



## The Effects of High Pressure Processing on Total Mesophilic Aerobic Bacteria Number and Color Properties of Frozen and Unfrozen Minced Beef

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### HIGHLIGHTS

- The increase in freezing process, pressure level and application time increased inactivation.
- Pressure level was the most effective factor in total mesophilic aerobic bacteria inactivation.
- The most effective inactivation at 300 MPa HPP in frozen and unfrozen samples occurred at -5 °C for 15 min.
- When freezing and pressure were applied together, the minced beef color was obtained closer to fresh characteristics.

### Abstract

High hydrostatic pressure processing (HPP) is a cold pasteurization technology that can be applied after packaging to products damaged by heat treatment. In this study, the effects of different levels (300, 350 and 450 MPa) of pressure applied at different temperatures (-5, 0 and 10 °C) and durations (5, 10 and 15 min) on the total mesophilic aerobic bacteria count (TMAB) and color values of frozen and unfrozen minced beef were investigated. The most effective factor on the number of TMAB was pressure. Freezing resulted in increased inactivation. In the application performed at constant temperature (10 °C), the difference in inactivation between frozen and unfrozen samples was seen maximum at 300MPa pressure application. Inactivation increased with increasing pressure level and time. At different application temperatures, the most effective inactivation of 300 MPa HPP in frozen and unfrozen samples occurred at -5 °C in 15 minutes. The increase in  $L^*$  value of frozen samples was less than that of non-frozen samples. This contributes to the preservation of freshness properties in meat. An increase in the  $L^*$  value was observed with the increase in pressurization time. Increasing the pressure level caused a decrease in the  $a^*$  value, and freezing caused an increase in the  $a^*$  value. It was determined that the 300-450 MPa HPP range was not large enough to observe changes in  $b^*$  values.  $\Delta E$  values in frozen samples were determined to be higher than in non-frozen samples. If freezing and pressure are applied in combination, a microbiologically safer product with a color closer to fresh properties can be obtained.

**Keywords:** Beef mince; Color of meat; High pressure treatment; Microorganisms in meat

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## 1. Introduction

The meat industry is trying to meet increasing consumer demands for high-quality raw meat with a long shelf life (Businesswire 2019; Rajendran et al. 2022). High hydrostatic pressure processing (HPP) is a non-thermal post-packaging technology that allows to extend shelf life, maintain high sensory and nutritional qualities and improve food safety in these products where other technologies such as heat treatment are not suitable (Grossi et al. 2014; Cava et al. 2021). It can reduce the presence of foodborne pathogens and spoilage microorganisms through HPP (Hayman et al. 2004; Patterson 2005; Simonin et al. 2012; Torres and Velasquez 2008). Therefore, it can be considered as an alternative to the use of chemical preservatives (Lerasle et al. 2014). The first effect of high pressure on microorganisms is the disruption of the membrane (Demir and Evrendilek, 2024). Inactivation depends on HHP conditions (pressure, time), properties of foods (pH, fat content, water activity, spices, etc.) (Bover-Cid et al. 2011). It also depends on different factors such as Gram type, strain and growth stage of the microorganisms (Garriga et al 2004; Rivas-Cañedo et al. 2009; Smelt 1998; Argyri et al. 2018).

In general, Gram-negative bacteria are more sensitive to pressure than Gram-positive bacteria, but there are large differences in pressure resistance among various strains of the same species (Cheftel and Colioli 1997). The application of high pressure in the range of 300–600 MPa has proven effective in inactivating vegetative cells. Unless HPP is performed at temperatures around 100 °C, more than 1000 MPa is needed to inactivate bacterial spores (Masana et al. 2015). Cells in stationary phase are more resistant to pressure. The growth phase of microorganisms may affect the HPP (Pagán and Mackey 2000; Mañas and Mackey 2004). The resistance of microorganisms to pressure increases with decreasing water activity (Cheftel and Colioli 1997; Jung et al. 2003). Although HPP is a non-thermal process, adiabatic heating provides a 2.5–4.8 °C/100 MPa increase in product temperature, depending on water and oil content (Patazca et al. 2007; Bozaris et al. 2021). In muscle food products, the increase in temperature combined with pressurization typically destabilizes the proteins. This results in enhanced drip loss and undesirable changes in color or other sensory properties (Gudbjornsdottir et al. 2010).

Ranges of HPP application (300–600 MPa) in pasteurization of fresh red meats can cause undesirable discoloration (Carlez et al. 1995; Realini et al. 2011). This defect can be reduced by combining of HPP with other processes, such as curing or freezing (Szerman et al. 2011; Vaudagna et al. 2012; Bulut 2014a). In their study Fernandez et al. (2007) evaluated the high pressure-low temperature process combined with freezing, and they concluded that freezing protects the meat from the harmful effect of pressure on color by regaining its original color after thawing. Although the initial investment is still high, pressure processes consume less energy than heat treatment. This is a factor that contributes to its commercial competitiveness (Garriga et al. 2004).

In this study, the effects of different degrees of pressure applied at different temperatures and times on the total microorganism number and color values of frozen and unfrozen minced beef were examined.

## 2. Materials and Methods

### 2.1. Material

In this study, beef tenderloin, which had the least amount of collagen, a homogeneously distributed fat tissue and water content, was preferred. *Longissimus costarum* muscle obtained from the dorsal region of Holstein cattle carcasses aged around 2 years was used (average 4% fat). After slaughtering, the carcasses are rested for 48 hours in cold storage at (1.0-4.0 °C). Then, they were brought to the laboratory in cold conditions in polyethylene bags in 1-2 kg masses. Fresh, lean, boneless meat samples were minced by passing them twice through a refrigerated meat grinder equipped with a 3 mm perforated plate, then kept at 4.0±2 °C until the experiment.

## 2.2. Method

### 2.2.1. Preparation of minced beef samples for pressurization process

Minced beef samples were homogenized using a food processor (Bosch, Germany). Then, the minced beef samples were packaged in air- and water-tight Polyamide/Polyethylene bags (Ege Plastik, İzmir), (90 µ thick, 83.7 g/m<sup>2</sup> weight, 10.4 cc/100 in<sup>2</sup>/day oxygen permeability and 0.55 g/100 in<sup>2</sup> /day moisture permeable) using a vacuum packaging machine (MV-20, Lipovak, Gebze, Turkey). Double packaging was done with bags of 10\*2.5 cm size. During packaging, the samples remained at room temperature for about 4 hours. Before HPP, half of the samples were frozen in a deep freezer (model: RT54QMSW, Samsung, Korea) at -21.0±5 °C in superfreezing mode for overnight (approximately 12–18 h). The other half of the samples were left in the refrigerator compartment of the device at +4.0±2 °C overnight (approximately 12-18 hours) and then the HPP process was applied. The frozen samples were not thawed before applying the HPP process. All samples were prepared in triplicate for each experiment. For convenience in the study, short names are given to the examples. F+ refers to frozen samples, F- refers to unfrozen samples.

### 2.2.2. Estimated temperature change in meat samples before HPP

Since the temperatures of the samples needed to be known before applying pressure, a thermocouple of the thermometer (Huato HE800, China) was placed from the upper end of the bag containing the sample to the center of the sample, and then frozen in the freezer with the thermocouple connected to the sample. After freezing for approximately 12-18 hours, the sample was removed from the freezer and placed in the pressure chamber. The non-frozen group was kept in the refrigerator for approximately 12-18 hours and was removed from the refrigerator and placed directly in the pressure chamber. Then the pressurization process was started. The pressurization temperature was adjusted according to the test temperature before pressurization using a cooler integrated into the system (model RE1050S, Lauda Dr R. Wobser GmbH & Co. KG, Germany). Temperature change was recorded every 30 seconds for up to 3 minutes.

### 2.2.3. High-pressure treatment and operating parameters

A high-pressure system used to process the samples had a 0.7-L working volume (model MSE-CIP-WB-5500, MSE Technology Ltd., Gebze, Turkey). Details of the system are given by Şayin Sert and Coşkun (2022). Pressure applications were carried out at 300, 350 and 450 MPa, in the temperature range of -5 °C and 10 °C, for 5, 10, 15 min. Ideally, the maximum working pressure was chosen as 450 MPa in this study, as it was aimed to reduce investment costs and minimize the change in the color and textural properties of the meat. All pressurization experiments were repeated three times for each parameter.

### 2.2.4. Microbiological analysis

Samples (10 g) were mixed with 90 ml PBS pH 7.1 and homogenized using a stomacher (model Seward 400, UK) at 200 rpm for 1 min. Serial dilutions were prepared using PBS pH 7.1. Plate Count Agar (PCA) (Merck, Germany) was used as the medium for total mesophilic aerobic bacteria (TMAB) enumeration. Incubation was carried out at 37 °C for 24-48 hours (Bulut 2014b). Microbial reduction was expressed as logarithmic reduction, corresponding to the logarithmic difference between the initial number of microorganisms before the pressure treatment and the number of surviving microorganisms after the pressure treatment.

### 2.2.5. Color analysis

Color measurements in ground meat were made using a Konica Minolta model CM-5 colorimeter (MinolTMABo, Ltd, Osaka, Japan). Three parallel samples were prepared for each experiment. After all samples were kept at approximately 25 °C for 20 minutes, they were placed in a petri dish and allowed to come into contact with oxygen by mixing with a spatula for approximately 1 minute until a homogeneous mixture was obtained. Then, the samples were placed in the petri dish of the device for color measurements at room temperature (25 °C) and measurements were made. CIE *L\** (brightness), *a\** (redness) and *b\**

(yellowness) values were measured three times on each sample and the average of the three readings was recorded. Total color difference ( $\Delta E$ ) was calculated using the following equation (Jung et al. 2003). Research was carried out in comparison with untreated control samples, and color values of unpressurized samples were used to calculate  $\Delta E$  (Bulut 2014a)

$$\Delta E = [(L^*-L_0)^2 + (a^*-a_0)^2 + (b^*-b_0)^2]^{1/2}$$

### 2.2.6. Statistical analyses

Statistical analysis of the data was carried out using SPSS v.16.0. (SPSS Inc., Chicago). Statistical analysis of the study was carried out in a random plots 3x3x3 factorial experimental design. Significant sources of variation were compared using Duncan's multiple comparison test. The significance of the variables is interpreted as a significant change on the dependent variables if P is less than 0.05.

## 3. Results and Discussion

### 3.1. Total mesophilic aerobic bacteria number

The total microorganism load in meat determines shelf life and spoilage due to metabolic activity. The presence of microbes, increases the risk of spoilage due to greening, off-flavor formation, gas production, or textural defects (Fougy et al. 2016; Vasilopoulos et al. 2015; Rajendran et al. 2022). Therefore, it is important to study the effect of combination technologies on the total number of viable bacteria in meat.

Table 1 shows the effect of pressure level and pressurization time on the TMAB number. According to the statistical analysis results, freezing situation, pressure, time, freezing situation\*pressure and pressure\*time factors were found to be significant ( $P < 0.05$ ). The most effective factor in TMAB inactivation was found the pressure factor ( $p < 0.05$ ). While there was a 2.24 log cfu/g decrease at 300 MPa according to all time averages, it was observed that there was a 3.35 and 3.97 log cfu/g decrease at 350 and 450 MPa, respectively. The biggest difference between the TMAB number of frozen and unfrozen ground meat before HPP was detected after 15 minutes of pressure application at 300 MPa. The smallest difference was obtained in 450 MPa applications. The reason for the high inactivation in frozen samples may be that ice crystals, which increase in size during frozen storage, mechanically damage the tissues (Koch et al. 1996).

In the study of Carlez et al. (1994), 200, 300, 400 and 450 MPa pressure was applied to ground meat for 20 minutes at 20 °C. While total bacteria were slightly affected at 200 MPa, a 0.5-3 log decrease was observed at 300 MPa, a 3 log decrease at 400 MPa, and a 3-5 log decrease at 450 MPa. This confirms that higher pressure level leads to a greater reduction in bacteria in meat (Shigehisa et al. 1991). The effect of high pressure on microorganisms depends on the type of microorganisms present and the composition of the food (Hoover et al. 1989). In Kim et al.'s (2018) study, marinated beef samples were subjected to HPP treatment at 550 MPa for 5 minutes at 10 °C. After treatment, the TMAB number of the treated samples (3.99 to 5.19 log cfu/g) was lower than those of the control samples (4.91–6.28 log cfu/g). In another study, while the TMAB number in fresh beef before pressurization was  $3.53 \pm 0.23$  log cfu/g, at 650MPa 10 min pressurization, there was a significant decrease in both unfrozen (20 °C) and frozen (-35 °C) samples ( $>2$  log cfu/g) occurred. After pressurization, the counts were below the limit of detection ( $<2$  log cfu/g) (Fernandez et al. 2007). Fernandez et al. (2007) emphasized that further research is needed to clarify whether combined treatments (conventional freezing process + HPP, low temperature) have an effect such as complete microbial inactivation or cell damage and to evaluate microbial growth during refrigerated storage. In Bulut's (2014a) study, as a result of applying pressure to minced beef at 300 MPa for 5 minutes at 10 and 20 °C, the average TAC reductions observed in frozen samples were log cycles of 2.4 and 2.2, respectively. These figures are approximately four times higher than the log reductions obtained in unfrozen samples after pressure treatment at the same temperatures of 10 and 20 °C, which are log cycles of 0.5 and 0.6, respectively.

**Table 1.** The effect of HPP (300, 350, 450 MPa, 5 min, 10 min, 15 min, 10 °C) applied to fresh minced beef on the logarithmic decrease in the number of TMAB

Pressure (MPa)	Time (min)	N <sub>0</sub> TMAB (log cfu/g)		Logarithmic Decrease (log cfu/g)	
		F-	F+	F-	F+
300	5	6.36±0.36	5.85±0.48	1.51±0.25 <sup>aA</sup>	2.24±0.89 <sup>aA</sup>
	10	6.36±0.36	5.85±0.48	1.64±0.11 <sup>aB</sup>	2.15±0.52 <sup>aB</sup>
	15	6.36±0.36	5.85±0.48	2.03±0.17 <sup>aC</sup>	3.85±0.52 <sup>aC</sup>
350	5	4.81±0.11	4.37±0.15	2.41±0.82 <sup>bA</sup>	2.94±0.49 <sup>bA</sup>
	10	5.81±1.10	5.93±0.69	3.76±1.09 <sup>bB</sup>	3.79±0.67 <sup>bB</sup>
	15	6.50±0.91	6.22±2.30	3.44±1.07 <sup>bC</sup>	3.54±0.99 <sup>bC</sup>
450	5	4.85±0.35	4.68±0.35	2.93±0.25 <sup>cA</sup>	2.87±0.54 <sup>cA</sup>
	10	5.26±0.82	6.08±0.51	4.25±0.60 <sup>cB</sup>	4.33±0.65 <sup>cB</sup>
	15	6.15±0.42	5.81±1.10	4.70±1.31 <sup>cC</sup>	4.75±1.17 <sup>cC</sup>

N= 6, Results are given as mean ± standard deviation. F-:Unfrozen, F+:Frozen

Lowercase letters indicate the difference between pressures.

Capital letters indicate the difference between periods

Microorganisms are more affected by high pressure applications outside optimum growth temperatures (Moussa et al. 2007; Ritz et al. 2000; Yuste et al. 1999). Because microbial cell membranes can deteriorate more easily at temperatures beyond optimum growth temperatures (Smelt 1998; Bulut 2014a). Microbial cell membranes, which are normally semi-crystalline gels, can become stiffer and sensitive to high pressure at lower temperatures (ter Steeg et al. 1999; Bulut, 2014a).

Table 2 shows the effect of pressurization temperature (-5, 0, 10 °C) and pressurization time (5, 10, 15 min) at constant pressure level. According to the results, the interaction factors freezing situation, temperature, time, freezing situation\*temperature, temperature\*time, freezing situation\*temperature\*time are significant in these study parameters ( $P<0.05$ ). It was observed that all factors had high impact values in inactivation. Maximum TMAB inactivation at 300 MPa (5 min) was observed at -5 °C (2.96 log cfu/g) in F+ samples (Table 2). At the same pressure level, inactivation increased as the temperature decreased and the time increased. There was a greater microbial reduction in F+ samples compared to F- samples in TMAB with 300 MPa HPP at all temperatures. Freezing increased inactivation at all times and temperatures. The highest death level occurred at -5 °C, considering all period averages. While the time change was not effective at 0 °C, the decrease in 15 min at -5 °C and 10 °C increased significantly ( $P<0.05$ ). According to the average of all temperatures in the F- group, the mortality level at 5, 10 and 15 minutes was 1.82, 1.81, 2.61 log cfu/g, respectively, while in the F+ group, it was 2.24, 2.41, 3.69 log cfu/g, respectively. It is thought that the temperature should be lowered below 0 °C to achieve higher inactivation at low temperatures.

**Table 2.** Effect of HPP (300 MPa; -5, 0, 10 °C; 5, 10, 15 min) applied to fresh minced beef on the logarithmic decrease in the number of TMAB

Pressure (Mpa)	Temperature (°C)	Time (min)	N <sub>0</sub> TMAB (log cfu/g)		Logarithmic Decrease (log cfu/g)	
			F-	F+	F-	F+
300	10	5	6.36±0.36	5.85±0.48	1.51±0.25 <sup>bA</sup>	2.24±0.89 <sup>bA</sup>
		10	6.36±0.36	5.85±0.48	1.64±0.11 <sup>bA</sup>	2.15±0.52 <sup>bA</sup>
		15	6.36±0.36	5.85±0.48	2.03±0.17 <sup>bB</sup>	3.85±0.52 <sup>bB</sup>
	0	5	6.30±0.56	5.83±0.48	1.58±0.49 <sup>aA</sup>	2.18±0.22 <sup>aA</sup>
		10	6.65±0.71	4.55±0.58	1.48±0.55 <sup>aA</sup>	2.07±1.32 <sup>aA</sup>
		15	6.86±0.42	4.60±0.65	1.97±0.13 <sup>aB</sup>	2.23±0.13 <sup>aB</sup>
	-5	5	6.30±0.58	5.70±0.71	2.37±0.30 <sup>cA</sup>	2.96±0.27 <sup>cA</sup>
		10	6.65±0.71	6.09±0.12	2.33±0.02 <sup>cA</sup>	3.04±0.02 <sup>cA</sup>
		15	6.65±0.71	6.09±0.12	3.84±0.06 <sup>cB</sup>	5.00±0.15 <sup>cB</sup>

N= 6 Results are presented as mean ± standard deviation. F-:Unfrozen, F+:Frozen

Lowercase letters indicate difference between temperatures

Capital letters indicate the difference between periods.

In one study, Malinowska et al. (2013) stated that the initial TMAB number in uninoculated pork and beef was 4.3 log cfu/g. They reported that this number was not changed by the 60 MPa level of HPP applied at -5 °C, and that 1.1 and 0.6 log cfu/g reduction was achieved in pork and beef, respectively, with the 193 MPa level of HPP (-20 °C). At room temperature, the TMAB level of beef was insignificantly affected by pressure treatments of 200 MPa or less. Fernandez et al. (2007) reported reductions in ATC (>2 log 10 cycles) with a count below detection limits after HHP treatment (at 650 MPa and -35 °C for 10 min) in frozen beef samples. Additionally, Carlez et al. (1994) reported that they achieved a 3 and 5 log cfu/g reduction in total flora with the application of 400 and 450 MPa high pressure, respectively. In our study, a 5 log cfu/g reduction was achieved in F+ samples with 300 MPa (-5 °C, 15 min) HPP. The study results are in agreement with the results of other researchers. Different results at similar pressure parameters may vary due to different initial microbial load levels and different microflora in fresh meat. Initial TMAB numbers in ground meat samples were in the range of approximately 4.85-6.86 log cfu/g. Under normal circumstances, a meat sample with these numbers is considered low quality in terms of microbial quality. Considering that the average TMAB numbers in beef are 4-4.5 log cfu/g, according to the results of this study, it is understood that the microbial load can be significantly reduced by the application of 300 MPa (-5 °C, 15 min) and 450 MPa (10 and 15 min) HPP. However, in these parameters, the color and texture changes of the meat and the parameters that can eliminate pathogenic microorganisms should be analyzed.

### 3.2. Effect of HPP on color parameters of fresh minced beef

The findings obtained as a result of 350/450 MPa (time effect at 300 MPa at 10 °C was not studied), 10 °C, 5, 10, 15 min HPP are shown in Figure 1. While the pressure factor was found to be effective on all dependent variables ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E$ ) in minced beef samples, freezing situation affected  $L^*$  and  $a^*$  variables the most ( $P<0.05$ ).

While the  $L^*$  values of unfrozen samples at 350 MPa 10 °C 5 min increased by 8.11 units compared to the control samples, the  $L^*$  value of frozen samples increased by 6.55 units. While the  $L^*$  values of the samples that were not frozen at 450 MPa 10 °C, 5 min increased by 13.35 units compared to the control samples, this value was 7.96 in the frozen ones. While the average  $L^*$  value difference between two pressure values increased in

F- samples, this difference disappeared in F+ samples. With increasing time, an increase in the  $L^*$  value was observed in general.

#### $L^*$

Researchers attributed the color lightening in meat as a result of HPP to the coagulation of myofibrillar and sarcoplasmic proteins, globulin denaturation, and the displacement or release of the heme group (Carlez et al. 1994). However and Xiong (2000) reported that color and texture deterioration in meat is related to protein oxidation. Although color criteria in fresh meat depend on HPP parameters, in HPP applications above 0 °C, there may be a decrease in redness ( $a^*$ ) or an increase in lightening ( $L^*$ ) in the color of meat (Vaudagna et al. 2012; Marcos et al. 2010).

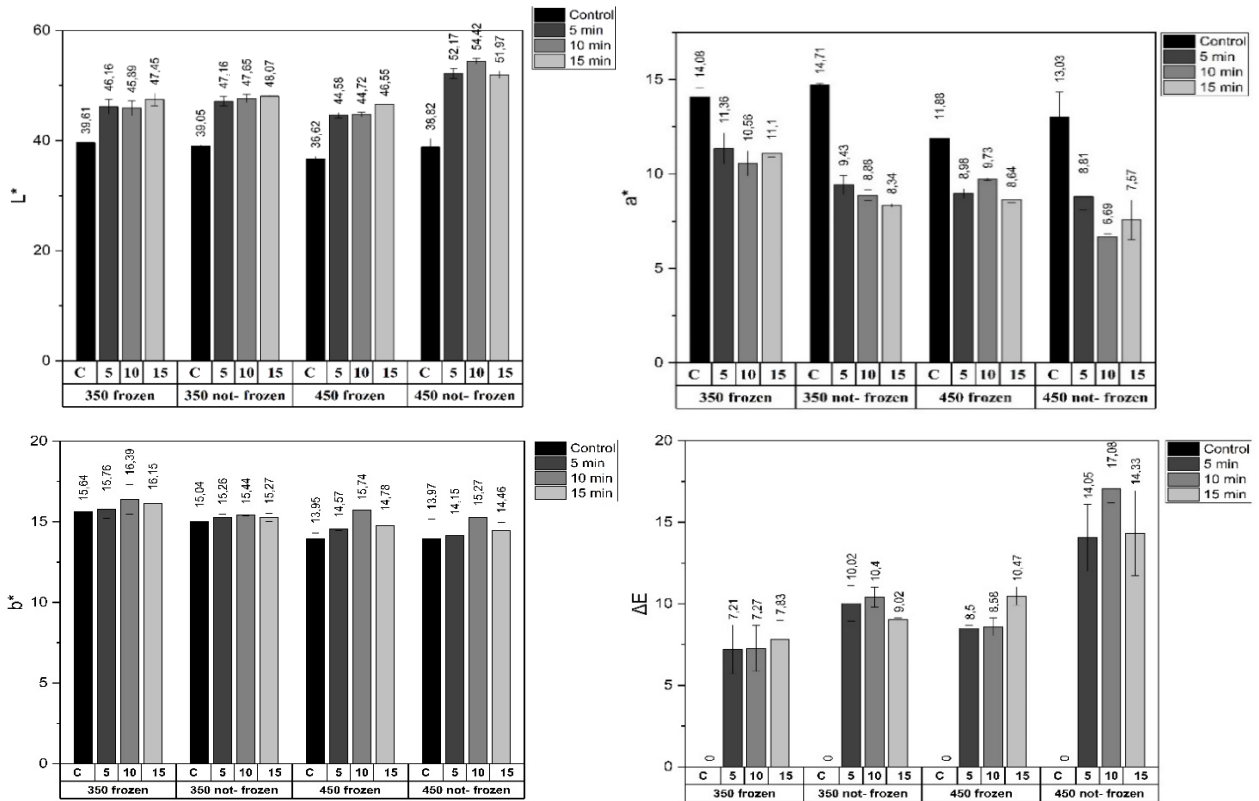
The study results regarding color lightening during HPP were found to be similar to the results of this study. As the pressure increased in unfrozen meat, the color lightening increased. Similar to our study, in the study conducted by Vaudagna et al. (2012), no significant change was observed in the  $L^*$  value of cured beef carpaccio in samples frozen at -30 °C as a result of application at 400 MPa for 1 to 5 minutes. An 8.57 br increase in  $L^*$  value was detected in unfrozen (20 °C) samples. Montero and Gomez (2005) reported that the increase in  $L^*$  value and decrease in  $a^*$  value depend on the critical pressure threshold value rather than time. In this study, freezing the samples contributed to the preservation of freshness properties on the  $L^*$  value of meat against the effects of pressure changes.

In this study, a significant color difference occurred in the meat color of F- samples at 450 MPa (10 °C, 10-15 min) pressure. It has been reported that HPP at low temperature is effective in reducing color change in meat (Fernandez 2007; Marcos et al. 2010; Vaudagna et al. 2012; Bulut 2014a). The effect of pressure on beef frozen at subzero temperature is likely to be milder and reversible. In this case, myoglobin may return to its natural conformation with thawing and, accordingly, normal color can be regained in fresh meat (De Alba et al. 2012).

#### $a^*$

Compared to pressure time, pressure level and freezing situation were more effective parameters for  $a^*$  value (redness). Similarly, Bulut (2014a) reported that in his study on minced beef at low temperatures of 300 MPa and subzero temperatures, only the freezing situation factor was effective regarding  $a^*$  values. Carlez et al. (1995) achieved a significant decrease in  $a^*$  values between 300 and 500 MPa by pressurizing ground meat. Jung et al. (2003) found that pressures above 350 MPa (10 °C, 20-300 sec) caused decreases in the  $a^*$  value. They thought that in this change in  $a^*$  values, some changes occurred in the content of myoglobin pigments and especially in the form of metmyoglobin during application. Regarding this, Ma and Ledward (2013) stated that myoglobin is denatured above 400 MPa.

Jung et al. (2003) concluded that only the effect of pressure was significant in the change in  $\Delta E$  (10 unit increase). In our study,  $a^*$  values of ground meat decreased with increasing pressure level. However,  $a^*$  values were found to be higher in frozen samples than in non-frozen samples. Mussa (1999) reported a decrease in redness in pork chops after 350 MPa (10–20 min, 25 °C) treatment. They reported that this decrease was due to the meat turning brown due to an increase in metmyoglobin ( $Fe^{3+}$ ). Vaudagna et al. (2012), as in our study, when the level of the pressure applied to cured beef carpaccio for 5 minutes was increased from 400 MPa to 650 MPa, a decrease in the  $a^*$  value was detected in both frozen and unfrozen samples. Color changes after HPP in fresh meat can be limited by optimizing HPP process parameters (Bajovic et al. 2012).



**Figure 1.** Changes in  $L^*$ ,  $a^*$ ,  $b^*$  and  $\Delta E$  values in fresh minced beef after HPP (350 and 450 MPa, at 10 °C, for 5, 10, 15 minutes)

$b^*$

There was a minimum increase in  $b^*$  values in both cases (F-, F+) when applying 350 MPa and 450 MPa pressure at 10 °C for 5 minutes. The increase continued as the pressurization time was increased to 10 minutes. A slight decrease in the  $b^*$  value was observed when the time was increased to 15 minutes at both pressure levels. Additionally, the effect of time and pressure level was not found to be significant for the  $b^*$  (yellowness) value of ground meat ( $P > 0.05$ ). It was thought that the 300-450 MPa HPP range was not large enough to observe changes in  $b^*$  values. However, in the study conducted by Vaudagna et al. (2012), when the pressure level was increased from 400 MPa to 650 MPa in cured beef carpaccio samples frozen at -30 °C for 5 minutes, an increase in the  $b^*$  value occurred, unlike in our study. In that study, a slight decrease in the  $b^*$  value was detected in unfrozen (20 °C) samples, as in our study. Since the curing was done in that study and the pressure intensities were different, it was thought that there may have been differences with the results in our study.

$\Delta E$

$\Delta E$  values were determined to be higher in frozen samples than in non-frozen samples. In the case of combined application of freezing and pressure, the color of the ground meat was obtained closer to fresh characteristics. In addition, in this study, the color difference remained below the limit values in all frozen samples at 10 °C with 350 and 450 MPa HPP. Researchers reported a  $\Delta E$  value of 10 units as significant color loss (Jung et al. 2003).

#### 4. Conclusions

HP technology is an alternative to pasteurization that can be applied to extend the life of meat without the use of chemical additives. With this application, the properties of meat are preserved better than



pasteurization. Various studies have been carried out to determine the most suitable parameters for the application of this technology. Although there are many studies on different pressure levels, time and temperature applications, there needs to be more studies on freezing the meat before application. The results of this study showed that freezing of minced beef before HPP was very effective in reducing the total mesophilic aerobic bacteria count. The color values of frozen minced beef were preserved better than non-frozen minced beef.

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