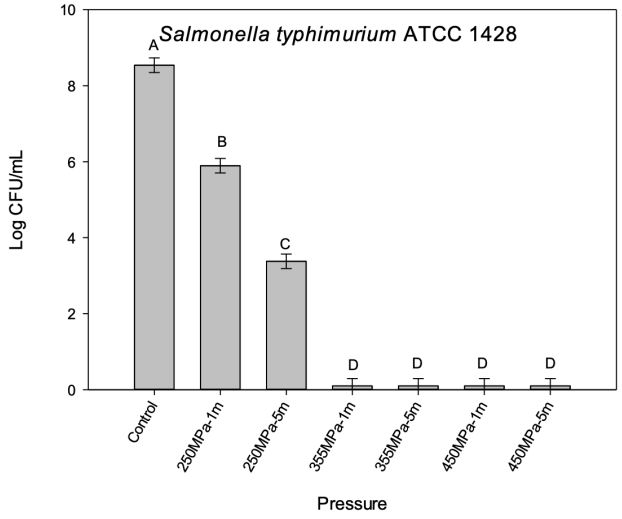

Figure 5. Changes in the counts of *S. typhimurium* ATCC 1428in acidified LRS after different high processing conditions. Means±SE denoted by different letters indicate significant differences.

Effectiveness of high-pressure treatments on physicochemical attributes

Bioactive constituents

More than 400 different chemical constituents have been identified such as triterpenoid saponins, chalcones, flavanone and their glycosides from Glycyrrhiza species (Ji et al., 2016). Roots contain significant amounts of flavonoid and phenolic contents. Major compounds of liquorice are glycyrrhizin, isoliquiritin and aglycones (Cheel et al., 2010). In our study, total phenolic and flavonoid contents of liquorice were independent of the power of the HPP ($p>0.05$) (Table 3). However, the control group had lower total flavonoid content compared to treated samples. The present findings are consistent with Plaza et al.'s (2011) findings which showed that HPP treatments (400 MPa-1m) increased the total flavanone content of the orange juices due to the higher extraction of phenolic compounds. In this work, the antioxidant capacity of the samples was not affected by different HPP treatments ($p>0.05$) (Table 3). Obtained results are in accordance with the report of Velázquez-Estrada et al. (2013) who showed that no significant difference was observed between fresh and HPP treated orange juices in the term of the anthioxidant capacity. In addition, Garcia et al. (2001a) showed that high pressure treatment did not change the antioxidant capacity of tomato puree compared to untreated samples. The study of Garcia et al. (2001b) also demonstrated that the anthioxidant potential of orange juices did not influence by high-pressure processing. However, our result differs from the studies of Del Pozo-Insfran et al. (2007) and Dede et al. (2007) who reported that antioxidant activity of muscadine grape juice and carrot juice were decreased after 400 MPa for 15m and 250MPa for 15 m HPP treatments, respectively. Inconsistency may be due to the different processing conditions or different chemical compositions. Another important finding was that the GA content of the HPP treated samples did not show any significant change compared to control samples (Table 3). This is also consistent with our earlier study (Aday et al., 2018) which showed that small molecules in liquorice such as saponins remain unaffected after HPP treatment.

Table 3. Effect of different HPP conditions on physicochemical properties of acidified LRS (Values are expressed as the mean ± SD).

Pressure (MPa)	Treatment Time (m)	pH	TSS	Total Phenolics(µg GA/mL)	Total Flavonoids (µg QE/mL)	Glycyrrhyzic acid (mg/L)	Antioxidant Capacity (TEAC)(µmolTrolox /mL)
0	1	4.21±0.09	0.50±0.00	308.28±42.71	6.56±4.29	174.57±38.17	3.49±1.10
0	5	4.21±0.09	0.50±0.00	308.28±42.71	6.56±4.29	174.57±38.17	3.49±1.10
250	1	4.21±0.09	0.53±0.06	312.83±39.28	7.13±3.76	221.80±68.18	3.47±0.88
250	5	4.23±0.11	0.53±0.06	309.39±41.88	7.28±3.29	216.21±57.49	3.45±0.97
355	1	4.24±0.13	0.50±0.00	313.17±57.78	7.36±3.51	214.55±73.92	3.44±1.15
355	5	4.23±0.12	0.53±0.06	312.28±50.97	7.00±3.06	216.00±85.56	3.62±1.31
450	1	4.29±0.06	0.55±0.07	320.67±35.12	7.21±4.18	270.12±89.32	3.55±1.88
450	5	4.22±0.11	0.53±0.06	308.28±29.35	7.74±3.00	220.91±84.18	3.38±1.31

pH and total soluble solids content

Acidity and total soluble solids (TSS) play a key role in food quality and consumer acceptability (Aday et al., 2013). From the data in Table 3, it can be seen that the pH and TSS content of the samples are not affected by HPP treatments ($p>0.05$). Our results are in agreement with the studies of Varela-Santos et al. (2012) and Barba et al. (2013) who reported that HPP treatments did not cause any significant changes in acidity and TSS values of pomegranate (350-550MPa for 30-150s) and blueberry (200-600MPa for 5-15m) juices, respectively.

Colour

Colour is an important parameter for determining food quality and affects consumers' purchase intention (Aday and Caner, 2014). Table 4 presents the L* (Lightness), a* (red-green) and b* (yellow-blue) parameters of the HPP treated and control liquorice samples. Statistical analyses revealed that interactions of two factors (pressure levels and treatment time) were significantly important regarding the L* values. Samples treated with 250 MPa for 1 m had significantly higher L* values as compared to the control group, but the differences among the HPP treated samples were not significant. Samples treated with 450MPa-5m pressure had higher L* values than control group. Obtained results are in accordance with the report of Calligaris et al. (2012) showed that an increase in L* values and a decrease in a* values of banana juices were observed as a consequence of high pressure treatment. Saldo et al. (2009) and Tribst et al. (2011) also reported that L* values of apple juice increased and * values of mango juice decreased after HPP treatments, respectively. In our study, exposure times were found significant when 450 MPa pressure was applied. However, that finding does not support the research of the Keenan et al. (2012) who reported that fruit smoothies pressurized with 450MPa-5m resulted in lower L* values than the smoothies pressurized with 450MPa-1m. This contradictory result might be due to the lower pH value (3.78) and composition (strawberry, apple, banana and orange) of the smoothie.

Statistical analysis of the redness (a*) values of the LRS showed that the interaction effect of factors was found statistically important. Samples treated with 450 MPa pressure had significantly lower a* values than control samples when the treatment time was applied as 5 minutes. A possible explanation for this might be that HPP treatments at higher pressure levels and treatment times may accelerate the degradation of anthocyanin and colour (Su et al., 2016). Flavonoid compounds are responsible for the yellow colour of sherbet. Pressure level and treatment time had a significant interaction effect on the yellowness of the LRS. However, this study showed that no differences were observed in the b* values of LRS when the holding time was chosen as 1 or 5 m. In addition, samples treated with 450MPa-5m pressure had lower b* values than control group. This is also consistent with the study of Patrigrani et al. (2019) who showed that HPP treatment caused a decrease in b* values of kiwifruit juice.

Table 4. Effects of the different high-pressure processing (HPP) conditions on colour values of acidified LRS. (Values are expressed as the mean ± SD).

	Pressure Levels (MPa) / L* values			
Treatment Time (m)	0	250	355	450
1	$72,30^{Aa}$+4,42	$78,99^{Ba}$+2,52	$77,65^{ABa}$+3,69	$72,39^{ABa}$+2,74
5	$72,30^{Aa}$+4,42	$77,76^{ABa}$+3,76	$77,96^{ABa}$+5,19	$82,04^{Bb}$+5,35
	Pressure Levels (MPa) / a* values			
Treatment Time (m)	0	250	355	450
1	$-0,76^{Aba}$+1,66	$-2,79^{Aa}$+0,99	$-2,57^{Aba}$+1,12	$-0,14^{Ba}$+1,05
5	$-0,76^{Aa}$+1,66	$-2,30^{ABa}$+1,39	$-1,99^{ABa}$+1,30	$-3,08^{Bb}$+1,16
	Pressure Levels (MPa) / b* values			
Treatment Time (m)	0	250	355	450
1	$37,46^{Aa}$+2,77	$32,26^{Aa}$+3,06	$31,70^{Aa}$+2,72	$38,93^{Aa}$+1,77
5	$37,46^{Aa}$+2,77	$33,97^{ABa}$+3,70	$28,37^{ABa}$+11,85	$27,98^{Ba}$+8,38

$^{A-B}$ Mean in the same row with different letters are significantly different ($p \leq 0.05$)
$^{a-b}$ Mean in the same column with different letters are significantly different ($p \leq 0.05$).

Conclusion

The results of this study showed that sherbets acidified by using citric acid only at pH 4.5 received higher liking scores based on aroma and flavor attributes. Pressures above 355MPa had a more pronounced effect on the inactivation of *E.coli* and *Salmonella* Typhimurium ATCC 1428 (> 6 log CFU/ml) in ALRS. Total phenolic, pH, total soluble solids (TSS), flavonoid contents, antioxidant capacity and GA contents of ALRS were not influenced by different HPP treatments. The acidification process caused an approximately seven-fold reduction in the glycyrrhizic acid content of ALRS compared to non acidified LRS. However, reduction in GA content of ALRS could be considered as an advantage, as the excess amounts of GA gives an unpleasant persistent aftertaste to LRS. Therefore, the acidification process and HPP treatments can be a realistic option to increase the consumer acceptability

and extend the shelf life of liquorice sherbets. Future research should concentrate on HPP treated non-acidified and acidified liquorice sherbet quality under different storage temperature conditions.

Compliance with Ethical Standards

Conflict of interest

The authors report no conflict of interest.

Author contribution

Serpil Aday: All analysis, Pressure treatments, Writing - review and editing.

Ciğdem Uysal Pala: Conceptualization, Supervision, Writing - review and editing.

Belgizar Ayana Cam: Sensory analysis.

Sami Bulut: Conceptualization, Pressure treatments, Review and editing.

Ethical approval

Not applicable

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Data availability

Not applicable

Consent for publication

Not applicable

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