



Effects of high hydrostatic pressure on the quality and safety of beef after the addition of conjugated linoleic acid



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ABSTRACT

We evaluated the effect of high pressure (HP) for infusing of conjugated linoleic acid (CLA) on the quality and storage stability of beef loin. To beef loins in a bag before sealing, CLA alone (1%; CLA), CLA + 0.15% lecithin (CL), or CL + 0.001% α -tocopherol (CLT) were poured into the bag, vacuum-packed, and HP-treated with 0.1, 300, 450, and 600 MPa for 5 min. CLA level, cooking loss, and pH were significantly increased, when pressure increased from 0.1 to 600 MPa. Increases in pressure levels and storage periods increased TBARS about 1.04 and 3.85 fold values. Total aerobic bacteria were not detected in HP treated samples (450 and 600 MPa). CL or CLT with HP (300MPa) caused higher overall acceptance and willingness to buy. Hence, the addition of CLA with HP (300MPa) can improve the nutritional and microbiological quality of beef loin with acceptable sensory quality.

Industrial relevance: High pressure (HP) processing is a safe and consumer-friendly preservation technology that can eliminate pathogenic and spoilage microorganisms and extends product shelf-life without detrimental effects of thermal processing or use of preservatives or additives. However, meat with lower intramuscular fat content can be rejected by some consumers due to the lack of suitable sensorial qualities. On the other hand, meat with higher intramuscular fat content, beef in particular, may also cause consumers' concern because of the elevated level of saturated fatty acids. This particular study demonstrated that HP in combination with the infusion of CLA can be used for the enhancement of nutritional and microbiological quality of beef. Based on the results, it can be suggested that not only the infusion of CLA but also other oils originated from plants in combination with HP treatment can modify fatty acid composition.

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1. Introduction

Recent trends in meat consumption patterns have shown that commensurate with higher national incomes and improved living standards, more emphasis has been placed on the quality and health aspects of meat products. Recently, research into the fortification of meat and meat products with various biological supplements has been conducted (Kearney, 2010).

There has been an increased interest in food containing higher levels of polyunsaturated fatty acids (PUFAs) as they are considered to be functional ingredients in the prevention of coronary heart disease and other chronic diseases (Russo, 2009). Dietary conjugated linoleic acid (CLA) is of particular interest due to its beneficial effects on human health (Poulson, Dhiman, Ure, Cornforth, & Olson, 2004; Schmid, Collomb, Sieber, & Bee, 2006). CLA has been recognized as having

anticarcinogenic and antioxidative properties in several animal models (Joo, Lee, Ha, & Park, 2002). In addition, Hur et al. (2004) reported that the CLA concentration was significantly increased by the addition of a substituted CLA source of fat. Substituted CLA fat sources improved the color stability, possibly by the inhibition of lipid and myoglobin oxidation (Fernández-Ginés, Fernández-López, Sayas-Barberá, & Perez-Alvarez, 2005).

Schmid et al. (2006) showed that the CLA content of pork, chicken, and horse meat is usually lower than 1 mg/g lipid. The highest CLA concentrations were found in beef (1.2 to 10.0 mg/g lipid) and lamb (4.3 to 19.0 mg/g lipid). Given that the daily ingestion of 3 g of CLA is effective for reducing body fat (Blankson et al., 2000), the CLA content in beef is considered insufficient to affect various physiological functions in the human body.

Lecithin is used in a wide variety of products including processed food, cosmetics, and pharmaceuticals. Commercial sources are predominantly vegetable oil seeds such as soybeans and sunflower seeds; however, for pharmaceutical and some dietary applications, egg yolk is also an important source of lecithin (Yamamoto & Araki, 1997). Lecithin is a source of omega-3 and essential fatty acids, which are low

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in most peoples' diets. The particular function of lecithin acts as an emulsifying agent within the digestive system, and it is added to food products as an emulsifier and stabilizer (Martín-Hernández, Bénet, & Marbin-Guy, 2005). Therefore, lecithin is expected to help CLA penetration, which can be inhibited by hydrophilic groups in meat such as sarcoplasmic protein or moisture.

High pressure (HP) processing is an attractive preservation technology that eliminates pathogenic and spoilage microorganisms and extends the shelf life of foods but has minimal effect on nutritional quality (Rubio, Martínez, García-Gachan, Rovira, & Jaime, 2007). It therefore has a good potential for application in the meat industry (Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004; Kruk et al., 2011). HP is accepted as safe and consumer-friendly due to its capacity to eradicate microorganisms, regardless of the geometry of the product, without the formation of heat damage modification and the use of preservatives or additives (Rastogi, Raghavaro, Balasubramaniam, Niranjani, & Knorr, 2007; Zhang & Mittal, 2008). Sorenson et al. (2011) reported that HP could improve the eating quality of a chilled ready meal manufactured using a low-value beef cut, such as beef brisket. Recently, Jung et al. (2012) and Kruk et al. (2014) reported that HP enabled the penetration of vegetable oils into beef loin changing the fat content, fatty acid composition, and sensory quality. However, HP processing may accelerate lipid oxidation and change the color of the meat (Kruk et al., 2011).

Therefore, the objective of this study was to investigate the effect of HP treatment after adding CLA to low-grade beef, in order to develop manufacturing methods of higher valued meat products with increased safety and enhanced functionality. We aimed to increase the effect of CLA penetration into the meat by using lecithin, and α -tocopherol (TP) was added to inhibit accelerated oxidation arising from the implementation of lecithin and HP.

2. Materials and methods

2.1. Sample preparation

Beef loin (Korean beef quality grade 2) was purchased from a local market in Daejeon, South Korea, CLA (79% purity) was obtained from Lipozen Co., Hwaseong, Korea, and lecithin (LC grade) was derived from Goshenbiotech Co., Namyangju, Korea. TP was purchased from Futurebiotics Co. (Torrance, USA). Vacuum-packed beef samples (-650 mm Hg in 10×10 cm low-density polyethylene/nylon vacuum bags with oxygen permeability of 22.5 mL/m²/24 h atm at 60% RH/25 °C and water vapor permeability of 4.7 g/m²/24 h at 100% RH/25 °C) were transported to the Korea Food Research Institute (Seongnam, Korea) in a cooled container. The vacuum pack was opened. For 20 g of beef loins in a bag before sealing, CLA alone (1%; CLA), CLA + 0.15% lecithin (CL), and CL + 0.001% TP (CLT) were poured into the bag, vacuum-packed again, and HP-treated with 0.1, 300, 450, and 600 MPa for 5 min. The control sample with no additive was also opened, vacuum-packed again, and treated by HP.

2.2. High pressure treatment

The vacuum-packed samples were immediately subjected to HP. Samples were placed in a pressure vessel, which was 9 cm in diameter and 32 cm in height with inner cylinder for loading sample (8 cm in diameter and 19 cm in height), submerged in hydrostatic fluid (Quintus food processor 6; ABB Autoclave Systems, Inc., Columbus, OH, USA) and pressurized at 300, 450 and 600 MPa for 5 min with the initial temperature of the pressure vessel set at 15 ± 3 °C. The hydrostatic fluid was a mixture of deionized water and water glycol type fire-resistant hydraulic fluid (Houghto-safe 620-TY, Houghton International Inc., Valley Forge, PA, USA). The rate of pressurization was 5–7 MPa/s and the pressure in the chamber was released within 10 s. Control samples were maintained under atmospheric pressure at 4 °C while the other

samples were treated. Immediately after treatment, all samples were transported to the laboratory in a cooled container. Microbiological and chemical analyses were conducted immediately and the samples for sensory evaluation were stored at 4 °C for 3 days.

2.3. Surface color

The surface color of the beef loins was measured using a spectrophotometer with Spectra Magic Software (CM-3500d, Minolta, Tokyo, Japan) after 30 min of opening the package. The color of each sample (4 cm in diameter and 1.5 cm in thickness) was measured at three different locations using a large size aperture (30 mm, 112 diameter). The average value from the three measurements was considered as an observation number for a replication and expressed as L^* (lightness), a^* (redness), and b^* (yellowness) on the Hunter color scale.

2.4. pH, cooking loss, fat and CLA content

pH measurements were carried out by adding 9 mL of distilled water to 1 g samples, homogenizing the mixture for 1 min at 1130 \times g using a homogenizer (T25 basic, Ika Co., Staufen, Germany) and results were recorded by a pH meter (750 P, Istek Co., Seoul, Korea). Cooking loss was obtained by submersing and heating samples in a hot water bath at 80 °C until the internal temperature reached 70 °C. The difference between the initial sample weight and the weight after cooking was considered as cooking loss. Crude fat content was measured according to the method of Soxhlet (AOAC, 1996).

The samples (1 g) were saponified with 1 N KOH in ethanol (20 mL) in a water bath (80 °C) for 1 h. After cooling, 10 mL of each sample was transferred to 50-mL tubes, vortexed with 6 N HCl (3 mL) and hexane (10 mL) for the extraction of fatty acid and then evaporated by N₂ gas (99.99%). After that, each mixture was methylated with 1 N H₂SO₄ in methanol (5 mL) in a water bath (50 °C) for 20 min. After cooling, 2 mL of 0.88% NaCl and hexane each was added to the same tubes, which were then centrifuged at 2090 \times g for 10 min. The top hexane layer containing FAME (fatty acid methyl esters) was transferred to another 15-mL tube, and dehydrated through anhydrous Na₂SO₄ in to a vial. CLA composition was then analyzed using a gas chromatograph (HP 7890, Agilent Technologies, Santa Clara, CA, USA). A split inlet (split ratio, 100:1) was used to inject the samples into a capillary column (30 m \times 0.32 mm \times 0.25 μ m; Omegawax 320, Supelco, Bellefonte, PA, USA), and ramped over temperature was used for the analysis [the initial temperature (60 °C) was increased to 190 °C at 30 °C/min and then increased to 200 °C at 1 °C/min and finally increased to 250 °C at 5 °C/min and maintained for 10 min]. The inlet and detector temperatures were 260 °C and 280 °C, respectively. N₂ gas was used as the carrier gas at a constant flow rate of 1.0 mL/min.

2.5. Lipid peroxidation (2-thiobarbituric acid reactive substances, TBARS)

Lipid peroxidation was measured by the method of Jung et al. (2012). Samples (3 g) were homogenized in 9 mL of extract solution (7.5% trichloroacetic acid, 0.1% EDTA, and 0.1% gallic acid) and then centrifuged at 2090 \times g, 15 min on a centrifuge (UNION 32R, Hanil Science Industrial Co., Ltd., Korea). The supernatant (5 mL) was transferred to a test tube with 5 mL TBA/TCA solution (20 mM thiobarbituric acid in 15% trichloroacetic acid) with 50 μ L 7.2% BHA. The mixture was heated in a water bath for 15 min at 90 °C. After cooling to 20 °C, the mixture was centrifuged (2050 \times g) for 15 min. The absorbance of the supernatant obtained after the centrifugation was determined by spectrophotometer (UV 1600 PC, Shimadzu, Tokyo, Japan) at 532 nm. Lipid peroxidation was expressed in mg malondialdehyde/kg meat.

2.6. Texture

Texture was measured by using an A-XT2 texture analyzer (Stable Microsystems, Surrey, UK) equipped with a 75-mm diameter probe. Samples were cut into pieces (diameter 3.0 cm, height 2.0 cm), and measured in triplicate. The measurement speed was set at 1.00 mm/s with the trigger force of 0.005 kg. The measured parameters included hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness.

2.7. Microbial analysis

The microbial testing of samples was carried out by the addition of sterile saline (9 mL) to the samples (1 g), mixing for 2 min using Bag mixer® (Model 400, Interscience, France), and diluting the mixture based on 10-fold serial dilution. Each diluent was inoculated to tryptic soy agar (Difco), and cultured for 48 h at 37 °C. The number of colonies produced was counted and expressed as colony-forming units per gram (CFU/g).

2.8. Sensory evaluation

Beef samples were cut into a similar size pieces (1-cm thickness, 5 g), cooked until the internal temperature reached 72 °C and served to the sensory panel. The semi-trained sensory panel consisted of ten panelists, who have had at least 1.5-years of experience in meat sensory analysis. Before the tasting session, panelists were familiarized with the assessment criteria and the attributes to be rated as well as had a test run on the control beef loin without additives and HP. The scoring of each sample was done on a single sheet using a 9-point hedonic scale. The sensory parameters scored were color (extremely light to extremely dark), aroma strength (very weak to very strong), texture (extremely gooey to extremely smooth), flavor (extremely unpleasant to extremely enjoyable), overall acceptance (disagreeable to enjoyable), and willingness to buy (definitely not to definitely yes) (Yun et al., 2012).

2.9. Statistical analysis

Experiment was conducted as three independent trials with four observations for treatment combinations per each trial. Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and when significant differences were detected, the differences among the mean values were identified by Student-Newman-Keul's multiple range test using SAS software with the confidence level at $P < 0.05$ (SAS, Release 8.01, SAS Institute Inc., Cary, NC). Mean values and standard error of the means are reported.

3. Results and discussion

3.1. Surface color

The surface color changes of the beef loin by HP treatment and the addition of CLA are shown in Table 1. The Hunter color L^* -value was increased, and the a^* -value was decreased by HP treatment. This trend was more pronounced when pressures of 450 and 600 MPa were applied. The addition of CLA, lecithin or TP did not significantly affect the Hunter a^* -value. The L^* -value was significantly higher in the groups treated with CLA at 0.1 and 300 MPa than in most other groups at the same pressure. However, no significant intergroup differences at 450 and 600 MPa were observed. A similar trend occurred with CL treatment; however, the differences within the 0.1 and 300 MPa groups were not statistically significant. As CLA and 600 MPa treatment significantly reduced the b^* value, there were no other significant differences between the HP groups for b^* values. The application of CL with at 450 MPa gave the largest reduction in the b^* value.

There have been several studies on the color stability of meat following CLA addition (Du, Ahn, Nam, & Sell, 2000; Hur et al., 2004). However, very few studies have examined the color stability mechanisms of meat treated by CLA, lecithin, and HP. The addition of CLA, lecithin and TP did not influence meat color, whereas HP treatment brightened the color which was observed by an increase in the L^* -value and a decrease in the a^* -value. These results agree with previous findings that HP treatment (100 to 500 MPa) on the *Semimembranosus* and the *Semitendinosus* brightened beef color with an increase in the L^* -value and a decrease in the a^* -value (Carlez, Veciana-Nogues, & Cheftel, 1995; Kim, Lee, Lee, Kim, & Yamamoto, 2007). The change in beef loin color is due to the alteration of myoglobin caused by heme transfer or separation from globin; iron separation from the porphyrin ring and oxymyoglobin oxidation and conversion into metmyoglobin (Carlez et al., 1995; Fuentes, Ventanas, Morcuende, Estevez, & Ventanas, 2010; Jung, Jung et al., 2012); water content changes due to drip loss (Jung, Ghoul, & de Lamballerie-Anton, 2003); or pressure-induced coagulation of sarcoplasmic and myofibrillar protein (Fernández et al., 2007). Jung, Kang et al. (2012a) reported that when chicken meat was treated by HP at 300 and 600 MPa, L^* -values were increased by 10 and 28% respectively, compared to a non-HP control.

The lack of a significant effect of combined HP treatment on b^* values shows that this color parameter is not always affected by the HP treatment (Kruk et al., 2011).

3.2. pH, cooking loss, fat, and CLA content

HP treatment significantly elevated pH values in all pressure groups (Table 2). However, the addition of CLA, lecithin and TP tended to reduce pH, particularly, the addition of lecithin, which significantly reduced pH in all pressure groups.

A similar trend occurred with cooking loss which increased with increasing pressure (Table 2). This effect was statistically significant when 450 and 600 MPa pressure was applied. However, no consistent effects within each pressure group were observed.

Table 1

Surface color of the beef supplemented with conjugated linoleic acid (CLA) and treated by high pressure.

Pressure (MPa)	Treatment ¹	Color value		
		L^*	a^*	b^*
0.1	Control	31.13 ^{bc}	17.31 ^A	15.89
	CLA	38.02 ^{ab}	15.89 ^A	18.16 ^A
	CL	34.14 ^{abB}	16.32 ^A	16.61
	CLT	36.53 ^{ab}	17.86 ^A	18.42
	SEM ²	1.079	1.524	0.973
300	Control	42.32 ^{bb}	14.81 ^B	17.48 ^{ab}
	CLA	47.41 ^{aA}	13.83 ^A	18.57 ^{aA}
	CL	42.47 ^{ba}	13.68 ^{AB}	16.74 ^b
	CLT	40.43 ^{bb}	15.91 ^A	17.29 ^{ab}
	SEM ²	1.198	0.801	0.396
450	Control	46.20 ^A	12.46 ^B	17.70 ^a
	CLA	49.97 ^A	10.47 ^B	17.60 ^{aAB}
	CL	48.13 ^A	10.14 ^B	16.63 ^b
	CLT	49.78 ^A	11.43 ^B	17.91 ^a
	SEM ²	1.200	0.674	0.238
600	Control	46.67 ^A	11.91 ^B	17.29
	CLA	49.01 ^A	9.36 ^B	16.63 ^B
	CL	47.66 ^A	10.03 ^B	16.62
	CLT	48.44 ^A	10.42 ^B	17.24
	SEM ²	0.953	0.579	0.241

^{a,b}Different letters within the same column with the same high pressure differ significantly ($P < 0.05$).

^{A-C}Different letters within the same column with the same treatment differ significantly ($P < 0.05$).

¹ CLA concentration was 1% (w/w). CL was prepared by addition of lecithin (0.15%, w/w) to CLA. CLT was prepared by addition of 0.001% α -tocopherol to the CL treatment.

² Standard error of mean ($n = 12$).

The pH results in this study agree with those reported in previous research. Angsupanich and Ledward (1998) showed that pH increased in cod treated with pressures of 100 to 800 MPa for 20 min at room temperature. Mcardle, Marcos, Kerry, and Mullen (2010) reported an increased pH in beef treated with pressure of 300 MPa for 20 min at higher temperatures (20 and 40 °C). It has been proposed that the mechanism behind pH change by HP might be related to protein denaturation. High pressure affects the rotation angles of single bonds in stable molecules, e.g., C–C, C–N and C–O, resulting in a loss of protons as a consequence of increased ionization by HP and consequent redistribution of ions, which increases the number of acid groups and consequently increases pH (Lerasle et al., 2014).

Cooking loss, an important component of the quality, taste, and yield of cooked meat, is related to water-holding capacity (WHC). The myofibril structure is a principal factor responsible for this effect. HP produces extreme contractions and changes in the myofibril, which then decreases the WHC (Jung, Ghoul, & de Lamballerie-Anton, 2000). Kim et al. (2007) performed HP treatments on beef *M. semitendinosus*, and reported that the cooking loss values began to increase at 100 MPa but WHC began to decrease significantly at 200 MPa. Kruk et al. (2011) reported that the cooking loss was increased significantly in chicken breasts treated at 450 MPa, which is consistent with the present study.

HP treatment did not affect the fat content. Kruk et al. (2011) reported that the fat content increased in chicken breasts treated with olive oil at 300 MPa. Jung, Jung et al. (2012) also showed increased fat content in beef treated by HP with vegetable oils (10% of the meat weight). However, in the present study, only 1% of CLA based on meat weight was used so this small amount may have been insufficient to influence the fat content.

The most common CLA isomer found in beef is cis-9 and trans-11 (Schmid et al., 2006). Although the fat content was not changed, the composition of CLA was significantly increased by the addition of CLA and was further increased by high pressure (Table 2) as we expected. The CLA content of the control sample ranged from 0.2 to 0.3 mg/g regardless of the treated pressure but increased to 5 mg/g by the addition of CLA without high-pressure treatment. When the treatment pressures were 300 and 600 MPa, the CLA content increased further to 7.79 and 8.46 mg/g, respectively. However, no significant differences

were found between 300 and 450 MPa of pressure. CL and CLT treatments did not show any indication on penetration efficiency.

3.3. Lipid peroxidation (2-thiobarbituric acid reactive substance [TBARS] value)

The increased pressure did not have any significant effect on TBARS values on days 0, 5, and 10, except 600 MPa, which significantly increased oxidation by approximately 2- and 3-fold on days 5 and 10, respectively (Table 3). CLA and CLT treatments reduced TBARS values on day 0 of storage; however, they increased oxidation on days 5 and 10 when pressures of 450 and 600 MPa were applied. Moreover, a significant increase in oxidation was also observed when the combination of CL and 600 MPa pressure was used.

Extended storage time was associated with higher TBARS values and pressure. Non-pressurized samples significantly increased TBARS values on day 10 regardless of the treatment, whereas HP-treated samples increased oxidation after 5 days of storage. This effect was especially pronounced at 450 and 600 MPa pressure where the oxidation levels differed significantly over 0, 5, and 10 days of storage. CL and CLT treatments caused more rapid oxidation than CLA treatment.

HP treatment of meat and meat products can trigger lipid oxidation. Similar results for the detrimental effects of pressure in terms of lipid oxidation were observed at over 400 MPa (Wiggers, Kroger-Ohlsen, & Skibsted, 2004), although HP at less than 300 MPa had no significant effect (Cheah & Ledward, 1996). With HP treatment, TBARS values increase with the acceleration of lipid oxidation, which is known to be caused by an increase in iron liberated by the deformation of the subcellular structure and the modification of heme-bearing protein. Bolumar, Skipsted, and Orlie (2012) described the kinetics of the formation of radical species under pressure in chicken breast. They found a 400-MPa threshold for the formation of radicals and proposed a possible link between the formation of radicals, an early event in lipid oxidation, and the induction of lipid oxidation.

Previous research has shown that the addition of ethylenediamine-tetraacetic acid, which can chelate metal ions, correlated with a reduction in lipid oxidation in meat processed by HP. This indicates that transition metal ion catalysis is the major cause underlying the

Table 2
pH, cooking loss, fat, and conjugated linoleic acid (CLA) content of the beef supplemented with CLA and treated by high pressure.

Pressure (MPa)	Treatment ¹	pH	Cooking loss (%)	Fat content (%)	c9, t11 C18:2n7 (mg/g)	t10, c12 C18:2n6 (mg/g)
0.1	Control	5.94 ^{bb}	41.25 ^{bc}	2.59	0.24 ^b	0.02 ^b
	CLA	5.90 ^{bc}	39.86 ^{cb}	2.44	2.57 ^{ay}	2.43 ^{ay}
	CL	5.83 ^{cc}	41.61 ^{bb}	2.20	1.41 ^{ay}	1.21 ^{ay}
	CLT	6.00 ^{ab}	42.52 ^a	2.71	1.83 ^a	1.77 ^a
	SEM ²	0.012	0.247	0.335	0.323	0.325
300	Control	6.16 ^{aA}	42.53 ^{bc}	3.37	0.18 ^c	0.02 ^c
	CLA	5.97 ^{bb}	41.66 ^B	2.67	3.97 ^{axy}	3.82 ^{axy}
	CL	5.98 ^{bb}	42.12 ^B	2.91	2.25 ^{bx}	0.19 ^{bx}
	CLT	5.93 ^{cc}	43.08	2.91	2.13 ^b	2.02 ^b
	SEM ²	0.007	0.531	1.321	0.199	0.193
450	Control	6.20 ^{aA}	43.68 ^{ab}	2.72	0.25 ^c	0.02 ^c
	CLA	5.99 ^{bb}	41.64 ^{bb}	3.03	3.73 ^{axy}	3.62 ^{axy}
	CL	5.96 ^{bb}	44.68 ^{aA}	2.58	2.71 ^{bx}	2.65 ^{abx}
	CLT	6.03 ^{ba}	44.06 ^a	2.58	2.25 ^b	2.19 ^b
	SEM ²	0.017	0.566	0.469	0.283	0.301
600	Control	6.16 ^{aA}	46.73 ^A	3.90	0.28 ^c	0.02 ^c
	CLA	6.14 ^{aA}	45.31 ^A	3.03	4.34 ^{ax}	4.12 ^{ax}
	CL	6.02 ^{ba}	45.64 ^A	2.80	2.53 ^{bx}	2.40 ^{bx}
	CLT	5.97 ^{bbc}	43.95	3.50	2.19 ^b	2.05 ^b
	SEM ²	0.023	0.974	1.480	0.240	0.247

^{a,b}Different letters within the same column with the same high pressure differ significantly ($P < 0.05$).

^{A-C}Different letters within the same column with the same treatment differ significantly ($P < 0.05$).

¹ CLA concentration was 1% (w/w). CL was prepared by addition of lecithin (0.15%, w/w) to CLA. CLT was prepared by addition of 0.001% α -tocopherol to the CL treatment.

² Standard error of mean ($n = 12$).

Table 3
2-Thiobarbituric acid reactive substance (TBARS) value (mg malondialdehyde/kg meat) of the beef supplemented with conjugated linoleic acid (CLA) and treated by high pressure.

Pressure (MPa)	Treatment ¹	Storage (day)			SEM ²
		0	5	10	
0.1	Control	0.22 ^{aby}	0.26 ^{yB}	0.33 ^{xB}	0.017
	CLA	0.17 ^{byAB}	0.27 ^{xyC}	0.34 ^{xC}	0.034
	CL	0.24 ^{ayAB}	0.25 ^{yB}	0.30 ^{xB}	0.010
	CLT	0.19 ^{aby}	0.24 ^{xyB}	0.35 ^{xB}	0.040
	SEM ³	0.015	0.015	0.043	
300	Control	0.24 ^a	0.24 ^B	0.30 ^{bB}	0.017
	CLA	0.14 ^{cyC}	0.26 ^{yC}	0.47 ^{axBC}	0.469
	CL	0.20 ^{bzAB}	0.26 ^{yB}	0.37 ^{abxB}	0.010
	CLT	0.19 ^{bz}	0.27 ^{yB}	0.37 ^{abxB}	0.015
	SEM ³	0.012	0.014	0.042	
450	Control	0.22 ^a	0.29 ^{bB}	0.39 ^{bB}	0.049
	CLA	0.17 ^{byBC}	0.49 ^{axB}	0.77 ^{axB}	0.090
	CL	0.19 ^{abzB}	0.38 ^{abyB}	0.52 ^{abxB}	0.021
	CLT	0.16 ^{bz}	0.46 ^{ayB}	0.76 ^{axB}	0.024
	SEM ³	0.011	0.047	0.079	
600	Control	0.21 ^{abz}	0.61 ^{byA}	1.09 ^{bxA}	0.075
	CLA	0.20 ^{bzA}	1.26 ^{ayA}	2.09 ^{axA}	0.089
	CL	0.27 ^{azA}	1.06 ^{ayA}	1.89 ^{axA}	0.169
	CLT	0.17 ^{bz}	1.09 ^{ayA}	1.95 ^{axA}	0.181
	SEM ³	0.020	0.122	0.202	

^{a,b}Different letters within the same column with the same high pressure differ significantly ($P < 0.05$).

^{x-z}Different letters within the same row with the same high pressure differ significantly ($P < 0.05$).

^{A-C}Different letters within the same column with the same treatment differ significantly ($P < 0.05$).

¹ CLA concentration was 1% (w/w). CL was prepared by addition of lecithin (0.15%, w/w) to CLA. CLT was prepared by addition of 0.001% α -tocopherol to the CL treatment.

² Standard error of mean ($n = 9$).

³ Standard error of mean ($n = 12$).

increased lipid oxidation (Cheah & Ledward, 1996; Ma, Ledward, Zamri, Frazier, & Zhou, 2007).

3.4. Texture

The texture profiles of beef loin were expressed by hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness (Table 4). The addition of CLA, lecithin and TP with pressures of 0.1

and 300 MPa did not affect the textural characteristics of beef loin. However, hardness was lower, except for CLT treatment. Gumminess was lower with CL with 450 MPa, and with CLA with 600 MPa treatments when compared with the non-additive control group. Park, Na, and Lee (2010) and Laack, Stevens, and Stalder (2001) reported that intramuscular fat was increased by HP treatment, which improved meat tenderness and lowered the hardness. According to the report of Kim et al. (2007), beef *Semitenosus* treated at 500 MPa showed a significant increase in shear force and hardness in comparison with cod, which was caused by the inactivation of Ca^{2+} and Mg^{2+} ATPase enzymes. However 300 MPa significantly decreased hardness. This result implies that the addition of CLA and lecithin may lower the hardness of beef loin treated by HP. However, these results are insufficient to identify consistent correlations between meat texture characteristics, pressure, and the additives.

3.5. Microbial analysis

The initial number of total aerobic bacteria in the control was 4.20 log CFU/g, which reduced to an undetectable level when treated at 450 MPa (Table 5). The microbial populations of the 0.1- and 300-MPa-treated samples significantly increased with an increased storage period at 4 °C. However, the microbial reduction was not consistent with the addition of CLA, lecithin and TP.

The efficacy of HP treatments for the inactivation of vegetative bacteria in meat has been reported previously. Kruk et al. (2011) and Jung, Jung et al. (2012) demonstrated that pressures 450 and 600 MPa almost completely eliminated the three major pathogens including *Salmonella Typhimurium*, *Escherichia coli*, and *Listeria monocytogenes* in chicken and beef. Microbial cellular membranes are affected by high pressure, resulting in osmotic changes, lysis, alterations of nuclear material, and other modifications, which can result in cell death (Mackey, Forestière, Isaacs, Stenning, & Brooker, 1994). Gola, Mutti, Manganelli, Squarcina, and Rovere (2000) reported that HP processing between 400 and 700 MPa increased the shelf-life of minced meat under refrigeration conditions. The application of combined hurdles together with HP has been proposed to increase the microbial effect of low pressure processes in order to minimize unwanted changes induced by HP in meat and meat products (Bajovic, Bolumar, & Heinz,

Table 4
Texture profile analysis of the cooked beef supplemented with conjugated linoleic acid (CLA) and treated by high pressure.

Pressure (MPa)	Treatment ¹	Hardness (kg)	Adhesiveness (g/s)	Springiness (mm)	Cohesiveness (%)	Gumminess (kg)	Chewiness (kg)
0.1	Control	45.99 ^A	-4.75 ^{AB}	0.66 ^{ab}	0.69 ^A	31.53 ^A	20.92 ^A
	CLA	44.12 ^{AB}	-12.39	0.62 ^{ab}	0.69	30.49 ^{AB}	18.73
	CL	39.48	-7.37	0.69 ^a	0.67	26.73	18.25
	CLT	47.37	-38.64	0.60 ^b	0.69	32.70	19.66
	SEM ²	4.254	14.595	0.024	0.012	3.194	2.027
300	Control	30.41 ^B	-1.01 ^A	0.67	0.62 ^{bB}	19.00 ^B	12.60 ^B
	CLA	41.71 ^{AB}	-17.43	0.59	0.67 ^{ab}	28.46 ^{AB}	16.73
	CL	43.54	-7.98	0.64	0.70 ^a	30.25	19.49
	CLT	37.33	-12.35	0.54	0.68 ^{ab}	25.71	15.02
	SEM ²	5.945	5.652	0.054	0.020	4.695	3.269
450	Control	38.53 ^{abAB}	-8.99 ^B	0.59	0.65 ^B	25.29 ^{abAB}	14.94 ^B
	CLA	51.52 ^{aA}	-16.12	0.72	0.79	41.22 ^{aA}	31.67
	CL	35.93 ^b	-5.78	0.63	0.66	23.64 ^b	14.76
	CLT	43.45 ^{ab}	-8.54	0.61	0.67	29.45 ^{ab}	17.65
	SEM ²	4.410	6.081	0.074	0.056	4.990	6.274
600	Control	41.86 ^{abA}	-4.69 ^{AB}	0.67	0.62 ^B	26.12 ^{abAB}	17.60 ^{abAB}
	CLA	33.03 ^{bB}	-3.15	0.65	0.63	20.70 ^{bB}	13.57 ^b
	CL	40.92 ^{ab}	-5.52	0.68	0.66	26.84 ^{ab}	18.18 ^{ab}
	CLT	44.26 ^a	-10.97	0.65	0.66	29.19 ^a	19.00 ^a
	SEM ²	2.650	4.313	0.036	0.016	2.093	1.543

^{a,b}Different letters within the same column with the same high pressure differ significantly ($P < 0.05$).

^{A-C}Different letters within the same column with the same treatment differ significantly ($P < 0.05$).

¹ CLA concentration was 1% (w/w). CL was prepared by addition of lecithin (0.15%, w/w) to CLA. CLT was prepared by addition of 0.001% α -tocopherol to the CL treatment.

² Standard error of mean ($n = 12$).

Table 5

The number of total aerobic bacteria (log CFU/g) of the beef supplemented with conjugated linoleic acid (CLA) and treated by high pressure.

Pressure (MPa)	Treatment ¹	Storage (day)			SEM ²
		0	5	10	
0.1	Control	4.20 ^{dzA}	5.68 ^{cyA}	6.80 ^{cxA}	0.105
	CLA	4.68 ^{czA}	6.45 ^{abyA}	7.51 ^{axA}	0.100
	CL	5.83 ^{azA}	6.76 ^{ayA}	7.26 ^{abxA}	0.131
	CLT	5.27 ^{bzA}	6.22 ^{byA}	7.09 ^{bxA}	0.066
	SEM ³	0.108	0.120	0.077	
300	Control	0.00 ^{dzB}	2.96 ^{cyB}	4.35 ^{bxB}	0.053
	CLA	2.26 ^{czB}	3.58 ^{byB}	5.04 ^{abxB}	0.170
	CL	2.78 ^{bzB}	3.68 ^{byB}	4.76 ^{abxB}	0.061
	CLT	3.62 ^{azB}	4.48 ^{ayB}	4.78 ^{abxB}	0.050
	SEM ³	0.076	0.058	0.139	
450	Control	ND ^{4B}	ND ^C	ND ^C	–
	CLA	ND ^C	ND ^C	ND ^C	–
	CL	ND ^C	ND ^C	ND ^C	–
	CLT	ND ^C	ND ^C	ND ^C	–
	SEM ³	–	–	–	
600	Control	ND ^B	ND ^C	ND ^C	–
	CLA	ND ^C	ND ^C	ND ^C	–
	CL	ND ^C	ND ^C	ND ^C	–
	CLT	ND ^C	ND ^C	ND ^C	–
	SEM ³	–	–	–	

^{a–d}Different letters within the same column with the same high pressure differ significantly ($P < 0.05$).

^{x–z}Different letters within the same row with the same high pressure differ significantly ($P < 0.05$).

^{A–C}Different letters within the same column with the same treatment differ significantly ($P < 0.05$).

¹ CLA concentration was 1% (w/w). CL was prepared by addition of lecithin (0.15%, w/w) to CLA. CLT was prepared by addition of 0.001% α -tocopherol to the CL treatment.

² Standard error of mean ($n = 9$).

³ Standard error of mean ($n = 12$).

⁴ Viable colony was not detected at detection limit $<10^1$ CFU/g.

2012). These results clearly demonstrate that HP treatment greater than 300 MPa was able to inactivate microbial populations and extend the shelf-life of beef loin regardless of the additives.

3.6. Sensory evaluation

Sensory panelists could not recognize the color difference between HP-treated or not treated samples (Table 6). This agrees with previously reported results (Jung et al., 2000; Jung, Jung et al., 2012). Aroma and flavor scores tended to decrease with the addition of CL and HP treatments; however, the differences were not statistically significant. The most significant effect of pressure and treatments was observed with chewiness, with CLT and pressures of 450 and 600 MPa reducing chewiness. It has been reported that HP treatment affects the flavor, juiciness and aroma of meat (Kruk et al., 2011). Rivas-Cañedo, Fernández-García, and Nuñez (2009) showed that the pressurization of minced beef and chicken breast using HP at 400 MPa significantly changed the levels of some volatile compounds by decreasing alcohols and aldehydes while increasing other compounds.

In the present study, however, significantly higher scores in both overall acceptance and willingness to buy were obtained when the samples were treated by CL or CLT with HP at 300 MPa, compared with controls. Overall acceptance and willingness to buy were significantly lower for CL or CLT with pressure of 600 MPa. This is an interesting finding as the CL and CLT samples were not significantly different from the control for any of the other sensory characteristics. This improved perception can be attributed to the application of lecithin as an emulsifying agent, which in combination with 300 MPa pressure increases the penetration of CLA into the beef loin. These results show that HP with the addition of CLT and lecithin is an effective technology for improving the sensory characteristics of beef loin and purchasing power of consumers.

4. Conclusions

Our results suggest that, although there were some undesirable changes including meat color and lipid oxidation, the addition of CLA followed by HP treatment is a good method for developing a safer meat product containing biologically active compounds as well as desirable sensory qualities.

Table 6

Sensory evaluation of the cooked beef supplemented with conjugated linoleic acid (CLA) and treated by high pressure.

Pressure (MPa)	Treatment ¹	Color	Aroma	Tenderness	Juiciness	Chewiness	Flavor	Overall acceptance	Willingness to buy
0.1	Control	6.29	6.29 ^a	4.86	4.71	4.71	6.00	5.57	4.86
	CLA	5.86	4.57 ^b	5.71 ^{AB}	5.14	5.71 ^{AB}	4.71	4.29	3.86
	CL	6.00	5.43 ^{ab}	5.71 ^A	5.57	5.57 ^B	4.57 ^B	5.00 ^B	4.57 ^B
	CLT	6.57	5.71 ^{ab}	5.86 ^{AB}	5.14	5.86 ^A	5.43	5.29 ^{AB}	5.14 ^{AB}
	SEM ²	0.476	0.452	0.590	0.550	0.531	0.612	0.639	0.656
300	Control	6.29	6.29	6.00	5.71	5.57 ^b	5.14	5.14	5.00
	CLA	6.14	5.43	6.43 ^A	5.57	6.29 ^{abA}	5.57	5.57	5.43
	CL	5.71	5.14	6.29 ^A	5.29	6.86 ^{aA}	6.14 ^A	6.29 ^A	6.43 ^A
	CLT	6.29	6.00	6.64 ^A	5.29	7.00 ^{aA}	5.57	6.00 ^A	6.14 ^A
	SEM ²	0.483	0.523	0.349	0.423	0.393	0.53	0.523	0.513
450	Control	6.57	6.00	5.00 ^b	5.14	4.86 ^b	5.00	5.43	5.00
	CLA	5.57	4.86	4.86 ^{abAB}	4.86	5.14 ^{abAB}	5.14	4.43	4.86
	CL	5.71	5.14	4.86 ^{abAB}	5.29	5.57 ^{abB}	4.29 ^B	4.71 ^B	4.57 ^B
	CLT	6.00	5.57	7.00 ^{aA}	5.14	6.71 ^{aA}	5.57	5.57 ^{AB}	6.00 ^A
	SEM ²	0.528	0.508	0.510	0.512	0.517	0.512	0.515	0.549
600	Control	6.57	5.29	5.00	6.14	5.43	5.43 ^a	5.43 ^a	5.14 ^a
	CLA	6.00	5.50	4.43 ^B	4.86	4.36 ^{AB}	5.00 ^{ab}	3.86 ^{ab}	3.71 ^{ab}
	CL	6.00	4.57	4.14 ^B	4.57	3.86 ^C	3.71 ^{bb}	3.43 ^{bc}	3.14 ^{bc}
	CLT	5.57	5.43	4.57 ^B	4.57	4.57 ^B	4.00 ^{ab}	4.00 ^{abB}	3.86 ^{abB}
	SEM ²	0.564	0.514	0.579	0.552	0.591	0.530	0.513	0.512

^{a,b}Different letters within the same column with the same high pressure differ significantly ($P < 0.05$).

^{A–C}Different letters within the same column with the same treatment differ significantly ($P < 0.05$).

¹ CLA concentration was 1% (w/w). CL was prepared by addition of lecithin (0.15%, w/w) to CLA. CLT was prepared by addition of 0.001% α -tocopherol to the CL treatment.

² Standard error of mean ($n = 28$).

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