Effects of Pressurization on Some Contamination Flora in Beef Pate

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Abstract: Pate meat is one of the food products at the top of the high-risk category since it is an undercooked product, which does not undergo any treatment to ensure its safety before consumption. In this study, pressurization, a nonthermal technology, was administered to the product to enhance its microbial safety for the 1st time. After being cooked, ground, and mixed with broth and spices, the pate meat was divided into 3 batches. Their additional fat contents were adjusted to 9.11%, 25.00%, and 35.00%, respectively. After the division of each batch again into 3 portions with different acidities (pH 5.65, 5.95, 6.40), they were contaminated with *Escherichia coli* American Type Culture Collection (ATCC) 11229 or *Listeria monocytogenes* ATCC 7644. Each batch was treated with 220 or 308 MPa at 21 or 43 °C for 5 or 15 min. Whereas the increase in pressure from 220 to 308 MPa enhanced the antimicrobial effect ($P < 0.01$), the increase in pH level and fat ratio decreased it. In the study, only the interaction between the fat ratio and acidity was found significantly important. The effect of temperature and time was not significant. The highest microbial inhibition with 1.65 log reduction was observed on *E. coli* when the pate meat with pH 5.65 and 9.11% fat was treated at 308 MPa at 43 °C for 5 min. The findings indicate the necessity of using higher pressure levels as ≥308 MPa with higher acidity levels (pH ≤ 5.65) and lower fat contents (≤9.11%) to achieve a higher microbial quality in pate meat.

Keywords: *Escherichia*, HHP, *Listeria*, meat, pate

Practical Application: Different combinations of pressure (308 and 220 MPa), additional fat (9.11, 25.00, or 35.00%), pH (5.65, 5.95, or 6.40), temperature (21 or 43 °C), time (5 or 15 min) on *Listeria monocytogenes* ATCC 7644 and *Escherichia coli* ATCC 11229 contamination flora were comparatively studied for the 1st time in pate meat. The findings are especially important for pate meat safety and industry since they indicate the necessity of using pressure levels as ≥308 MPa with higher acidity levels (pH ≤ 5.65) and lower fat contents (≤9.11%) to achieve a higher microbial quality in pate meat, which is highly prone to microbial spoilage.

Introduction

Pate meat products are one of the food product groups at the top of the high-risk category in food safety. On the other hand, ready to eat foods including pate do not undergo any treatment to ensure its safety before consumption (Cabedo and others 2008). Pate, as an undercooked food product with its rich nutrition and higher water contents, was associated with *Listeria* meningitis (the inflammation of brain membranes), which has always been an important threat in food industry and public health. Cumber and others (1991) informed that although a 63-y-old man with myeloid leukaemia had followed the Department of Health advice and avoided soft cheeses, cook-chill meals and salads, he suffered with *Listeria* meningitis because he had consumed pate which was contaminated with the bacteria. The same authors also stated that pate should be in the list of foods to be avoided by immunocompromised patients. Northern Ireland, England, and Spain are also faced with Listeriosis due to contaminated pate consumption (Lauchlin and others 1991; Dominguez and others 2001). In these incidences, few people were contaminated with the bacteria, but the consequences of the illness were severe like meningitis, meningoencephalitis, septicemia, stillbirth, and abort (Kaczmarski and Jones 1989). Due to its survival capability in cold temperatures, *Listeria monocytogenes* has also been reported as a potential hazard in refrigerated foods, particularly in food products like pate, which is consumed as refrigerated without further cooking (Azevedo and others 2005). Finally, Gillspie and others (2006) recommended avoiding high-risk foods such as pate, especially in the diets of the immunocompromised, the elderly, and the pregnant.

Similarly, certain strains of *Escherichia coli* might induce diarrheal diseases, meningitis, hemolytic uremic syndrome (which has become a pandemic in Central Europe since May 2011), and rheumatoid arthritis (Olsvik and others 1991). Shooter and others (1970) mentioned the harmful potential of *E. coli* and determined large numbers of the organism especially in the hospital foods, the hospital kitchen, the meat on arrival at the hospital, the meat and the environment in an abattoir, which can result in urinary-tract infections. The important harmful potential of the bacteria has been attributed to the insufficient hygiene, the contaminated equipment and personnel, its resistance to certain antibiotics, namely nalidixic acid, ciprofloxacin, and gentamicin, which make the bacteria an important threat for nursing home

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Ministry of Health, Ankara, Turkey and which contained 4 × 10^3 cfu/mL *L. monocytogenes* ATCC 7644) was added to each of the 9 subdivided portions (4 × 10^4 cfu/666.00 g) with different pH levels (Figure 1) and fat ratios and then mixed homogenously under aseptic conditions. Finally, artificially contaminated batches were incubated at room temperature for 20 min to allow *L. monocytogenes* penetration.

Contamination of pate batches with *E. coli* ATCC 11229

The 9 subdivided portions with different fat ratios and pH levels were produced once more and all of the aforementioned procedures were repeated. This time, the 9 subdivided portions were contaminated with *E. coli* ATCC 11229. Similar to the contamination process of pate meat *by L. monocytogenes*, a 10 mL aliquot from an enriched *E. coli* ATCC 11229 stock suspension (which

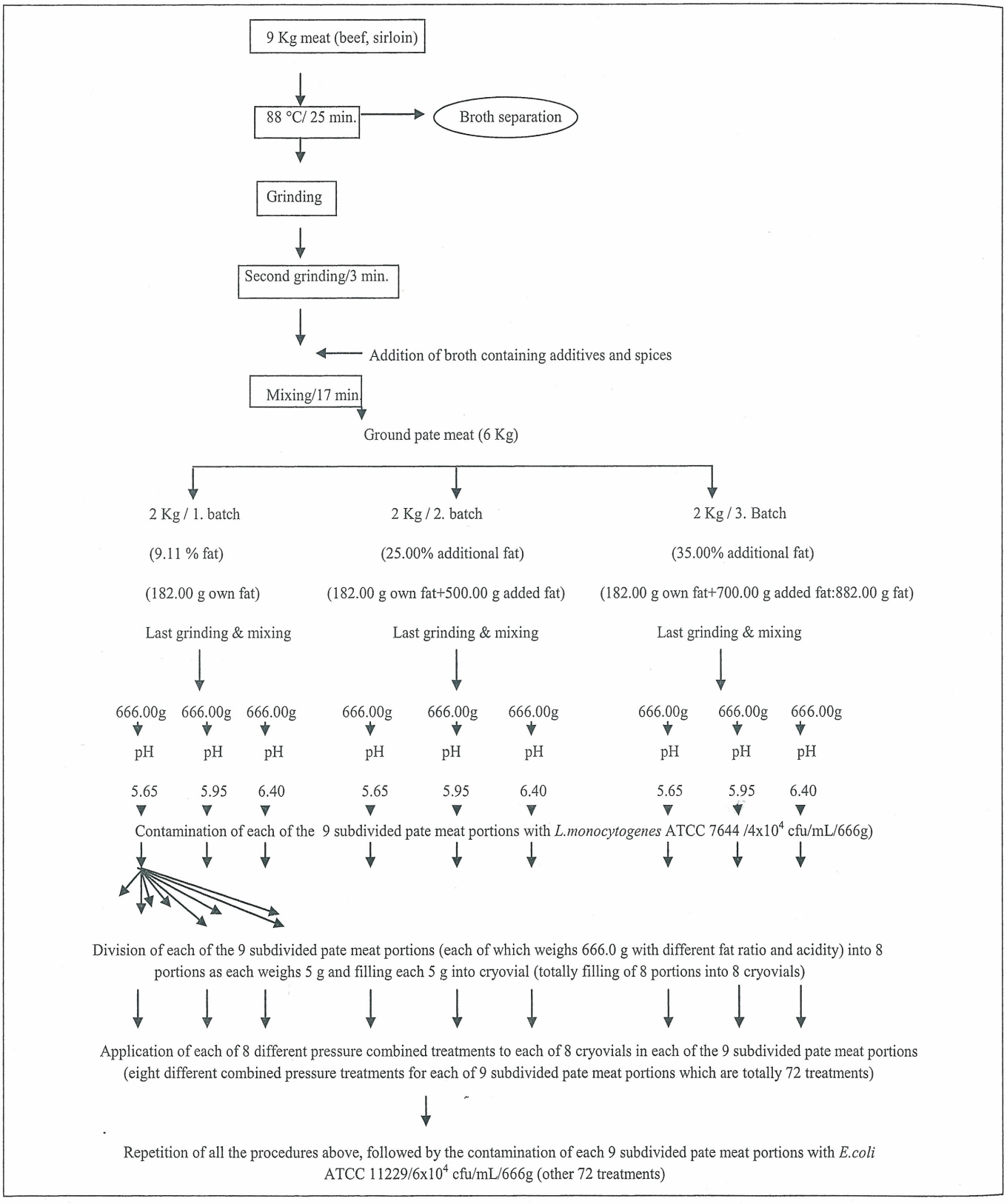


Figure 1–Process line of beef pate with the treatments of the subdivided pate meat portions.

residents (Setiabudhi and others 1997; Saenz and others 2001; Barza 2004; Sun and others 2006; Jackson and others 2007; Lund and O'brien 2009). Both microorganisms, *E. coli* and *L. monocytogenes*, are listed as indirect indicators of pathogens in processed foods in the food safety standards of European community (Todd 2003).

In order to eliminate pathogens, high hydrostatic pressure (HHP) treatment applies pressure (between 200 and 800 MPa) to food products at the temperatures below 0 °C to above 100 °C (San Martin and others 2002). One of the advantages of high-pressure treatment in comparison to thermal processing is the uniform transfer of pressure throughout the mass of the liquid or solid food independent of size, shape, and food composition (Farkas and Hoover 2000). HHP denatures and modifies the proteins and enzymes without affecting hydrogen bonds and protein structures (Meersman and others 2006). The primary meat products which have currently been subjected to the treatment are ham, pork and poultry cuts, bacon, salami, smoked and nonsmoked sausages, delicatessen, oyster, fruit juice, Guacamole, jam, sauce, cake, and dessert (O'Reilly and others 2000; San Martin and others 2002; Aymerich 2008). On the other hand, the application of HHP to pate meat to increase its microbial quality has not been a common process except for a few companies. In this study, HHP was applied to pate in order to eliminate the aforementioned pathogens for the 1st time.

In spite of the great importance of both *E. coli* and *L. monocytogenes* bacteria in food safety, no research has been done on the antimicrobial effect of HHP treatment on these bacteria species in pate. Most of the research regarding the antimicrobial effects of pressure on other meat products has only studied the effect of either time (Patterson and others 1995; Simpson and Gilmour 1997), or pH (Liberti and others 1996), or pressure (Shigehisa and others 1991), without analyzing the interactions among pH, time, pressure, and heat during pressurization.

What makes this study unique is that, for the 1st time, a study investigated the comparative antimicrobial effects of various combinations of HHP, heat, time, additional fat, and pH on *L. monocytogenes* American Type Culture Collection (ATCC) 7644 and *E. coli* ATCC 11229 contamination flora of beef pate.

Materials and Methods

Meat selection for the pate production

The primal cuts were purchased from a commercial meat integrated plant (Benge-Makro-Meat and meat Products, Ankara, Turkey). After the slaughtering process in the plant, the beef sirloin primal cuts were taken to refrigeration at (−4 °C), under 85.00% relative humidity and at an air speed of 0.50 to 1.00 m/s and then, the primal cuts were transferred to the pate process unit on the 3rd day of the refrigeration period at the fabrication stage.

Cooking and grinding

Nine kilograms of meat (beef, sirloin) was cooked in autoclave at 88 °C for 25 min. Then, the cooked meat was separated from its broth and placed and ground in the cutter (Shark, Model SY 12 to 80, 220 V, stainless steel with 150 to 180 kg/h production capacity of grinder with 220 r/min, Zhejiang, China) after the cutter was cleaned with 0.50% alkali detergent solution containing NaOH 60.00%, trisodium phosphate 25.00%, and sodium metasilicate 15.00% and the detergent residues were rinsed with cold water.

Second grinding and mixing with seasonings

Cooked and ground meat was taken into a 2nd grinding process. Then, seasoning mixture consisting of meat broth (32.00%), lecithin (15.00%), phosphate (0.16%), nitrite (0.50%), starch (0.50%), black pepper (0.050%), salt (1.00%), and ginger (0.050%) was prepared and added to the ground meat. Later, the ground meat with seasoning mixture was mixed for 17 min in the mixer (Model MX50, 220 V, 50 kg capacity, Food Machinery of America, Niagara Falls, NY, U.S.A.).

Preparation of beef pate application batches with different fat ratios

The pH level of the 6.00 kg of ground, cooked, and spiced pate meat (3.00 kg of 9.00 kg of meat was lost due to the grinding and cooking process in autoclave) was determined as 5.91 by using a digital pH meter (Heidolph-Schott, Maintz, Germany) after homogenization of 10.00 g pate meat in 90.00 mL deionized water with a stomacher-4000 (AJ Steward Co. Ltd., London, U.K.). Then, the cooked, ground and spiced meat was divided into 3 equal batches, each of which weighs 2.00 kg (Figure 1). No fat was added into the pate meat in the 1st batch and the pate meat in this batch contained only its own fat as 9.11% (182.00 g/2.00 kg). 500.00 g tallow fat was externally added to the 2nd batch to make the additional fat content 25.00% (682.00 g/2.00 kg). Finally, 700.00 g tallow fat was externally added to the 3rd batch to make the additional fat content 35.00% (882.00 g/2.00 kg) (Figure 1). To mix the meat and fat globules homogenously, the ground pate meat with 25.00% additional fat in the 2nd batch and the ground pate meat with 35.00% additional fat in the 3rd batch were taken into the final mixing process. Therefore, 3 different pate meat batches in 3 different additional fat concentrations, 9.11%, 25.00%, and 35.00%, were produced.

The production of the subdivided pate meat portions with different fat ratio and acidity

Each of the three 2.00 kg pate batch with different additional fat ratio was divided again into 3 equal portions, each of which weighs 666.00 g (Figure 1). The pH level of the 1st subdivided portion was adjusted to 5.65 by using 0,10 N lactic acid (5% at pH 2.90, Shanghai Trustin Chemical Co, Ltd, China), while the pH level of the 2nd subdivided portion was adjusted to 5.95 and the pH level of the 3rd subdivided portion was adjusted to 6.40 pH by using 0.10 N sodium hydroxide (Merck KGaA, pH 12.7, 1.00 g/cm^3). In other words, totally 9 different pate meat batches each of which weighs 666.00 g with different fat ratio and acidity were produced. These are; 1st batch: pate meat with pH 5.65, fat 9.11%; 2nd batch: pate meat with pH 5.95, fat 9.11%; 3rd batch: pate meat with pH 6.40, fat 9.11%; 4th batch: pate meat with pH 5.65, additional fat 25%; 5th batch: pate meat with pH 5.95, additional fat 25%; 6th batch: pate meat with pH 6.40, additional fat 25%, 7th batch: pate meat with pH 5.65, additional fat 35%; 8th batch: pate meat with pH 5.95, additional fat 35% and finally 9th batch: pate meat with pH 6.40, additional fat 35% (Figure 1).

After the pH and additional fat ratio adjustments of the 9 subdivided portions in pate batches, they were separately contaminated with *L. monocytogenes* ATCC 7644.

Contamination of pate batches with *L. monocytogenes* ATCC 7644

A 10 mL aliquot from an enriched *L. monocytogenes* ATCC 7644 stock suspension (which was purchased from Hıfzısıhha Refik Saydam Research Center, Department of Culture Collection,

was purchased from Hıfzısıhha Refik Saydam Research Center and which contained 6×10^3 cfu/mL *E. coli* ATCC 11229) was added to each of the 9 subdivided pate meat portions (6×10^4 cfu/666.00 g) with different pH levels and fat ratios (Figure 1) and then mixed homogenously under aseptic conditions. Then, artificially contaminated batches were incubated at room temperature for 20 min to allow *E. coli* penetration.

Eight combined treatments of 2 different pressurization levels (220 or 330 MPa), 2 different time intervals (5 or 15 min), and 2 different heat levels (21 or 43 °C) were applied to each of the 9 subdivided pate meat portions each of which weighs 666.00 g and which was at one of the different pH levels (5.65, 5.95, or 6.40) and additional fat ratios (9.11%, 25.00%, or 35.00%) and which were contaminated with *L. monocytogenes* ATCC 7644 or *E. coli* ATCC 11229. So, 8 combined treatments for each of the 9 subdivided pate meat portions, in other words $8 \times 9 = 72$ different combined treatments of different pressure, heat, time, fat and acidity were applied to pate meat with different additional fat ratio, acidity and microorganism (Figure 1). The application and control batches with their codes are given in Table 1.

The applications of the different combined pressure treatments to the subdivided pate meat portions

For the application of the each of the 8 combined treatments in each subdivided portion from the 9 subdivided portions, 5 g of meat from each of the 9 subdivided portion which weighs 666.00 g at different pH level and additional fat ratio was transferred into cryovial and the cryovial was placed in the pressurization apparatus. Then, one of the 8 different combined treatments (2 heat levels: 21 or 43 °C × 2 time intervals: 5 min or 15 min × 2 different pressurization levels: 220 or 330 MPa = 8 treatments) of the different time intervals, heat levels, and pressurization levels was applied to the cryovial. After the treatment, each of the other 7 cryovials was treated with one of the other 7 different combined treatments of the different time intervals, heat levels, and pressurization levels. As a result, 8 different combined treatments of the different time intervals, heat levels, and pressurization levels were applied to the 1st subdivided pate meat portion. The same 8 different treatments were also applied to each of the other 8 subdivided pate meat portions each of which weighs 666 g. So, totally 8 different treatments were applied to each of the 9 subdivided pate meat portions. In other words, totally, 72 treatments were applied by using combinations of 2 different heat levels (21 or 43 °C), 3 different fat ratios (9.11%, 25.00%, 35.00%), 3 different pH levels (5.65, 5.95, or 6.40), 2 different time periods (5 or 15 min), and 2 different pressure levels (220 or 330 MPa) (2 [the number of heat levels] × 3 [the number of additional fat ratios; 9.11%, 25.00%, and 35.00%] × 3 [the number of pH levels; 5.65, 5.95 and 6.40] × 2 [the number of time periods; 5, 15 min] × 2 [the number of pressure levels; 330, 220 MPa = 72]) (Figure 1).

Table 1–The abbreviations which are used for the codes of pate batches with some code samples.

m:	Minute
C:	°C
MPa:	Abbreviation for the megaPascal unit where a Pascal is a unit of measure of pressure.
Some examples from the codes of pate batches, used in figures:	
15m21C308MPa:	. Pate under pressurization at 308 MPa for 15 min at 21 °C.
C15m21C308MPa:	Control of pate coded "15m21C308MPa", but without pressurization process at 308 MPa for 15 min at 21 °C.

Pressurization

The equipment for pressurization had a pressure chamber with 2 end closures, a pressure pump, a hydraulic unit to generate high pressure for compression, and a temperature control device. The pressure vessel was made of hot galvanized carbon steel and piston was hard chrome plated and polished to mirror finish (steel-type heat-treated special K) which was processed into the required sizes at Electrical and Electronic Engineering Department of Middle East Technical University, Ankara, Turkey. To keep the temperature constant at the liquid–solid interface, the heat levels were regulated by means of a laterally surrounding isolated coil and ethylene glycol was used as the thermoregulating fluid. Before pressurization, the sample was placed directly into the pressure vessel and the initial temperature was set by using a regulating device (Figure 2). The vessel was thermoregulated by means of a laterally surrounding isolated coil and ethylene glycol was used as the thermoregulating fluid to keep the temperature constant at the liquid–solid interface (Figure 2). During pressurization of beef pate samples in cryovials, pressure level, time, and temperature were all controlled and recorded.

Seventy-two cryovials, which were filled with the pate meat which was previously contaminated with *L. monocytogenes* ATCC 7644 and which had different additional fat ratios and acidities, were immediately transferred to the enrichment and then microbial analysis after their 72 different combined treatments of pressure, heat, time, fat, and acidity while their 72 controls with the same additional fat ratio, acidity and microorganism were taken directly to the enrichment and then microbial enumeration without any treatment. Each pate meat in each cryovial after the application of one of the 72 different combined treatments represented a different combined treatment of pressure, time, heat, acidity, and fat. The microbial inhibition, which was caused by the treatment, was calculated by comparing the bacterial count of the treated cryovial with that of its control cryovial (Log [cfu/g (Control)] – [cfu/g (Treatment)]). On the other hand, 72 cryovials which were filled with the pate meat which was previously contaminated with *E. coli* ATCC11229 and which had different additional fat ratio and acidities were similarly transferred to the enrichment and then microbial analysis after their 72 different combined treatments of pressure, heat, time, fat, and acidity while their 72 controls with the same additional fat ratio, acidity, and microorganism were taken directly to the enrichment and microbial enumeration without any treatment. Because of the huge number of the treatments (72 treated pate meat cryovials which contained the pate meat which was contaminated with *L. monocytogenes* ATCC 7644 and their 72 controls, totally 144, and similarly, 72 treated pate meat cryovials which contained the pate meat which was previously contaminated with *E. coli* ATCC 11229 and their 72 controls, another 144) and because the antimicrobial effects of most of the treatments were found as statistically not important, only the 2 treatments with the highest and the 2 treatments with the lowest antimicrobial effect with their controls at each fat concentration and acidity were included to the figures. Each column in the figures shows a microbial load which is belong to one of the 144 cryovials which was treated with a different combined pressure application (for this reason, each of 144 cryovials represents a

different treatment as indicated above) or one of the 144 controls which was not treated as opposite to its treated match (treated cryovial) with the same fat ratio, acidity, and microorganism. In other words, one of the columns, which were placed in figures as in pairs is belong to the treatment and the column next to it is its (treated cryovial's) control. The difference between these 2 columns in the figures corresponds to the antimicrobial potential of the treatment.

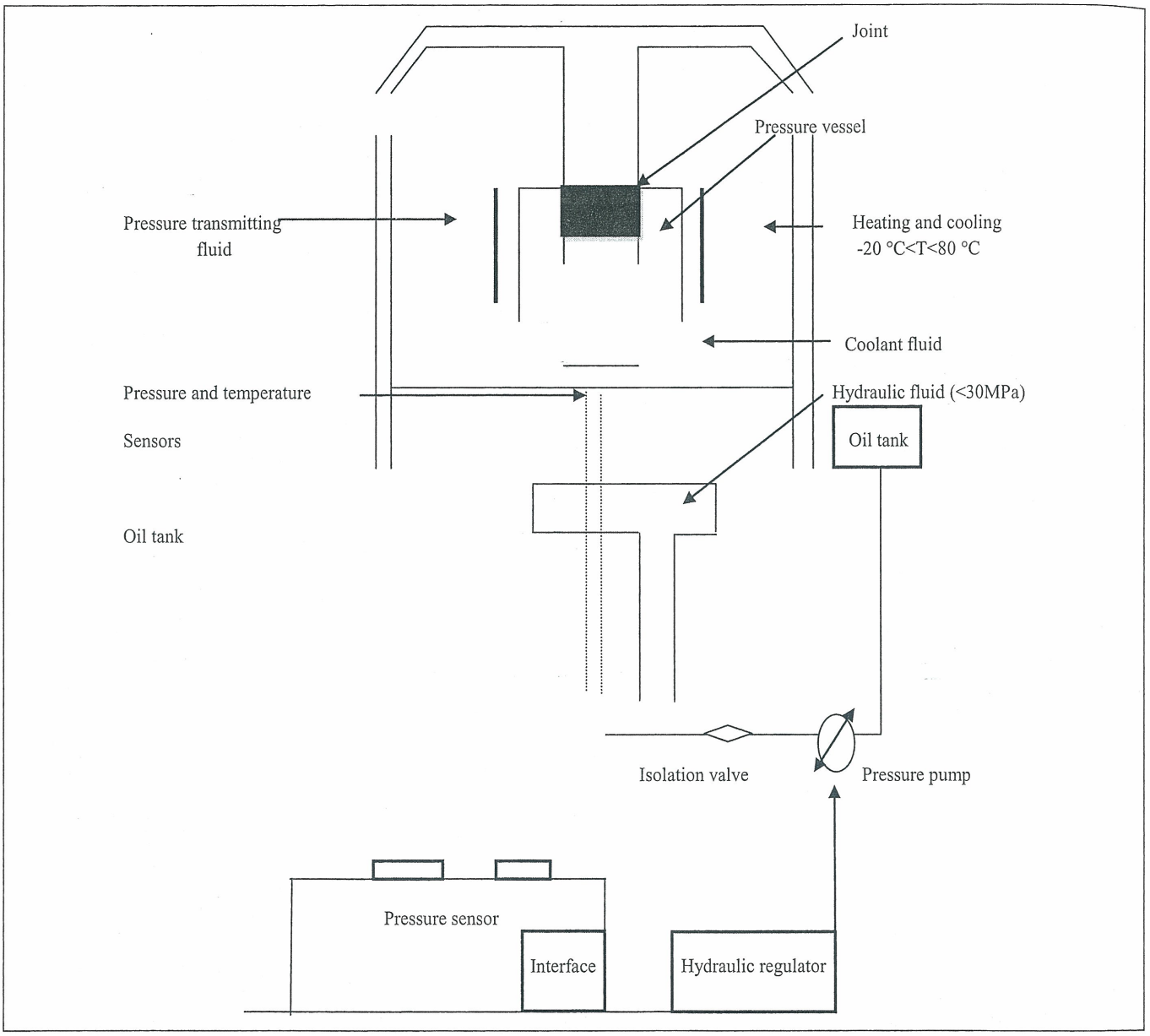


Figure 2–The diagram that shows the main parts of the pressure equipment.

Table 2–Effect of heat, bacteria, fat ratio, pressure, and time on microbial inhibition with Duncan multiple choice test results of the most and least effective applications.

pH	S*0.000	5.65	5.95	6.40
Increase in heat	0.080	NE	NE	NE
Increase in pressure	0.001	E	E	E
Bacteria (Most inhibited)	0.000	*E. coli*	*E. coli*	*E. coli*
Fat increase	0.000	E	E	E
Time extension	0.719	NE	NE	NE
Application most effective		5m43C308MPa (Fat 9.11%) on *E. coli*	15m43C308MPa (Fat 9.11%) on *E. coli*	15m43C308MPa (Fat 9.11%) on *E. coli*
Application least effective		5m43C220MPa (Fat 35.00%) on *L. monocytogenes*	15m43C220MPa (Fat 35.00%) on *L. monocytogenes*	15m21C220MPa (Fat 35.00%) on *L. monocytogenes*

NE, noneffective; E, effective.
*Statistical Significance (which indicates to a significant importance if it is less than 0.05).

The enrichment step of the treatments and their controls before the main microbial enumeration

After pressurization, all treatments (treated cryovials) and their controls (their matches with the same additional fat ratio, acidity, and microorganism but nontreated cryovials) were enriched at 30 °C for 24 h by using TSB (Triptic soy broth) to recover *E. coli*, prior to *E. coli* enumerations; and Fraser broth to recover *L. monocytogenes*, prior to *L. monocytogenes* enumerations, (Fraser and Sperber 1988; Clavero and Beuchat 1996). Briefly, in order to be able to see the real antimicrobial effect of the pressurization not only on viable cells but also on the injured ones, each of 72 treated pate meat cryovials which contained the pate meat which was previously contaminated with *L. monocytogenes* ATCC 7644 and their 72 controls and similarly, 72 treated pate meat cryovials which contained the pate meat which was previously contaminated with *E. coli* ATCC 11229 and their 72 controls, were all

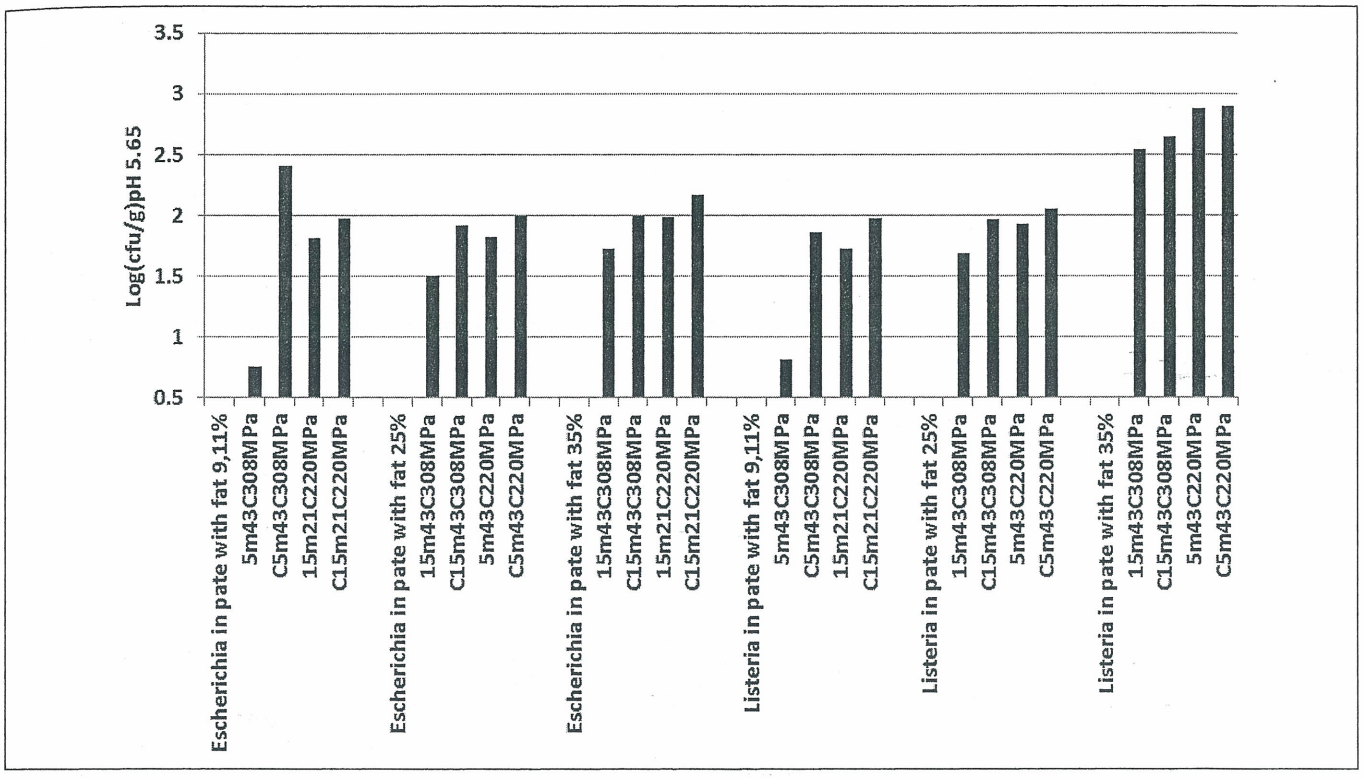


Figure 3–The log values of *E. coli* and *L. monocytogenes* counts (cfu/g) belonging to various heats, times, fat ratios, and pressure combined applications in beef pate with pH 5.65.

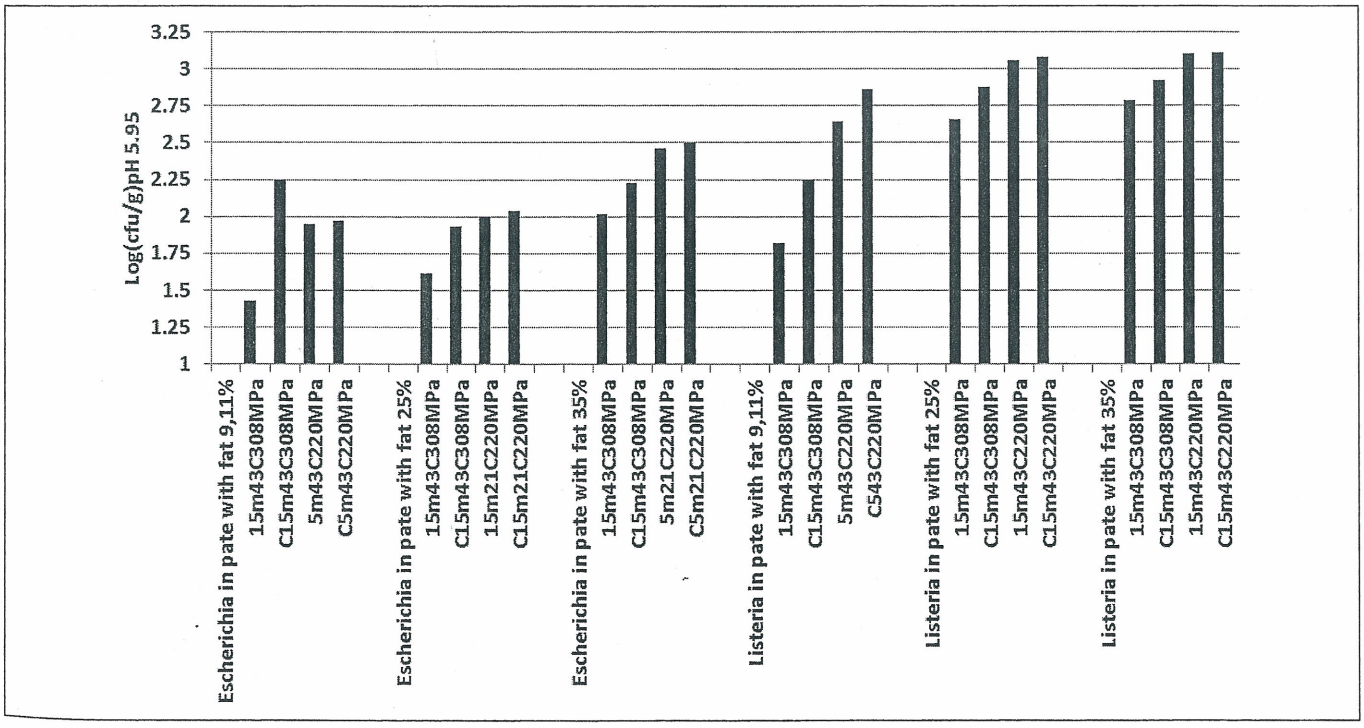


Figure 4–The log values of *E. coli* and *L. monocytogenes* counts (cfu/g) belonging to various heats, times, fat ratios, and pressure combined applications in beef pate with pH 5.95.

enriched before microbial enumerations. After the enrichments, the treatments and their controls were taken to the main microbial enumeration. Previous studies about pressure and its antimicrobial effect had different approaches as related to the enrichment and injured cell counting. Some of them only determined the amount of injured cells by taking the difference between the counts of only the living cells followed by the elimination of the injured ones by the selective agent like NaCl in selective agar and the counts of all cells including both injured and living ones by the nonselective agar namely Tryptose Soy Agar or Sorbitol MacConkey Agar (Bozoglu and others 2004; Somolinos and others 2008). Some studies did not make any enrichment and instead, preferred to count directly only the viable cells without any enrichment (Kumar and others 2009; Malinowska-Panczyk and Kolodziejska

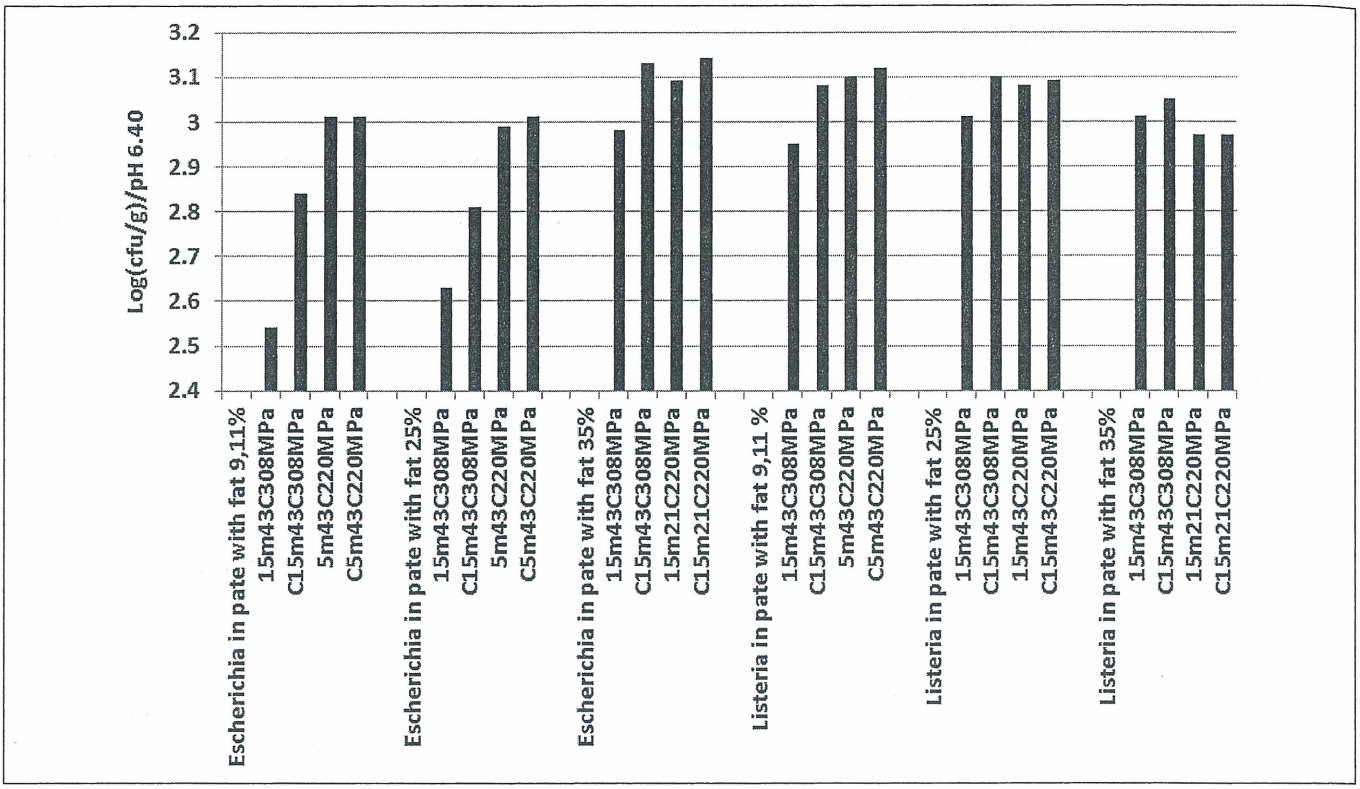


Figure 5–The log values of *E. coli* and *L. monocytogenes* counts (cfu/g) belonging to various heats, times, fat ratios, and pressure combined applications in beef pate with pH 6.40.

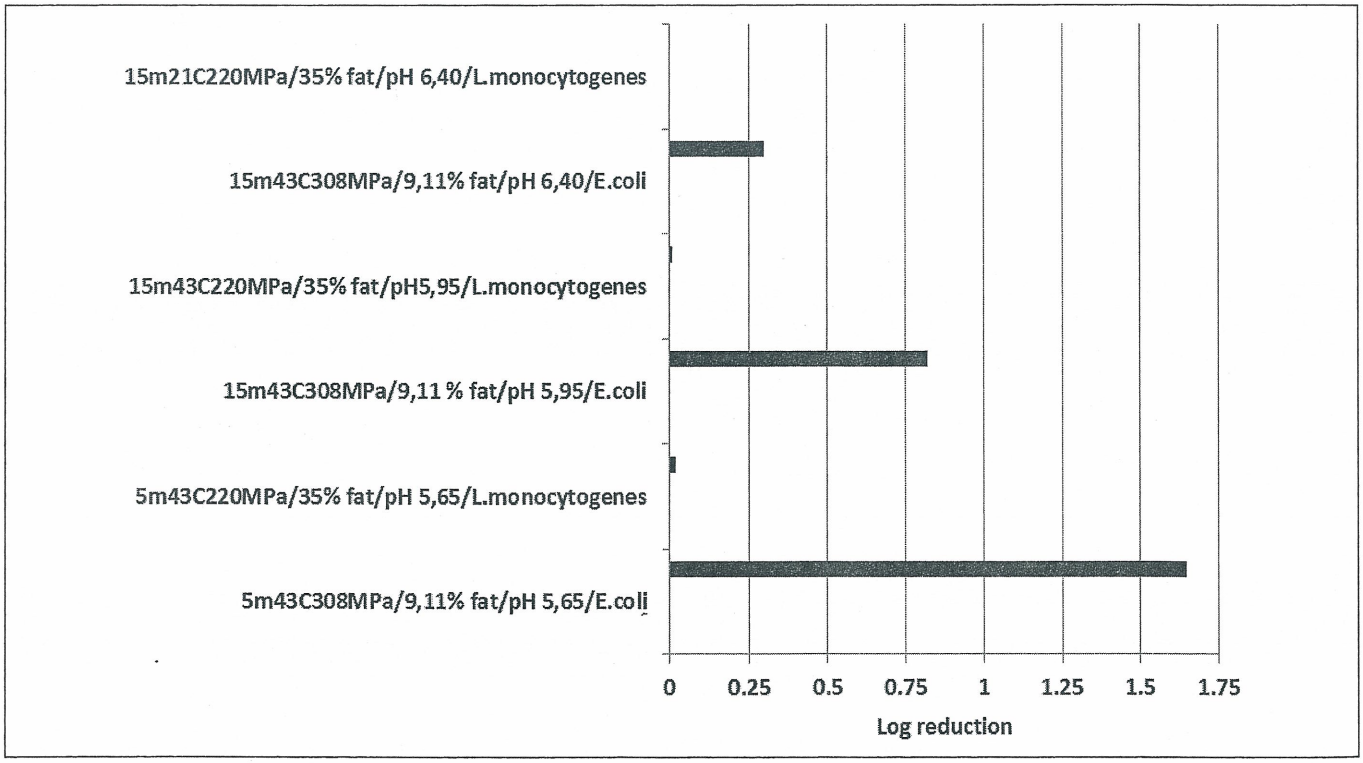


Figure 6–The maximum and minimum bacteria count Log reductions* belong to the most and least effective combined pressure applications. (*the differences between bacteria counts belong to application and its control).

2009; Han and others 2010) by disregarding the possibility of the injured cell existence due to pressure. Finally, some studies like this study, made enrichment before the main enumeration to give a chance to the injured cells to recover their wounds caused by the pressure (Oblinger and Kraft 1973; Wuytack and others 2002; Lee and others 2012). In this study, as indicated, the enrichment process was intentionally made for all pressure treatments and their controls to be able to include the wounded bacteria (by the means of TSB to recover *E. coli* and Fraser broth to recover *L. monocytogenes*) into the bacteria counting. Because, one of the most important effect of pressurization is not only the killing of the bacteria, but also the wounding. The wounded bacteria may be active in the pressurized food during other process and marketing steps until consumption. Such possibility requires the application of an enrichment step before the main microbial enumeration to be able to detect the wounded bacteria and include them to the total microbial counting. Such bacteria can heal themselves and form colony by the means of the enrichment so that they can be detected after the enrichment. For these reasons, all microbial enumerations were made after the aforementioned enrichments. On the other hand, in addition to the direct effect of pressure, also the survival of the normal and wounded bacteria in the initial cooking, the contamination of the food after the cooking process with such bacteria, the recovery and multiplication of the heat-damaged and wounded bacteria especially *L. monocytogenes* during the cold storage are the other initial factors which increase the risk for the high number of the bacteria including the wounded ones in cooked-chilled food products such as pate (Lacey 1992). For these reasons, this study preferred to follow up an approach which includes the load of wounded bacteria into microbial counting with an additional enrichment step before the main microbial enumeration by considering the hazard potential of the wounded bacteria after the pressure treatment and during the marketing, cooking and consumption periods. After the aforementioned enrichments, only the number of living bacteria (including the ones which were injured previously but healed by the enrichment) was counted.

Table 3–The variables and the interaction effects among them which were determined by Mixed Linear Model (using SPSS 17.0 version).

Main effects and two-way interactions	Significance
pH level * fat	0.000
pH level * min	0.993
pH level * temperature	0.471
pH level * pressure	0.574
Fat * min	0.716
Fat * temperature	0.862
Fat * pressure	0.218
Min * temperature	0.763
Min * pressure	0.833
Temperature * pressure	0.067
pH level	0.000
Fat	0.000
Min	0.719
Temperature	0.080
Pressure	0.001

Bacterial enumerations

Note that 1.00 mL of sample, for the microbial enumeration, which was taken from each of 72 enrichment broths that are belong to the 72 treated pate meat cryovials which contained the pate meat as contaminated with *L. monocytogenes* ATCC 7644 and treated with a different combined pressure treatment and from each of 72 enrichment broths that belong to their 72 controls was put into 9.00 mL, 0.10% peptone water. Similarly, 1.00 mL of sample which was taken from each of 72 enrichment broths that belong to the 72 treated pate meat cryovials which contained the pate meat as previously contaminated with *E. coli* ATCC 11229 and treated with a different combined pressure treatment and from each of 72 enrichment broths that belong to their 72 controls was put into 9.00 mL, 0.10% peptone water. Serial dilutions were prepared from the homogenized samples in peptone water (0.10%) for microbial analyses. Enumeration of both *L. monocytogenes* and *E. coli* was made by using direct plating method (pour plating).

Violet Red Bile (VRB) Agar (1.01406.0500) was used as the solid media for the enumeration of *E. coli*. The agar was dissolved in distilled water and the pH level was adjusted to 7.40. The agar was heated in water bath but it was not autoclaved.

Followed by the sterilization of PALCAM Agar in the media preparation for *Listeria* counting, 1 vial PALCAM *Listeria* selective supplement (Merck 1.12122.0001) was dissolved in 1 mL distilled water and this distilled water was added into the PALCAM Agar (Merck 1.11755.0500). Inoculated agar plates were finally incubated at 37 °C for 48 h and the number of colonies was enumerated.

The bacterial enumeration of each treatment or its control was performed in 4 replicates and each replicate was performed in duplicates. Then, the average of 8 duplicates was taken into the statistical analysis. Each average, which belongs to the treatment or its control shows the microbial load of the treatment or its control. The research with its 144 different treatments and 144 controls of these treatments was performed once.

Linear Mixed Model was employed to determine which main effects and interaction effects of different temperature (21, 43 °C), time (5, 15 min), pH (5.65, 5.95, or 6.40), pressure (220, 330 MPa) and fat ratio (9.11, 25.00, or 35.00%) are important, using SPSS 17.0 version (Howard and Steven 2000). Two ways of interactions were examined since the other type of interactions larger than 2 ways are useless.

Results and Discussion

The overall evaluation of the findings

Increasing the temperature from 21 to 43 °C was not found significantly important on the both pressure levels and both contamination flora of pate batches at any pH levels ($P < 0.05$) (Table 2). Increase in additional fat ratio reduced the antibacterial effect of pressurization in pate meat at all pH levels ($P < 0.01$) (Figure 3 to 6 and Table 2). Increasing the pressure enhanced the

Table 4–Means and variation of measurement values.

	pH			Fat (%)			Time (min)		Temperature (°C)		Pressure (MPa)	
	5.65	5.95	6.40	9.11	25.00	35.00	5	15	21	43	220	308
Mean	1.89	2.34	2.97	2.17	2.39	2.65	2.39	2.42	2.45	2.36	2.51	2.30
Variance	0.25	0.25	0.02	0.44	0.32	0.23	0.38	0.36	0.32	0.41	0.32	0.40

antimicrobial effect at all pH levels ($P < 0.01$) (Figure 3 to 6 and Table 2). Confirming this, Patazca and others (2006) and Reineke and others (2011) observed the antimicrobial enhancing effect of the increase in pressure on the spores of *Geobacillus stearothermophilus* ATCC10149 and *Bacillus subtilis*. Prolonging time from 5 to 15 min did not make any changes at any pH levels (Table 2). Pressurization at 308 MPa at 43 °C for 5 min (5m43C308MPa) of pate batches with 9.11% fat at pH 5.65 with 1.65 log reduction was the most inhibitory treatment combination on *E. coli,* while the same application made 1.05 Log reduction on *L. monocytogenes*. (Figure 3 and 6; Table 2). Pressurization for 15 min of the batches at 308 MPa and 43 °C with 9.11% fat content (15m43C308MPa) with 0.82 Log reduction was the application which increased the antimicrobial effect of pressurization the most at pH 5. 95 on *E. coli*. It was also the most inhibitory application on *L. monocytogenes* at this pH level with 0.43 Log reduction. (Figure 4 and 6; Table 2). Moreover, the highest microbial inhibition at pH 6.40 occurred in the same application on *E. coli* with 0.30 Log reduction; the same application was also the most effective one to inhibit *L. monocytogenes* with 0.13 Log reduction (Figure 5 and 6; Table 2) at the same pH level. As a result, these findings indicated that the pH increase in pate meat at all additional fat ratios reduced the antimicrobial effect of pressure ($P<0.01$) (Figure 3 to 6 and Table 2).

The Log reductions on the microbial flora in this study are lower than those in some other studies (Amanatidou and others 2000; Castillo and others 2004; Usajewicz and Nalepa 2006; Jackowska and others 2008; Jofre and others 2008). The higher reductions (3 to 6 Log) in these studies may be due to the higher pressure levels (400 to 600 MPa), vacuum packaging, controlled atmosphere, nisin; however, none of them were applied in this study except for the pressurization. Another study which was carried out by Ariefdjohan and others (2004) obtained 2.00 Log reductions in *E. coli* and 1.10 Log reductions in *L. monocytogenes* counts in Alfalfa seeds by 575 to 475 MPa, which are very close to the findings of this study.

As mentioned above, this study showed that pH increase in pate meat at all additional fat ratios decreased the antimicrobial effect of pressure ($P < 0.01$) (Figure 3 to 6 and Table 2). The reason for the increase in the antibacterial effect of pressurization due to the increase in acidity may be the synergistic effect of higher acidity and pressurization. The optimum pH level for both microorganisms falls between 6.50 and 7.50 (Coffey and others 1996; Presser and others 1997). Among 3 batches of beef pate, the batch nearest to optimum pH for the growth of both microorganisms is the batch group with pH 6.40 while others (with pH 5.65 and 5.95) are more acidic. The lower acidity (pH 6.40) of pate meat may have contributed to forming a much more suitable media for microbial growth and the higher microbial loads at such lower acidities may have clouded and lessened the antimicrobial effect of pressure. The findings of Stewart and others (2000) confirm these findings in that they observed only a 0.50 log reduction on *Clostridium sporogenes* spores by pressurization (404 MPa) at pH 7.00 while the figure was 2.50 Log at pH 4.00.

When a protein molecule is denatured by high pressure, it undergoes an increase in hydration due to the electroconstriction of water molecules. Once the pressure or any other denaturant is removed and when the amount of free water is too low, like in the emulsified food products, such as pate, the protein may not have enough support from water molecules to retain its denatured confirmation and may return to its native state (Mozhaev and others 1994), which may be the main reason for the higher bacteria numbers because of the increase in fat ratio. In this study, similarly, the increase in additional fat ratio might have significantly decreased the free water content, so the protein structures in microorganism might not have found enough support from free water molecules to retain its denatured confirmation and might have returned to its native state which may be the reason for the higher microbial numbers in the pressured pate samples with higher fat ratios. Grasso and others (2010) applied 600 MPa for 5 min at 45 °C to inhibit *Salmonella typhimurium* ATCC 53647 contamination flora in peanut flour/water and peanut flour/oil mixtures. Confirming the reducing effect of fat on the antimicrobial effect of pressurization in this study, they could obtain only 1.7 to 1.0 Log reductions in pressurized peanut flour/oil mixtures whereas pressurized peanut flour/water mixtures had a 6.7 Log reduction. The authors confirm the same effect of oil that protects microorganisms from the antimicrobial effect of pressure. He and others (2006) applied pressurization to oyster (Crassostrea gigas) at 207 to 310 MPa for 0.1 and 2 min Their findings as related to the antimicrobial inhibitory potential of pressurization on aerob and anaerob loads of oyster indicated to a 2 to 3 Log reductions which are also close to the findings in this study. Finally, the reason for the stronger antimicrobial effect of pressurization on *E. coli* may be the structural differences in Gram-negative cell wall, which was also mentioned, in previous studies (Paul and others 1997; Ponce and others 1998; Wuytack and others 2002).

The statistical evaluation of the data

Two types of bacteria were examined based on 5 explanatory variables, namely, pH, fat, pressure, time, and temperature. Those variables consisted of factorial levels; for example, pH had 3 levels (5.65, 5.95, or 6.40). In order to examine the main variables and the interaction effects among those variables, Mixed Linear Model was employed by using SPSS 17.0 version. As a result, the important main factors that accounted for the difference between the 2 types of bacteria were pH level, fat, and pressure. Moreover, the interaction effects were examined by the model. Therefore, "pH level * fat" interaction was found statistically significant. The other two-way interactions, which were insignificant, are denoted in Table 3. Other interactions, such as three-ways and four-ways, were also examined. However, those interactions were not found significantly important since their significance values were greater than 0.05. The significance level, which is called α in general, was chosen as 0.05 throughout the analysis. The means and variation of measurement values are presented in Table 4.

Briefly, the important main factors affecting the antimicrobial potential of pressurization on both bacteria are: "pH level," "additional fat ratio," and "pressure level." The decrease in pH level and additional fat ratio enhanced the antimicrobial effect of pressurization, whereas the increase in pressure enhanced this effect ($P < 0.01$). Among all interactions of pH level, time, pressure, and additional fat ratio, only "pH level and additional fat ratio" interaction was found statistically significant ($P < 0.01$).

Conclusions

The comparative antimicrobial effects of the different pressure treatments on pate meat may be summarized as follows: (1) Heat increase from 21 to 43 °C did not enhance the antimicrobial effect of pressurization at any of the pH levels. (2) Increasing pressure from 220 to 308 MPa significantly enhanced the antimicrobial effect of pressurization, especially on *E. coli* rather than *L. monocytogenes*, at all pH levels. (3) The increase in additional fat ratio from 9.11% to 25.00% or 35.00% (of the total meat weight) directly reduced the antimicrobial effect of all different pressure applications

on both microorganisms. (4) Prolonging the pressurization time of the pate from 5 to 15 min did not make any changes in the antimicrobial potential of the pressurization. (5) Although heat increase in pate batches with higher acidities (pH 5.65 or 5.94) and prolonging time from 5 to 15 min did not have any influence on antimicrobial potential of pressure, the combination of higher acidity (5.65), and higher heat level (43 °C) was able to make strong inhibitions on both contamination flora of the pate with the lowest fat ratio 9.11%. Confirming this, the application with the greatest antimicrobial effect was the pressurization of the pate meat containing 9.11% fat at pH 5.65 at 308 MPa at 43 °C for 5 min on *E. coli* and *L. monocytogenes* with 1.65 and 1.05 Log reductions, respectively.

The findings of this study may be of great importance and use for the possible future applications of pressurization in pate meat technology and meat hygiene as they indicate the necessity for the administration of a minimum 308 MPa or higher pressure levels combined with higher acidities (pH $\leq$ 5.65) and lower fat ratios% ($\leq$9.11) for a better microbial inactivation in pate meat products.

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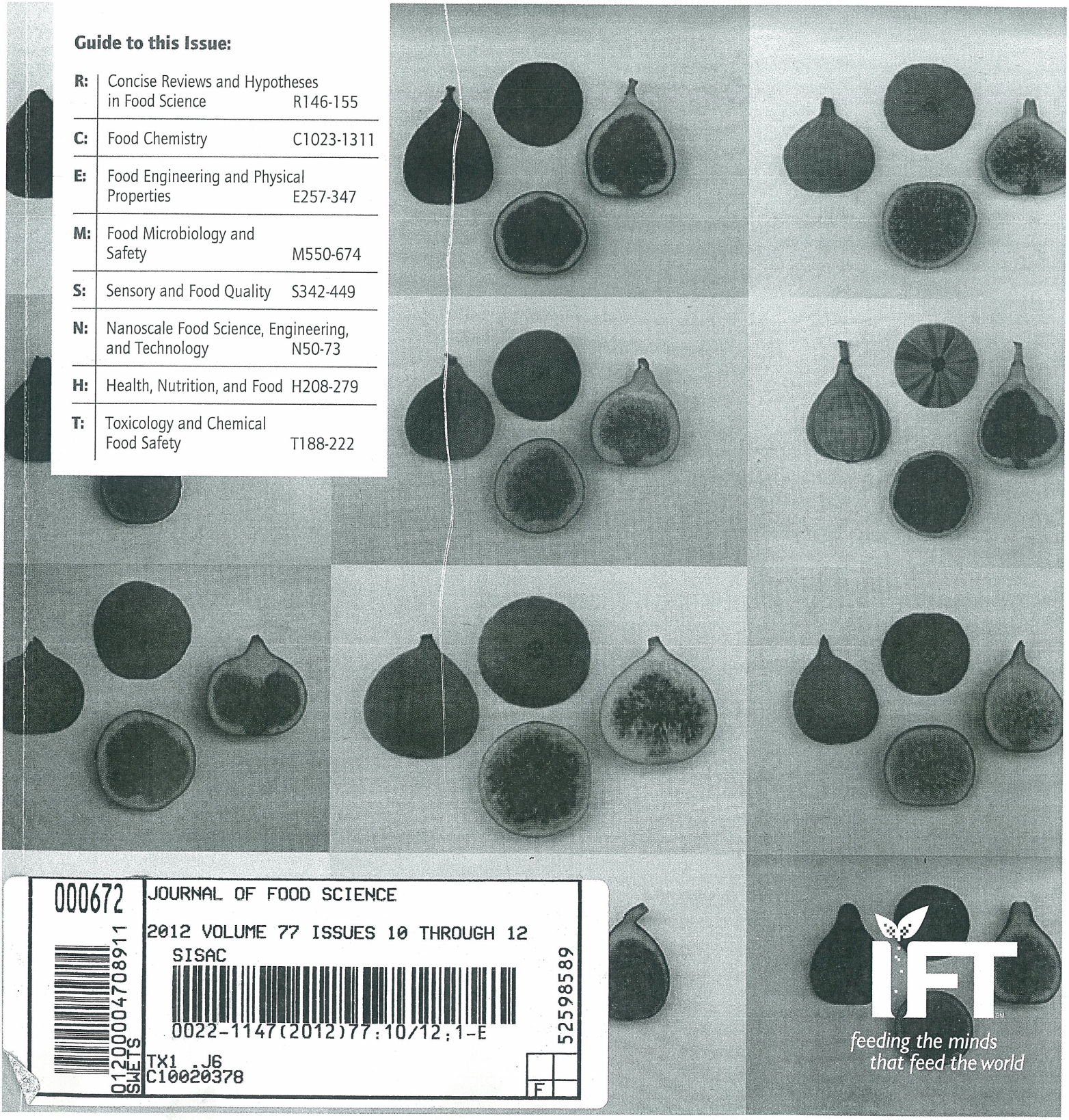
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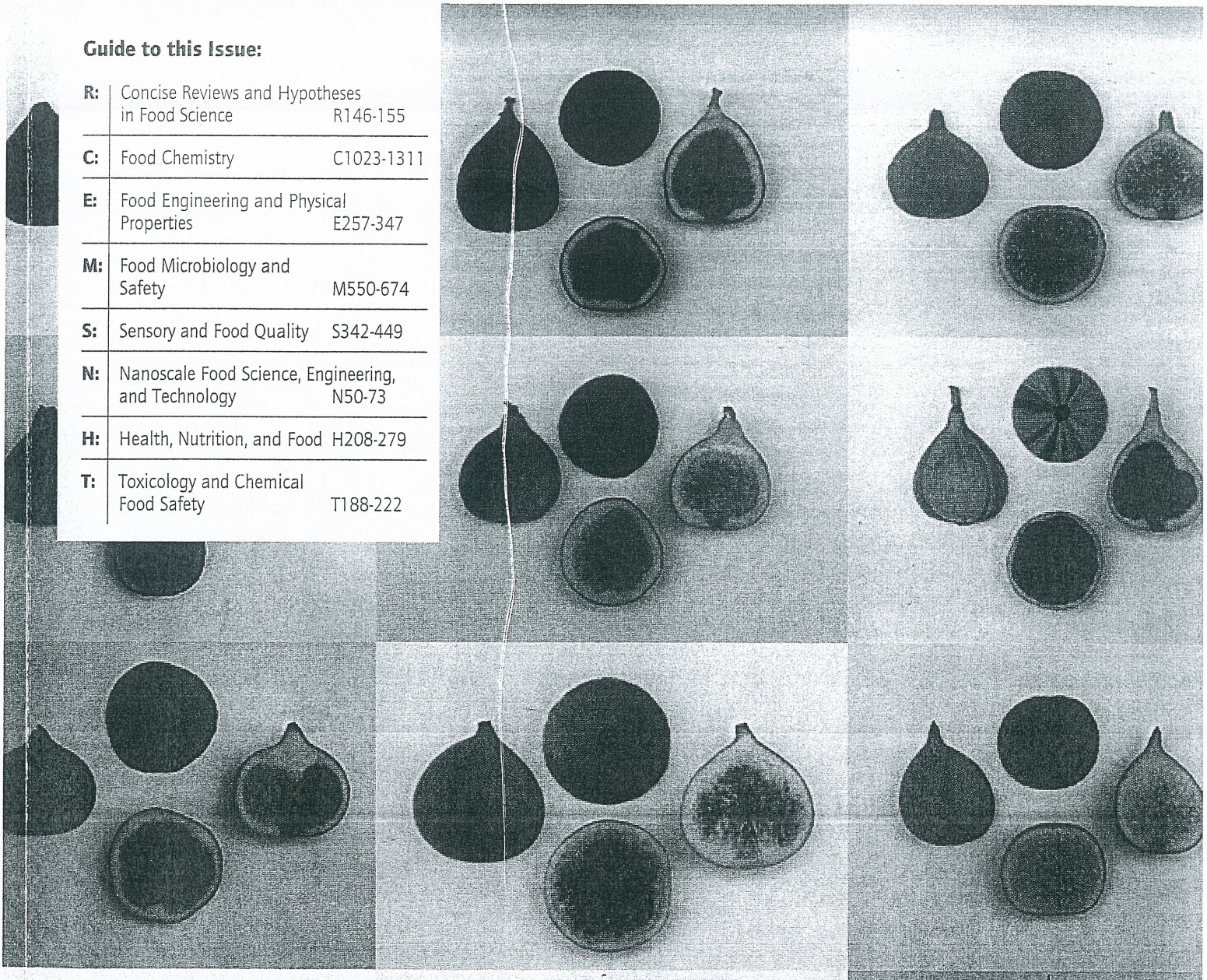
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