Effect of high hydrostatic pressure processing on the lipid and protein characteristics of different lamb cuts

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Abstract

HPP treatment is a popular non-thermal food processing technique, which can improve food quality and extend shelf-life. This study investigates the effects of high hydrostatic pressure processing (HPP) treatments of different lamb cuts on lipid oxidation, as well as fatty acid and free amino acid content. Eight different lamb cuts, tenderloin (T), rump (R), knuckle (K), inside (I), heel (H), Flat (F), eye of loin (E) and bolar (BL) were analysed. The cuts were treated at 600MPa (42°C), 400MPa (28°C), 300MPa （21°C）, 200MPa (14°C) and 0MPa (7°C)for 5 seconds.

In general, the higher levels of pressure (400MPa and 600MPa) employed resulted in higher oxidation values exceeding 0.5 mg MDA/kg in E, F, H and T cuts. On the other hand, lower pressures of 200MPa and 300MPa, resulted in oxidation values less than 0.5 mg MDA/kg for all cuts except for the K cut that had the highest oxidation value (0.522 mg MDA/kg) at 300 MPa.HPP treated R and H cuts had higher SFA and MUFA ($P < 0.0001$) content, and lower polyunsaturated fatty acid/saturated fatty acid ratio ($P < 0.001$) compared to control sample. Polyunsaturated fatty acid content was not significantly different to control for both cuts suggesting that little or no oxidation occurred. On the other hand, saturated fatty acid and monounsaturated fatty acid content were lower in HPP treated I, B, K, E, T and F cuts at all pressures compared to control samples. Polyunsaturated fatty acid content was also lower in HPP treated I (at all pressures), K (400 and 600 MPa), E (300 MPa) and T (600 MPa) cuts compared to control. The decrease in Polyunsaturated fatty acid was due to the decrease of mainly C18:1 n9 and C18:3 n3 fatty acids.

Seventeen free amino acids, including 9 essential and 8 non-essential amino acids were identified in the eight lamb cuts. High pressure processing increased the total free amino acids composition significantly ($P<0.05$) compared to control at all pressures for almost all cuts except I and E cuts. As for the E cut, a significant decrease in total amino acids was observed at 600 MPa with a highly significant ($P<0.0001$) decrease in the essential amino acids leucine and isoleucine.

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Chapter 1 Introduction and Objectives

Lamb is a common meat source, which contains high biological value protein and fats (Scollan et al., 2006). New Zealand is one of the largest producers and dominant exporter of lamb meat. There are many processing methods available to improve the quality or extend the shelf life of meat and meat products, traditionally thermal treatments have been widely used. However, thermal treatments can reduce food quality by damaging nutritional components and flavour compounds in foods. Therefore, a non-thermal treatment technique applied on foods, like high pressure processing (HPP) may overcome this problem without sacrificing food quality.

During processing, lipid oxidation can seriously reduce the quality of meat and meat products. Meat lipid oxidation and its relative interaction can cause several negative effects on meat colour, flavour, nutrition and safety during processing. Hence analysis of lipid oxidation in high-pressure-treated meat is necessary for successful implementation of high-pressure technology in the meat industry. In beef muscle, the pressure required to initiate lipid changes was reported to be lower (200 MPa) than that required for pork and chicken (Ma, Ledward, Zamri, Frazier, & Zhou, 2007). However, in some cases, pressure induced lipid oxidation during subsequent storage of the meat (Beltran, Pla, Yuste, & Mor, 2003, 2004; Dissing, Bruun, & Skibsted, 1997; Orlien, Hansen, & Skibsted, 2000). The mechanism by which high pressure induces lipid oxidation is still not fully understood and studies have shown that the release of haem molecules through membranes disruption somehow triggers lipid oxidation (Orlien et al., 2000).

There have only been a few studies to date that investigated the effects of high pressure processing on fatty acid composition. In general, high pressure treated samples oxidised more rapidly than the control samples with little changes in fatty acid composition in most studies. Although all foods studied are susceptible to pressure-induced lipid oxidation, the critical pressures that influence oxidation varies with food type. Yagiz et al. (2009) reported no significant ($p > 0.05$) difference between control and HPP treated Atlantic salmon dark muscle in terms of total saturated fatty acids, monounsaturated fatty acids, n-3 PUFA and n-6 PUFA fatty acid profile. Kang et al. (2013) further reported that fatty acid content in Korean black goat meat was not significantly ($P>0.05$)

different between control and HPP treated samples for all fatty acids detected. Only Wang et al. (2013) reported that 600 MPa treatment induced a lower ($p < 0.05$) percentage of polyunsaturated fatty acids in pressure-treated yak body fat. McArdle, Marcos, Mullen, and Kerry (2013) on the other hand reported that the PUFA/SFA ratios of pressurised samples were significantly higher, with the exception of the milder treatments (20 °C at 200 and 400 MPa) compared to non-treated samples. Ono, Berry, and Paroczay (1985) found an increase in the PUFA/SFA ratio in cooked beef samples when compared to raw beef meat. They hypothesised that unsaturated fatty acid especially PUFAs are less affected by cooking as they are part of the membrane structure and that proportional change in fatty acid composition may be explained by the breakdown of SFAs. Pressurising with higher temperatures may breakdown SFAs in a similar way to cooking.

Muscle proteins are also susceptible to oxidative reactions that involve the loss of essential amino acids and decrease protein digestibility. The mechanisms and reaction pathways for the oxidation of lipids and proteins are different but are directly linked as both are influenced by similar pro oxidant and antioxidant factors (Simonin, Duranton, & Lamballerie, 2012). High pressure between 100 and 300 MPa for 10 min at 25 ˚C increased the overall autolytic activity of raw beef meat leading to a higher concentration of free amino acids (Ohmori & Hayashi, 1991). Suzuki et al. (1994) reported that serine, glutamic acid, glutamine, glycine and alanine content gradually increased with increasing pressure applied to lean beef meat up to 200 MPa. However, no significant differences in all 10 amino acids were observed suggesting that high pressure treatment had no adverse effect on amino acid composition. Campus, Flores, Martinez, and Toldrá (2008) investigated the effect of pressures of 300, 350, 400MPa on sliced and vacuum packaged commercial dry-cured pork loin. Only the untreated samples showed an increase in free amino acid content during vacuum storage. In fact, high pressure (300 to 400 MPa for 10 min at 20 ◦C) was found to stabilize the free amino acid content during storage due to reduction in amino peptidase activities.

While researchers have assessed the impact of HPP on meat quality, only limited information is available on the effect of high pressure processing on lamb. In this study, different lamb cuts were used to investigate the possibility of adding value to and increasing processing opportunities for different muscle types. Hence the aim of this study was to analyse the effects of varying HPP pressure (200MPa, 300MPa, 400MPa

and 600MPa) on different lamb cuts (tenderloin (T), rump (R), knuckle (K), inside (I), heel (H), flat (F), eye of loin (E) and bolar (BL)) in terms of lipid oxidation, as well as fatty acid and free amino acid content.

This thesis is composed of five parts. Chapter one introduces the study and provides the study objectives. Chapter two summarizes past research on HPP treatment and their effects on chemical composition of meat. Chapter three presents materials and methods employed in this project as well as data analysis. Chapter four summarizes and discusses results obtained. Finally, chapter five provides a conclusion for this study with some suggestions for future study.

Chapter 2 Literature review

Lamb meat is a protein rich and ‘‘low’’ carbohydrate product that contributes to a low glycaemic index. Lamb meat and its products are also favour able in terms of their micronutrient profile, and contain a wide range of minerals (eg. heme iron, zinc) and vitamins (eg. Vitamin A, B12) (Biesalski, 2005; Kouvari, Tyrovolas, & Panagiotakos, 2005).

According to Kegalj, Krvavica, Vrdoljak, Ljubičić, and Dragaš (2011) lamb meat is widely consumed for sensorial or nutritional reasons, rather than religious or traditional reasons. New Zealand has a long history as a producer of quality lamb meat. The wider meat industry makes an important contribution to employment and foreign exchange earnings through export, and also as more tourists enjoy the experience of eating New Zealand lamb meat (Beef and Lamb New Zealand, 2016).

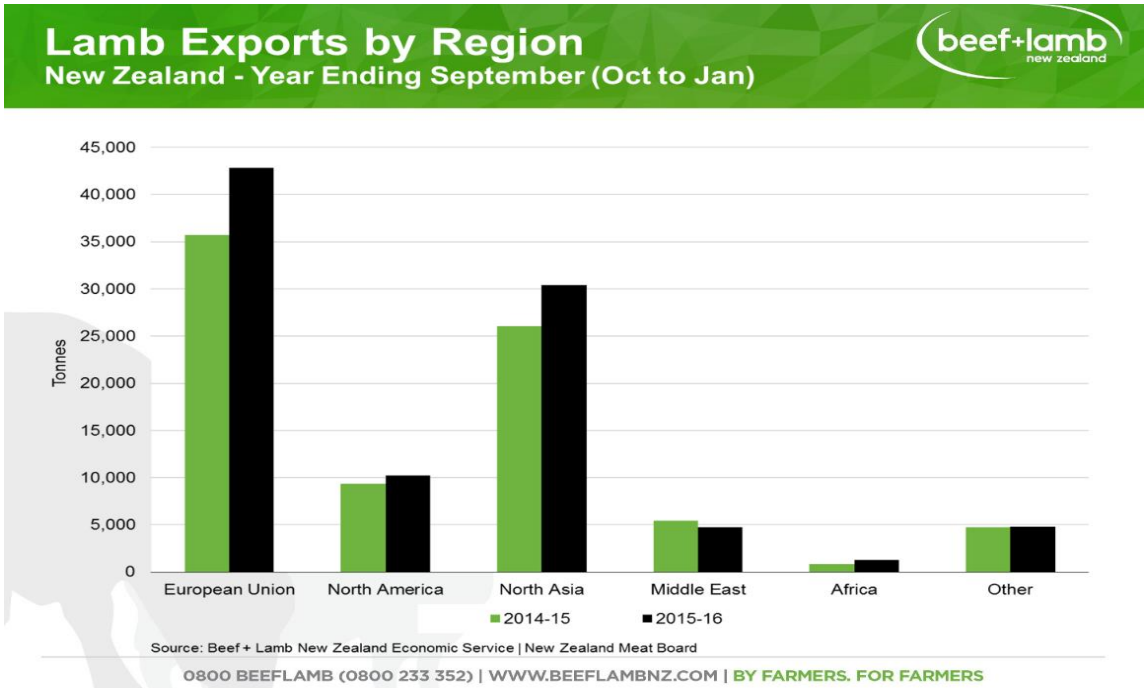


Figure 1 New Zealand sheep export data by regions (Beef and Lamb New Zealand, 2016).

With the highest density of sheep per unit area in the world, the export value of New Zealand lamb meat as shown in Figure 1 increased from 2014 to 2015. New Zealand is one of the main exporters of lamb meat with a total value of NZD 2.6 billion (Beef and Lamb New Zealand, 2016), making sheep farming one of its most important agricultural industries.

2.1 Lamb cuts

Like many other types of meat, lamb exhibits variation in appearance and eating quality that is perceptible by the consumer (e.g. colour, flavour, texture, and juiciness). It is well established that this difference is attributable to the combined effects of ante mortem factors such as sex, breed, age and feeding background (Berge et al., 2003). However, in the same animal, different cuts vary in colour, flavour, texture, and juiciness. A primal cut is also known as a wholesale cut of an animal. Meat cutting separates cuts into tender and less tender cuts, and lean and fatty cuts (Vaclavik & Christian, 2008). With a trend from producing primal cuts to more complex retail cut specifications for many markets, the production process requires more flexibility to determine the best cutting strategy for each carcass, and thus to maximize the revenue from the carcasses available each day. Improvements in the prediction of primal and retail cut yields are needed to drive process optimization (Ngo et al., 2016).

Vaclavik & Christian (2008) reported that less exercised skeletal muscles that provide support, like cuts of meat along the backbone (e.g. loin), are usually tenderer than skeletal muscles used in locomotion. Ultimately, tenderness is a function of how meat is torn by the teeth, not just the cut, age, and so forth. In this project eight different cuts of lamb: tenderloin (T), rump (R), knuckle (K), inside (I), heel (H), flat (F), eye of loin (E) and bolar (BL) cuts will be analysed (Figure 2). According to Vaclavik & Christian (2008) rump, tenderloin, eye of loin, bolar and flat cuts are more tender compared to knuckle, heel and inside round cuts.

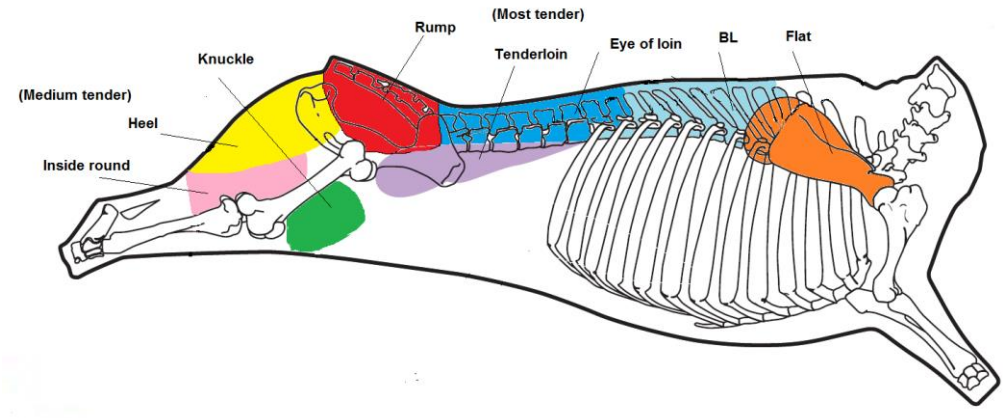


Figure 2 Diagram of lamb meat cuts used in this project.

2.2 Composition of lamb meat

2.2.1 Lipid

Lipids are significant in food and biological systems (Edwin, 2014; Pereira & Vicente, 2013; Nigel et al., 2014; Shahidi & Zhong, 2010). They influence food quality and contribute to flavour, odour, colour, and texture of foods. Nutritionally, lipids are a concentrated source of calories (approximately 9 Kcal per gram). They also provide essential nutrients, such as linoleic acid, linolenic acid, and fat-soluble vitamins (A, D, E and K) (Edwin, 2014; Shahidi & Zhong, 2010). Lipids contribute to the structural components of cell membranes and are important signalling agents in biological systems (Shahidi & Zhong, 2010). Overconsumption of lipids especially the "bad" lipids (e.g. certain saturated lipids and trans fats) however has been associated with a number of diseases and health conditions, including obesity, hypertension, cardiovascular disease and cancers (Pereira & Vicente, 2013; Shahidi & Zhong, 2010).

Fat in meat contributes to the eating quality of meat (Webb, 2006; Wood & Fisher, 1990). Studies confirmed that there is a chemical perception of dietary fat in the oral cavity, which could affect the taste of food (Hiraoka, Fukuwatari, Imaizumi, & Fushiki, 2003). It is also widely accepted that the amount and type of fat in meat influence two major components of meat quality notably tenderness and flavour (Webb & Neill, 2008).

2.2.1.1 Lipid oxidation

Lipid oxidation is one of the most important chemical reaction that affects meat quality and acceptance (Beltran et al., 2003; Chaijan, 2008). Lipid oxidation is a chain reaction comprising initiation, propagation, and termination reactions, and involves the production of free radicals. Oxidation of lipids is very important after post-slaughter period, during handling, processing, storing and cooking (Chaijan, 2008). This lipid oxidation leads to discolouration, drip losses, off-odour and off-flavour development, texture defects and the production of potentially toxic compounds in meat. Food products can become rancid due to lipid oxidation and the changes associated with it (Ahn, Grün, & Mustapha, 2007; Ladikos & Lougovois, 1990). Chemically lipid oxidation products comprise of: fatty acids, malondialdehyde and cholesterol oxidation products (Medina-Meza, Barnaba, & Barbosa, 2014). These products can affect our health and lead to the development of many diseases; disruption of cell membranes; limitation of enzyme activity; and damage of proteins (Niki, 2009).

According to Linares, Berruga, Bórnez, and Vergara (2007) lipid oxidation in foods is strongly encouraged by the presence of inorganic and biological catalysts, such as metal ions and enzymes, normally present as their constituent. Therefore, the strategy adopted in emerging technologies is to limit the extent of lipid degradation by either avoiding high temperature for long times, or controlling such catalysts by inactivation (enzymes) or removal (metal ions).

Meat products are highly susceptible to lipid degradation due to higher unsaturated lipid fractions. Most studies employ the thiobarbituric acid reactive substances (TBARS) method (Edwin Nessim Frankel, 2014; Linares et al., 2007; Maqsood, Abushelaibi, Manheem, Al Rashedi, & Kadim, 2015; Shahidi & Zhong, 2010; Vázquez, Torres, Gallardo, Saraiva, & Aubourg, 2013) to determine products derived from lipid oxidation. Malondialdehyde (MDA) is reported to be the most important biological breakdown product from 5-membered cyclic peroxides of linoleate and linolenate (Esterbauer, Schaur, & Zollner, 1991). The reaction of malondialdehyde (MDA) with thiobarbituricacid (TBA) is the basis of the most common method used to assess lipid peroxidation in biological materials. These markers are frequently used in many laboratories due to simplicity and low cost (Lykkesfeldt, 2007; Niki, 2009).

Thiobarbituric acid reactive substances (TBARS) assay is the most frequently used method for the quantification of MDA in foods and biological fluids and tissues (Addis, 1986). Treatment of biological and food samples with TBA under appropriate conditions results in the formation of pink-coloured products, which absorb in the 500–550 nm range (Addis, 1986; Esterbauer et al., 1991; Frankel & Neff, 1983).

2.2.1.1 Fatty acid

Straight chain aliphatic carboxylic acids with 4 or more carbon atoms are called fatty acids. In nature, they occur with an even number of carbon atoms, with few exceptions (Webb & Neill, 2008; Wood et al., 2008; Wood et al., 2004). The systematic and trivial names of frequently occurring fatty acids according to Hoffmann (2013) are shown in Table 1.

Table 1 Systematic and trivial names of important fatty acids in meat and meat products (Hoffmann, 2013).

Systematic name (acid)	Trivial name (acid)	Chain length (ω notation)	m.p. (°C)	Triacylglycerol m.p.α (°C)
Decanoic	Capric	10:0	31.6	31.5
Dodecanoic	Lauric	12:0	44.4	46.4
Tetradecanoic	Myristic	14:0	54.3	57.0
Hexadecanoic	Palmitic	16:0	62.9	
Octadecanoic	Stearic	18:0	70.0	73.1
9-Octadecenoic	Oleic	18:1, ω-9	13.0	5.5
9-trans-Octadecenoic	Elaidic	18:1, ω-9	36.0	42.0
13-Docosenoic	Erucic	22:1, ω-9	33.5	30.0
9,12-Octadeca-dienoic	Linoleic	18:2, ω-6,9	-3	-13.1
9,12,15-Octa-decatrienoic	α-Linolenic	18:3,ω-3,6,9	11.9	-24.2
4,8,12,15,19-Doco-sapentaenoic	Clupanodonic	22:5,ω3,7,10,14,18		
12-Hydroxy-9-octadecenoic	Ricinoleic	18:1,ω-9(OH)	5.5	

Note: m.p.α stands for the melting point of fatty acids.

A study conducted by Enser, Hallett, Hewitt, Fursey, and Wood (1996) found differences in the fatty acid composition of beef, lamb and pork. The total fat content of steaks (obtained by dissection) was highest in lamb. In addition, the total fatty acid composition of the longissimus muscle, including some fat attached to the perimysium, was also highest in lamb and the least in pork.

Table 2 summarizes the fatty acid composition of different lamb muscles. The studies investigated the effects of diet, breed, sex, age and anatomical location. Fatty acids in different lamb muscles mainly comprised C18:1 (35-54%), C16:0 (19-29%). C18:0 (10-19%), and C18:2 (1-9%). Other fatty acids present in lower concentrations include C10:0, C12:0, C15:0, C15:1, C17:0, C17:1, C20:1, C20:3, C22:0, C24:0, C22:4, C22:5

and C22:6 fatty acids. When these fatty acids were present, the levels were included in the sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) in the studies reported.

The ratios of PUFA to SFA and n-6 to n-3 (polyunsaturated omega-6 fatty acids: polyunsaturated omega-3 fatty acids) have been widely used as an indicator of healthy dietary fat (Scollan et al., 2006). According to Wood et al. (2004) the recommended ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (PUFA: SFA) should be above 0.4. Beef and lamb naturally have a PUFA: SFA ratio of around 0.1 (Badiani et al., 2002; Enser, Richardson, Wood, Gill, & Sheard, 2000). A higher PUFA to SFA ratio (≥ 0.4) can prevent the negative effects of saturated fat, hence decreasing the risks of cardiovascular disease and metabolic syndrome (Haffner, 2006). Consumption of higher levels of MUFAs, in conjunction with reduced levels of SFAs, is also believed to prevent an increase in blood cholesterol levels (Garaffo et al., 2011).

Meat has been implicated in causing imbalanced fatty acid intake of today's consumers. For this reason, ways to improve the PUFA: SFA ratio during meat production are required. The only way to improve the P/S ratio in ruminant meats is by preventing ruminal biohydrogenation or by feeding protected PUFA supplements (Smet, Raes, & Demeyer, 2004; Scollan, Enser, Gulati, Richardson, & Wood, 2003). The increase of PUFA: SFA ratio in human dairy intake are promoted by three factors; selective breeding and feeding practices in animal, preference of leaner carcasses reinforced by marketing practices and meat classification systems, and advances in technology for trimming practices (Higgs, 2000).

Table 2 Fatty acid composition (%) of total lipids in different lamb muscles (mean% of pooled data).

Lamb meat cuts/fatty acid compound	Rump	LD	SM	TB	LD	Lean	TB	LD	GB	lean
14:00	2.02	1.85	1.73	1.88	4.17	3.13	3.17	3.99	3.23	-
16:00	23.4	22.71	21.81	21.63	28.77	22.82	19.4	20.9	20	-
16:1 cis	3.02	1.74	1.74	1.88	2.03	3.58	2.05	2.19	2.09	-
18:00	11.1	16.28	15.44	14.89	16.13	13.87	17.9	17.5	18.6	-
18:1 cis-9	53.2	41.75	41.67	42.28	45.3	42.73	36.59	35.73	35.83	-
18:2n-6	1.2	5.22	6.26	5.89	3.6	8.05	3.43	3.24	3.28	4.9
18:3n-3	1.4	0.55	0.61	0.61	-	1.57	2.31	1.94	2.31	2
20:4n-6	0.2	-	-	-	-	1.12	1.19	1.12	1.16	1.2
Others	-	9.84	10.66	10.49	-	2.68	2.51	2.25	2.44	2.1
SFA	36.52	40.8	38.97	38.4	49.07	41.96	40.47	42.39	41.83	45.6
MUFA	56.6	43.58	43.49	44.6	47.33	47.2	38.64	37.92	37.92	44
PUFA	3.8	5.77	6.87	6.49	3.6	10.74	9.41	8.55	9.19	10.2
PUFA:SFA	0.1	0.14	0.18	0.17	0.07	0.26	0.23	0.2	0.22	0.22
n-6:n-3	0.86	9.49	10.26	9.66	-	5.13	1.48	1.67	1.42	2.45
References	Duncan, Orskov, and Garton, 1976	Solomon, Lynch, Paroczay, and Norton, 1991	Solomon et al., 1991	Solomon et al., 1991	Marinova, Shindarska, and Banskalieva, 1992	Rhee and Chow, 2000	Enser et al., 1998	Enser et al., 1998	Enser et al., 1998	Li, Ng, Mann, and Sinclair, 1998

Note: LD: *longissimus dorsi* refers to eye of loin in our study; SM: *semimembranosus* refers to inside round in our study; TB: *infraspinatus* refers to flat in this study; GB: *gluteo biceps* refers to bolar in our study.

2.2.1.2 Unsaturated fatty acids

Unsaturated fatty acid includes monounsaturated fatty acids and polyunsaturated fatty acids. Monounsaturated fatty acids (MUFA) are primarily palmitoleic (C16:1) and oleic (C18:1); and polyunsaturated fatty acids (PUFA) consist largely of linoleic (C18:2), linolenic (C18:3) and arachidonic (C20:4) acids (Banskalieva, Sahlu, & Goetsch, 2000). Theoretically, substrates necessary for meat deteriorative reaction include unsaturated fatty acids, oxygen and chemical species that accelerate oxidation and are abundant in meat displayed aerobically or in high oxygen modified atmosphere packaging. However, oxygen molecule and polyunsaturated fatty acid (PUFA) cannot interact with each other due to thermodynamic constraints (Faustman, Sun, Mancini, & Suman, 2010). Most PUFAs are found in the triacylglycerols of oils of vegetable origin, and in the oils of aquatic animals. The latter contain fatty acids with 4, 5 and even 6, mainly isolated double bonds (Hoffmann, 2013).

As with the PUFA: SFA ratio, meats can also be manipulated towards a more favourable n-6:n-3 ratio.The increasing awareness of the need for diets to contain higher levels of n-3 PUFA has focused on the importance of meat as a natural supplier of these to the diet. The ratio of n-6:n-3 PUFA is particularly beneficial (low) in ruminant meats, especially from animals that have consumed grass containing high levels of 18:3 fatty acids. Ruminants also naturally produce conjugated linoleic acids (CLAs) which may have a range of nutritional benefits in the diet (Enser et al., 2001). CLA is a group of polyunsaturated fatty acids that appear in dairy products and are thought to have beneficial effects on health (Belury, 2002).

2.2.1.3 Saturated fatty acids

Meat is seen to be a major source of fat in the diet and especially of saturated fatty acids, which have been implicated in diseases. Diseases include various cancers and coronary heart disease. In the UK, the HMSO (1994) recommended that fat intake be reduced to 30% of total energy intake (from about 40%) with a figure of 10% of energy intake for saturated fatty acids (from 15%). “Saturated” means that all the carbon valencies (except in the carboxylic acid group) are satisfied independently (Hoffmann, 2013; Nollet & Toldrá, 2008; Schmid, 2010; Wood et al., 2008). According to Banskalieva et al. (2000) saturated fatty acid are mainly myristic (14:0), palmitic (C16:0) and stearic

(C18:0) in lamb meat. Saturated fatty acids increase hardness of fat and easily solidifies upon cooling influencing meat palatability (Banskalieva et al., 2000).

2.2.2 Protein

Protein nutrition is undoubtedly the core of most studies in the field of animal nutrition (Guillaume, 2001). Proteins are polymers of amino acids that perform diverse functions. They make up the enzymes that catalyse biochemical reactions to sustain life (Nollet & Toldrá, 2008). Therefore, a continuous supply of proteins with balanced amino acids is needed for maintenance of body metabolism and growth of animals. In muscle foods, proteins are the major non-water ingredients and constitute almost 20% of the weight of lean muscle tissue (Nollet & Toldrá, 2008). The variable side chains of protein units give each protein chain its distinctive character (Hepler & Gilman, 1992). In lamb meat, there are three kinds of proteins: myofibrillar (50-55%), sarcoplasmic (30-34%) and connective tissue proteins (10-15%) that directly affect the taste of meat products, and structure of cooked meat (Pereira & Vicente, 2013).

Meat protein content can vary significantly. According to Williamson, Foster, Stanner, and Buttriss (2005), protein content in meat can vary substantially depending on animal species and fat content. In general, protein content decreases with increase in fat content. Red meat contains, on average, 20-24% protein when raw, and 27-35% protein when cooked. Sheep meat, such as lamb and mutton, have higher fat levels with a decreased protein content compared to cattle meat including beef and veal (Williams, 2007; Williamson et al., 2005).

2.2.2.1 Amino acids

Chemically, amino acids (AA) are defined as organic substances containing both amine ($-NH_2$) and carboxylic acid (-COOH) functional groups, usually along with a side-chain specific to each amino acid. Because of variations in their side chains, AA have remarkably different biochemical properties and functions. AA were traditionally classified as nutritionally essential (indispensable) or non-essential (dispensable) for humans and animals (Munro, 2012). In mammals essential amino acids are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan,

valine; and non-essential amino acids are: alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, taurine, tyrosine (Wu, 2009).

2.2.2.2 Free amino acid

Little is known about free amino acid (FAA) variations in meat muscles. Free amino acids are important flavour precursors. They produce amines by decarboxylation (Hernández, Izquierdo, Veciana, & Vidal, 1996) and play a role in Maillard reactions resulting in the production of numerous volatile products (Bailey, 1994). Better knowledge of their concentrations, which is closely associated with muscle types, could contribute to the understanding of variability in meat properties.

Muscle content of several amino acids are closely related to the metabolic type of muscle (Cornet & Bousset, 1999). Franco et al. (2010) analysed the fraction of FAA found in the different muscles of fresh veal calves of the Blonde Galician breed. They reported that in general the free amino acid composition (mg/100g protein) showed high variability in the amino acids of different muscles (ST, *semitendinosus*; BF, *biceps femori*; SM, *seminebranous*; LD, *longisimus dorsi*; MS, *masseter*; CM, *cardiac muscle*) with significant differences in histidine, alanine ($P < 0.05$), aspartic and glutamic acid, where histidine was the most abundant. The sum of histidine, alanine, aspartic and glutamic acids represented values of around 50% of the total free amino acid, and even reached 77% in the case of CM as reported by Franco et al. (2010) and shown in Table 3.

Moreover, Madruga, Elmore, Oruna, Balagiannis, and Mottram (2010) analysed the free amino acid (mg/100g protein) composition in raw and cooked goat meat shown in Table 3. They reported the most abundant amino acids to be glycine, alanine, glutamine and arginine. There were highly significant differences ($p < 0.01$) in the concentrations of all the free amino acids between beef lamb and chicken, suggesting that genetic factors may be important. The concentrations for most of the free amino acids were lower than the values reported for calves (Franco et al., 2010) except for glycine and alanine (Table 3).

Table 3 Free amino acids content (mg/100g protein) in beef and lamb meat.

Free amino acid	Raw goat meat	Cooked goat meat	Calves ST	Calves BF	Calves SM	Calves LD	Calves MS	Calves CM
Histidine	4.7	3.4	37.6	41.1	20.9	9.7	49.4	96.2
Isoleucine	4.8	3.2	10.4	5	5.6	2.2	6.6	3.3
Leucine	7.9	5.1	6	2.9	2.4	1.6	3.4	1.8
Lysine	5.6	3.7	7.5	4.4	4.5	1.6	6	3.5
Methionine	1.7	1.2	4	1.6	1.8	0.9	1.8	1.1
Phenylalanine	3.9	2.5	7.6	3.8	4.1	2.5	4.6	3
Threonine	6.4	3.8	11.8	14	15.1	5.9	7.6	4.5
Valine	7.6	5.1	8.5	4.1	4.7	1.5	5.5	2.7
Arginine	12.2	9.3	10.9	21.8	18.9	n.d	11.35	1.9
Alanine	38.7	30.4	21.2	20.8	19	5.2	26.3	28.1
Aspartic acid	1.2	1.2	2.8	2.6	2.4	2.4	3	4.3
Cysteine	0.04	0.01	8.6	6.1	7.4	2.6	2.5	n.d
Glutamic acid	11	6.3	20.1	8.8	11.2	5	15.9	28.6
Glycine	56.7	38	9.4	6.8	7.8	3	5.5	4.3
Proline	4.2	3	5.9	3.6	4.1	2.6	4.3	3.9
Serine	8.9	5.1	10.1	5.5	5.8	3	9.6	7.3
Tyrosine	3.7	2.3	6.9	30.8	3.5	1.5	4.1	2
References	Madruga et al., 2010	Madruga et al., 2010	Franco et al., 2010	Franco et al., 2010	Franco et al., 2010	Franco et al., 2010	Franco et al., 2010	Franco et al., 2010

Note: LD: *longissimus dorsi* refers to eye of loin in our study; SM: *semimembranosus* refers to inside round in our study; ST: *semitendinosus* refers to heel in our study; BF: *biceps femoris* refers to knuckle in our study; CM: cardiac muscle.

2.3 High Hydrostatic Pressure Processing

High hydrostatic pressure processing (HPP) has emerged as a new non-thermal technology for preservation of cooked and fresh meat in the last decade (Cheftel & Culioli, 1997; Zhou, Xu, & Liu, 2010). However, there are undesirable changes in meat quality associated with this technology, such as decolouration (Jung, Ghoul, & de Lamballerie, 2003), change in food matrix, texture (Ma et al., 2007; Sikes, Tornberg, & Tume, 2010), change in muscle protein (Mor & Yuste, 2003), and production of volatiles from lipids (Rivas, Fernández, & Nuñez, 2009; Simonin, Duranton, & Lamballerie, 2012). Commercially, pressure levels used is in the range of 100MPa to 1000MPa at different temperature and processing times for different applications as shown in Figure 3 (Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008). In most of cases this technology has been used to reduce microbial colony counts (Sikes et al., 2010).

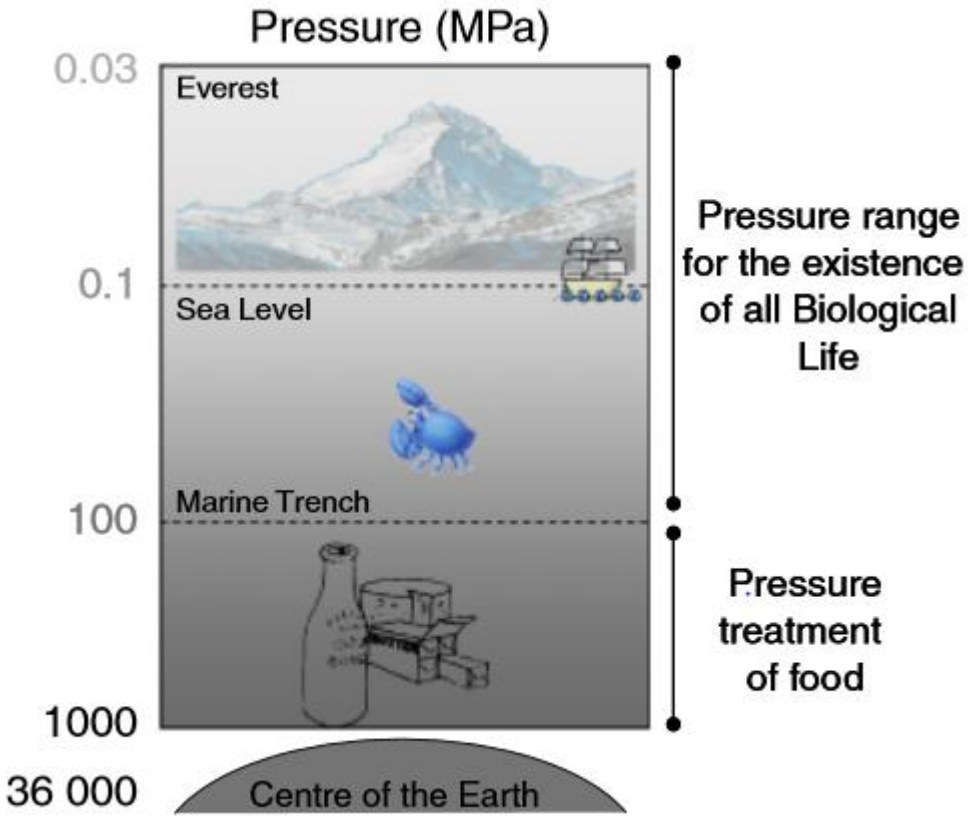


Figure 3 Schematic diagram of pressure involved in HPP (Considine et al., 2008).

2.3.1 Principles of HPP

The pressure applied to food products such as meat is distributed uniformly and instantaneously across the food matrix. Hence the pressure distribution during HPP is independent of food sample size and form as opposed to thermal processing (Considine et al., 2008; Linton, Patterson, & Patterson, 2000; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). The effects of high pressure on chemical reactions in food is governed by the Chatelier's principle. When a system at equilibrium is disturbed by an external force, the system then responds in a way that tends to minimise the disturbance. The effect of HPP will be significantly observed when there is a decrease in reaction volume by pressure such as change in phase, chemical reactivity, change in molecular configuration and chemical changes (Rastogi et al., 2007). This imparts the stability of chemical bonds, disrupts the structure of microorganisms, and changes the stability of enzymes, lipids and small molecules (Considine et al., 2008; Linton et al., 2000; Rastogi et al., 2007).

During pressure build up, elevating pressure in a closed vessel results in adiabatic heating, which temperature increases during pressurisation. This temperature increase could be useful to inactivate enzymes that are responsible for generation of off flavours (Otero, Ramos, Elvira, & Sanz, 2007; Toepfl, Mathys, Heinz, & Knorr, 2006). An example of a HPP instrument used is shown in Figure 4 (Urrutia, 2005).

Figure 4 An example of a HPP instrument used (nc hyperbaric, hyperbaric 55).

2.3.2 Effects of high hydrostatic processing on oxidative and chemical properties of food

HPP is a relatively new process in the meat industry. A brief overview of various high pressure processing conditions on the oxidative and chemical properties of different foods are summarized in Table 4. In general, high pressure treated samples oxidised more rapidly than the control samples with little changes in fatty acid composition in most studies (Angsupanich & Ledward, 1998; Beltran et al., 2003; Cava, Ladero, González, Carrasco, & Ramírez, 2009; Cheah & Ledward, 1996; Dissing et al., 1997; Ma et al., 2007; McArdle et al., 2013; Orlien et al., 2000; Yagiz et al., 2009). Although all foods studied are susceptible to pressure-induced lipid oxidation, the critical pressures that influence oxidation varies depending on meat type (lamb, beef, pork, chicken, fish, oyster etc.).

Table 4 Effects of high pressure processing on lipid oxidation, as well as fatty acid and amino acids content in meat.

Type of meat	High pressure Processing parameters	Chemical measurements	Results	References
Minced pork	800 MPa for 20 min at 20°C prior to storage at 4°C.	Lipid oxidation	• High pressure treated samples oxidised more rapidly than the control samples. • The rate of lipid oxidation of high pressure treated samples was similar to that induced by heat (80 °C for 15 min). • No significant increase in rate of oxidation was observed in minced meat samples treated at 300 MPa, but above this pressure the rate increased with intensity.	Cheah and Ledward, 1996
Turkey thigh muscle	100 MPa, 200 MPa, 300 MPa, 400 MPa or 500 MPa for 10 min or 30 min.	Lipid oxidation	• All pressure treatments affected lipid oxidation compared to non-treated meat. • Only the most intense pressure treatments (500 MPa for 30 min) resulted in lipid oxidation that was comparable to that induced by heat treatment (100°C for 10 min). • Pressure treatment at 400 MPa and lower pressures for 30 min (and for 10 min) resulted in a lower oxidation level.	Dissing et al., 1997
Cod (*Gadus morhua*) muscle	0, 200MPa, 400MPa, 600MPa and 800MPa in room temperature	Lipid oxidation	• After treatment at pressures above 400 MPa, the oxidative stability of lipids in cod (*Gadus morhua*) muscle were markedly decreased and oxidation ratio increased.	Angsupanich and Ledward, 1998

Type of meat	High pressure Processing parameters	Chemical measurements	Results	References
Chicken breast muscle	300, 400, 500, 600, 700 or 800 MPa for 5 min or 10min, or to heat treatment (8 to 7 °C for 10 min)	Lipid oxidation	• The pressure treatment at 800 MPa for 10min was found to enhance lipid oxidation to the same extent as heat treatment. • Pressure treatment at 600MPa and 700 MPa resulted in less oxidation compared to heat treatment. • Chicken breast muscle exposed to pressure at or below 500 MPa showed no indication of rancidity, similar to untreated meat during chill storage.	Orlien et al., 2000
Chicken breast	300 and 500 MPa for 30 min at 20°C;Cooked (90 °C for 15 min)	Lipid oxidation	• Increased lipid oxidation level with increased pressure. • Generally, pressurized samples had less oxidation compounds than cooked samples. • Compared to traditional thermal processing (90°C, 15mins), non-thermal processing (300 and 500MPa 30mins in HPP at 20°C) resulted in little change in nutritional value of meat products.	Beltran et al., 2003
Beef and chicken muscles	200MPa,400MPa, 600MPa and 800 MPa; 40°C 50°C 60°C and 70°C, for 20mins	Lipid oxidation	• The increase in lipid oxidation values were more marked after treatment at pressures ≥400 MPa than lower pressures in beef. • Pressure treatments of 600 MPa and 800 MPa increased rates of lipid oxidation in chicken muscle. • Oxidative stability of minced chicken breast muscle was not affected by pressures up to 500 MPa and this was believed to be related to the integrity of the cell membrane (Kruk et al., 2011).	Ma et al., 2007

Type of meat	High pressure Processing parameters	Chemical measurements	Results	References
Atlantic salmon dark muscle	150 MPa and 300 MPa for 15 min at 25 °C	Fatty acid profile and lipid oxidation	• After 4 days and 6 days of storage, 150MPa samples resulted in a higher oxidation rate than control samples but was not significantly different compared to control samples. However, 300MPa samples resulted in significantly lower oxidation rate compared to control samples. • There were no significant ($p > 0.05$) difference between control and HPP treated samples in terms of total saturated fatty acids, monounsaturated fatty acids, n-3 PUFA and n-6 PUFA fatty acid profile.	Yagiz et al., 2009
Sliced dry-cured Iberian ham and loin	200 MPa 15 min, 200 MPa 30 min, 300 MPa 15 min, 300 MPa 30 min at room temperature	Lipid oxidation	• An increase in lipid oxidation value was reported with an increase in pressure in dry-cured ham. • Pressurization of vacuum packed slices of dry-cured loins did not increase the extent of lipid oxidation at Day 0, but resulted in an unexpected reduction in lipid oxidation compared ton on-pressurized samples.	Cava et al., 2009
Pork loin, inside portion of the ham and cushion.	HPP of 215MPa for 15s with water temperature at 33°C	Lipid oxidation	• HPP inhibited the rate of lipid oxidation in ground pork samples. This might due to the low pressure (215Mpa) employed.	Souza et al., 2011
Vacuum packaged cold-smoked cod	400, 500and 600 MPa for 5 and 10 min	Lipid oxidation	• No significant differences in lipid oxidation after pressurization.	Montiel, Alba, Bravo, Gaya, and Medina, 2012

Type of meat	High pressure Processing parameters	Chemical measurements	Results	References
The subcutaneous fat and kidney fat of yak	0 min at 100, 200, 400, or 600 MPa under 4°C and 15 °C	Lipid oxidation and fatty acid composition	• At 4 °C and 5days storage, 400MPa and 600MPa yak fat sample showed a lower oxidation rate (P<0.05) compare to 200MPa sample. • Fatty acid composition analysis shows that 600 MPa treatment induced a significantly lower (p <0.05) percentage of polyunsaturated fatty acids. • Different pressure treatments affected the composition of fatty acids. High pressure significantly affected the PUFA:SFA ratios.	Wang et al., 2013
Lamb (*M. pectoralis profundus* muscle)	20mins at 200,400 and 600 MPa, and temperatures of 20, 40 and 60 °C	Lipid oxidation, fatty acid.	• Samples pressurised at 400 & 600 MPa at 60 °C resulted in the highest TBARS values. • The PUFA/SFA ratios of pressurised samples were significantly higher when compared to non-treated samples, with the exception of the milder treatments (20 °C at 200 and 400 MPa). However there were no significant effects on the n6:n3 ratios.	McArdle et al., 2013
Korean native black goat	100 MPa for 24h at 20 °C	Fatty acid	• Fatty acid content in goat meat was not significantly (P>0.05) different between control and HPP treated samples for all fatty acids detected.	Kang et al., 2013
Beef rounds	100 to 300MPa for 10mins at 25 °C	Free amino acids	• High-pressure treatment modulates the proteolytic activities of meat to improve its quality resulting in increased free amino acid content. Tryptic digestibility of the beef extract was increased at pressures higher than 400 MPa.	Ohmori and Hayashi, 1991

Type of meat	High pressure Processing parameters	Chemical measurements	Results	References
Lean beef meat	2°C with ice and water, and Pressure was applied at 100, 150, 200, 300 or 400 MPa for 5 mins.	Free amino acids	• No significant differences were observed in the amino acid and peptide content, suggesting that high-pressure treatment had no adverse effect on the components responsible for brothy and meaty flavours, and cooked flavour of meat.	Suzuki et al., 1994

2.3.2.1 Effects of HPP on lipid oxidation

Since the 1990s, HPP has been used as an alternative to thermal treatments to pasteurize food products (Dissing et al., 1997; Ma et al., 2007; Montiel et al., 2012; Nishiwaki, Ikeuchi, & Suzuki, 1996; Orlien et al., 2000; Rivalain, Roquain, & Demazeau, 2010). Foods having a high content of lipids (include trialglycerols and cholesterol-derivative) are sensitive to oxidation (Beltran et al., 2003; Cheftel & Culioli, 1997; Orlien et al., 2000; Souza et al., 2011). Over the last decade, there has been increasing interest in the response of lipid components to HPP, especially considering the deleterious outcomes that secondary oxidation-derivative molecules have on the final product (Medina-Meza et al., 2014). Ma, Ledward, Zamri, Frazier, and Zhou (2007) reported that heat and pressure were responsible for damage to cell membrane and this contributed to increased lipid oxidation.

In beef muscle, the pressure required to initiate lipid changes was reported to be lower (200 MPa) than that required for pork and chicken (Ma et al., 2007). Wang et al. (2013) further reported that yak fat was very sensitive to lipid oxidation, and showed a marked increase in malondialdehyde at 200 MPa. In contrast, in turkey meat, oxidation was promoted only if samples were treated at more than 400 MPa for 30 min, which is comparable to the effects of heat treatment (Dissing et al., 1997). In chicken breast muscle, there was no effect of HPP on lipid degradation until at 500 MPa. Only treatment at 800 MPa resulted in oxidation similar to heat treatment at 80°C (Omana, Plastow, & Betti, 2011; Orlien et al., 2000; Rivas-Cañedo et al., 2009; Wiggers, Kröger, & Skibsted, 2004).

Cheah and Ledward (1996) determined POV (peroxide value) and TBARS values in sardine oils treated by high hydrostatic pressure of up to 506 MPa for 60 min. Both indicators of the degree of oxidation did not change after treatment. However, when cod muscles were exposed separately to high hydrostatic pressures of 202, 404 and 608 MPa for 15 and 30 min, the peroxide value of the extracted oils increased with increasing hydrostatic pressure and processing time. Even more pronounced effects of high-pressure treatment were observed for mackerel muscle lipids (Ohshima, Nakagawa, & Koizumi, 1992), proving that intrinsic components of muscle could enhance lipid oxidation. The POV levels of oils extracted from the pressurized and subsequently refrigerated cod and mackerel muscles were higher than non-pressurized and

refrigerated samples (Ohshima et al., 1992). The presence or absence of oxygen was less significant in malondialdehyde formation, since cod muscles packed in nitrogen had similar oxidation state to muscles packed in oxygen (Angsupanich & Ledward, 1998). In fish products, lipid autoxidation occurred at lower pressure, since a slight increase on TBARS value was observed at 200 MPa in turbot fillets, but no triglycerides hydrolysis is revealed (Chevalier, Le Bail, & Ghoul, 2001), suggesting that the pressure treatment applied did not affect the hydrolysis mechanisms. Similarly, Gómez, Montero, Giménez, and Gómez (2007) reported that there was little but not significant increase in TBARS values of cold-smoked sardine when a pressure of 300 MPa at 20 °C was applied. More recent studies showed non-significant increase in MDA value of smoked-salmon treated up to 900 MPa for short times (0–5 min) (Gudbjornsdottir, Jonsson, Hafsteinsson, & Heinz, 2010; Montiel et al., 2012). Atlantic salmon treated at 150 and 300 MPa for 15 min however showed a marked increase in malondialdehyde (from 3.8 μmol MDA/kg to 116 μmol MDA/kg) after 6 days of storage at 4 °C (Yagiz et al., 2009).

Pressure treatment of pork fat had little effect on lipid oxidation at below 300 MPa, but increased linearly at pressures above this value. 300 to 400 MPa appeared to be a critical pressure for inducing marked changes in meat (Cheah & Ledward, 1995; Cheah & Ledward, 1996; Cheftel & Culioli, 1997). Rendered pork fat (a_w = 0.44) subjected to hydrostatic pressure of 800 MPa for 20 min showed a shorter induction time of TBARS (3 days) with respect to lower pressures (4 days, at P<800 MPa), estimated as the time necessary to reach an exponential increase of peroxide values and TBA values (Cheah & Ledward, 1995). Washed muscle fibres and minced pork treated with HPP in a range of 300–800 MPa for 20 min showed a linear relationship between pressure and TBARS number (Cheah & Ledward, 1996). Addition of citric acid (0.02%) inhibited the increased rate of lipid oxidation pork meat, eliminating the catalytic effect of pressure treatment. It is possible that metal ions ($Fe^{+2/+3}$, $Cu^{+2/+3}$) were released during HPP, generating free radicals (via the Fenton's reaction), and the addition of citric acid effectively chelated the released ions (Cheftel & Culioli, 1997).

Cava et al. (2009) reported that TBARS values of dry-cured ham and loins significantly increased even at lower pressure (200 and 300 MPa) regardless the time of treatment. However, the positive effect of holding time and pressure in secondary product formation was amplified during storage at 4 °C for 60 days at low pressures (200 and

300MPa). In another study (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010), HPP treatment at 600 MPa for 6 min at 12 °C dry-cured ham enhanced the formation of lipid derived aldehydes, such as pentanal, hexanal, heptanal and nonanal, which are responsible for off-flavours.

All the studies above confirm that HPP promoted lipid oxidation, and acted as a catalyst for lipid oxidation (Bajovic, Bolumar, & Heinz, 2012; Ma et al., 2007; Orlien et al., 2000; Vázquez et al., 2013; Wang et al., 2013). However, the mechanism by which high pressure induces lipid oxidation is still not fully understood. Studies have shown that the release of haem molecules through membranes disruption somehow triggers lipid oxidation (Cheah & Ledward, 1996; Orlien et al., 2000). The change in protein structure of metmyoglobin under high pressure makes haem molecule more accessible, which in turn eventually results in high lipid oxidation. Cheah and Ledward (1996) also concluded that 300-400 MPa of pressure constitutes the minimum critical pressure required to initiate catalysis of lipid oxidation.

The major disadvantage of lipid oxidation in HPP conditions is the loss of volatile flavours of meat. Lipids and fatty acids are precursors for the generation of volatile flavour compounds in meat. Hence increased lipid oxidation rate can reduce sensory quality (Mottram, 1998; Toldrá, Flores, & Sanz, 1997). Studies carried out to reduce lipid oxidation during HPP processing is summarized in Table 5. HPP treated chicken meat with added chelating agent (Beltran et al., 2004), rosemary extract (Bolumar, Andersen, & Orlien, 2011) and tomato extract (Alves, Bragagnolo, Silva, Skibsted, & Orlien, 2012), as well as limited oxygen exposure (Bolumar, Skibsted, & Orlien, 2012) can all reduce lipid oxidation.

Table 5 Methods employed to reduce lipid oxidation during high pressure processing.

HPP processing parameter	Method	Result	Reference
300 and 500 MPa for 30 min at 20°C; Cooked (90 °C for 15 min) - chicken breast meat.	Addition of EDTA	Acts as a chelating agent for transition metal ions. It has the ability to sequester iron ion and thus prevents lipid oxidation. EDTA significant reduced the lipid oxidation level in all treated samples.	Beltran et al., 2004
800MPa at 5°C for 10min -chicken meat.	Rosemary extract	Rosemary extract is a phenolic compound that neutralizes free metal ions by donating a hydrogen ion. This interferes with the pathway that haem molecules trigger for lipid oxidation. After long time storing (10-25 days) samples treated with anti-oxidant were shown a significant lower lipid oxidation rate compare to untreated samples.	Bolumar et al., 2011
0.1, 200, 400, 600, and 800 MPa at 5, 25, and 40°C for 5 and 10 min - chicken breast sample.	Limited oxygen exposure	Limited oxygen supply during high pressure processing can directly affect the lipid oxidation rate. Low levels means low oxidation rate.	Bolumar et al., 2012
300, 600, or 800 MPa - chicken breast meat.	Tomato extract	A waste product from industrial tomato paste production was found to yield efficient protection against lipid oxidation in pressurized (especially 800MPa) chicken meat.	Alves et al., 2012

2.3.2.2 Effects of HPP on fatty acid composition

Fatty acid composition of meat is important in determining the quality traits of meat such as nutritional value, flavour, and textural properties. It varies widely depending on species, degree of trimming, nature of processing/cooking, and on the preservation techniques employed (Gerber, Scheeder, & Wenk, 2009). Many researchers have studied the influence of HPP on meat quality (Cava et al., 2009; Cheah & Ledward, 1995; Cheah & Ledward, 1996; Chevalier et al., 2001; Ma et al., 2007; Mor & Yuste, 2003; Ohshima et al., 1992; Tanzi et al., 2004; Wang et al., 2013). McArdle et al. (2013) reported that the fatty acid composition of non-treated lamb M. *pectoralis profundus* was similar to other livestock species reared for meat production The majority of fatty acids in muscle lipids were oleic (C18:1), palmitic (C16:0) and stearic (C18:0). The most abundant fatty acid was oleic acid (C18:1) (Kanatt, Chander, & Sharma, 2006; Kang et al., 2013).

McArdle, Marcos, Kerry, and Mullen (2010) and Kang et al. (2013), observed no significant differences in the fatty acid composition of non-treated and pressurised samples of beef *Pectoralis profundus* muscles and Korean native black goat meat respectively. McArdle et al. (2010) explained that the variability in fatty acid composition among animals could have influenced these results. As fatty acids are a major contributor to various aspects of meat quality and are central to the nutritional value of meat (Wood et al., 2008), it is vital to investigate the effects of HPP on fatty acid composition. Changes in fatty acid composition can affect the sensory perception and nutritional value of meat.

The ratio of omega 6 to omega 3 PUFAs (n6: n3) ratio is also important, as it is a risk factor in cancer and coronary heart diseases (Enser et al., 2000). The recommended ratio is less than 4. The n6: n3 ratio is particularly beneficial in ruminant meats, especially from animals that have consumed grass, which contains of 18:3 (Wood et al., 2004). According to McArdle et al. (2013), high pressure (400 MPa at 40 °C) had no significant effect on n6: n3 ratios with the exception samples treated. Moreover, the n6: n3 ratios in all treated samples remained within the recommended levels, with ratios of n6:n3 between untreated and pressurised samples in the range of 1.03–2.12. Fatty acid profiles monitored over 30 days of storage (-2 °C) were also not significantly different.

Yagiz et al. (2009) reported on the fatty acid profile of pressure treated, cooked and control Atlantic salmon dark muscle during 6 days of storage at 4 °C. The source of total saturated fatty acids was mainly from 14:0, 16:0 and 18:0 fatty acids. There was no significant difference in total saturated fatty acid composition between control and pressure treated (150 MPa, 300 MPa) samples. The saturated fatty acid composition was also not significantly affected by storage at 4 °C for 6 days. However, cooked samples were significantly lower in total saturated fatty acids than pressure treated and control samples. The pressure levels 150 MPa and 300 MPa did not lead to significant differences in 20:1n-7 and 22:1n-11 fatty acids at days 0, 2, and 4 compared to control samples. However, cooked samples had significantly higher levels of these monounsaturated fatty acids than both pressure treated and control samples during the entire storage period.

The polyunsaturated/saturated fatty acid (PUFA/SFA) ratios for lamb and beef is typically 0.1 (Badiani et al., 2002; Enser et al., 2000) but it can be higher in some muscles (Wood et al., 2004). Factors that affect this ratio include animal breed, sex and nutrition (Enser et al., 2000). The PUFA: SFA ratios of both untreated and pressurised samples were in the range of 0.21–0.54. McArdle et al. (2013) reported that the PUFA: SFA ratios of pressurised lamb M. *pectoralis profundus* muscle) samples were significantly higher when compared to non-treated samples, with the exception of samples subjected to milder treatments (20 °C at 200 and 400 MPa).

McArdle et al. (2010) indicated that while some alterations of individual fatty acids were observed in lamb M. *pectoralis profundus* muscles, high pressure had no effect on polyunsaturated/saturated fatty acid (PUFA/SFA) or n-6/n-3 (n6/n3) ratio. Moreover, the temperature at which HPP was applied had a significant effect on the sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. HPP at 40 °C showed higher SFA and PUFA, and lower MUFA compared to HPP at 20 °C. These results show that high pressure at low or moderate temperatures improves the microbiological quality of the meat with minimal effects on meat quality. Similarly, no differences in n6/n3 ratios were found in oysters pressure treated at 260, 500 and 800 MPa for 3–5 min (Cruz, Kerry, & Kelly, 2008).

Wang et al. (2013) analysed variations in SFAs, MUFAs, and PUFAs composition in yak fat during 4 °C storage after HPP processing. The ratio of PUFA: SFA decreased continuously, particularly at the end of storage, and the most prominent decrease was after 600 MPa treatment. A significant decrease at the end of storage was observed in the PUFA/SFA values for all samples, and a larger decrease ($p < 0.01$) in the PUFA/SFA values after 600 MPa treatment was observed. Considerable effort has been invested to improve the PUFA/SFA ratio in meat, either by improving the animal diet or by adding ingredients with high PUFA content during meat processing (Serra et al., 2007; Wood et al., 2004).

2.3.2.3 Effects of HPP on free amino acid content

High pressures between 100 and 300 MPa for 10 min at 25 ˚C increased the overall autolytic activity of raw beef meat leading to a higher concentration of free amino acids (Ohmori & Hayashi, 1991). However, with higher pressure treatments at 400 MPa and 500 MPa, the concentration of free amino acids was identical to that of control sample over one week of chilled storage.

Suzuki et al. (1994) reported that serine, glutamic acid, glutamine, glycine and alanine content gradually increased with increasing pressure applied to lean beef meat up to 200 MPa. However, some amino acids, especially glutamine and alanine, decreased in the muscle pressurized at 300 MPa. However, no significant differences in all 10 amino acids were observed suggesting that high pressure treatment had no adverse effect on the components responsible for brothy and meaty tastes and cooked flavour of meat.

Campus et al. (2008) investigated the effect of pressures of 300, 350, 400MPa on sliced and vacuum packaged commercial dry-cured pork loin. Only the untreated samples showed an increase in free amino acid content during vacuum storage. High pressure (300 to 400 MPa for 10 min at 20 ◦C) was found to stabilize the free amino acid content during storage due to reduction in amino peptidase activities.

Chapter 3 Materials and Methods

3.1 Preparation of lamb samples used in this study

Eight cuts, inside, heel, knuckle, rump, tenderloin, eye of loin, bolar and flat were used in this study. All meat samples were obtained from Ag Research (Hamilton, New Zealand) (four animals mean cold carcasses weight of 140.5-150.5 kg). All samples were stored at 4 °C for 48 hours after slaughter. Each cut was individually then packed and hermetically sealed in high density polyethylene bags at 4°C ± before HPP processing (n=3).

3.2 HPP processing

Pressurization of lamb was conducted using an industrial scale HPP equipment (HPP 055, Multivac, Multivac Sepp Haggenmüller GmbH & Co., Wolferschwenden, Germany). Water was used as the pressure-transmitting medium, with the initial temperature around 7-8^{o}C. The temperature reached after pressure build up was less than 25^{o}C. The rate of pressure build up was conducted at 125 MPa/min. Packaged Lamb samples were loaded in a cylindrical loading container and HPP-treated at 200, 300 400 and 500 MPa. Pressure was held for one minute once the targeted pressure was achieved. After depressurisation, all samples were transported and stored at - 20 °C ± for further analysis.

3.3 Lipid oxidation

Samples were thawed at room temperature (24 ± 2 ^{o}C) for between 1-2 hours. Lipid oxidation was determined using the 2-thiobarbituric acid reactive substances (TBARS) method according to Nam and Ahn (2003). The method was modified by measuring the amount of malondialdehyde (MDA) present in the sample. Minced meat sample (3.0 g) was homogenised using a homogenizer mixer (Janke Kunkel IKA Labortechnik Ultra Turrax T25, Breisgau, Germany) in 9.0 mL deionised distilled water at 14,000 rpm for 30 seconds. Lamb homogenate (1 mL) was obtained and transferred to a disposable test tube. This was followed by addition of 50 µL of butylated hydroxytoluene (BHT) (7.2% w/v in ethanol) and 2 ml thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA and 15% (w/v) TCA). The mixture was vortexed and then incubated in a

90 °C water bath for 30 min until pink colour was present. Samples were then cooled down in a water and ice bath for 10 min, and centrifuged at 3500rpm for 15 min at 5 °C. The absorbance of the resulting upper layer was measured at 531 nm using a spectrophotometer (Ultraspec 7000 Pro spectrophotometer, Biochrom Ltd, Cambridge, England) as shown in Figure 5, and the absorbance measured against a blank prepared with 1 ml deionised water and 2 ml TBA/TCA solution.

Figure 5 Ultraspec 7000 Pro spectrophotometer used in this study

The results are expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde (MDA) per kg of meat using a standard curve constructed using tetraethoxypropane (TEP). Mean values are then obtained for duplicate samples (n = 2).

TEP (Tetraethoxypropane) standard curve preparation

The following solutions were prepared using a stock solution of 41.76µM TEP (100µL of 4.176mM TEP + 10mL of double distilled water (DDH_2O). Further dilutions were made from the stock solution (Table 6).

Table 6 Serial dilution for construction of the MDA standard curve.

Standard	TEP solution(µL)	DDH_2O(mL)	MDA concentration
1	0	2	0.000
2	40	1.96	0.2023
3	120	1.88	0.6069
4	240	1.76	1.2138
5	500	1.5	2.529
6	800	1.2	4.046
7	1200	0.8	6.069

An aliquot of 500 µL of 6M HCl was added to each standard dilution, and then 2mL of thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA and 15% (w/v) TCA) were added. The mixture was vortexed and then incubated in a 90 °C water bath for 30 min until the solution turned pink in colour. Samples were then cooled down in a water and ice bath for 10 min, and centrifuged at 3500 rpm for 15 min at 5 °C. The absorbance of the resulting upper layer was measured at 531 nm.

3.4 Fatty acids analysis

Quantification of total fatty acids was carried out according to Juárez et al. (2008) by acid hydrolysis of lipids in lyophilized samples to release free fatty acids. This is followed by in situ esterification to fatty acid methyl esters (FAMEs) and their extraction into toluene for analysis by a Shimadzu GC2010 GLC gas chromatography (GC).

Samples were thawed at room temperature (24 ± 2 ^{o}C) for between 1-2 hours, and further lyophilized for 48 hours until completely dried. Then approximately 20mg samples were weighed into 10 ml test tubes, and the weight was recorded. A 10µL aliquot of 2g/L tridecanoic acid in toluene was added as internal standard followed by further addition of 490µl of toluene and 750µL of freshly prepared 5% methanolic HCl. The mixture was mixed using a vortex and the headspace of each tube was filled with

nitrogen. The tubes were then sealed and incubated in a water bath at 70°C for 2 hours. After tubes were cooled down to room temperature, 1 mL of 6% aqueous K_2CO_3 and 500 µL toluene were added. The mixture was vortex gently to mix, and then centrifuged at 2000 rpm for 5 mins. The organic phase was then removed using a glass Pasteur pipette for analysis of FAME content.

For FAME analysis, a Shimadzu GC2010 GLC equipped with a Flame Ionisation Detector (FID) (Figure 6), a split injector and an AOC-20i auto-injector was used. The Phenomenex Zebron ZB-WAX capillary column (0.25mm x 30m x 0.25µm) was used with Nitrogen as a carrier gas. The pressure was set to 43 Pa, the flow rate was 7 mL/min, and the initial oven temperature was 140 °C, increased to 245 °C at a rate of 5 °C/min, and held for 15 minutes at this temperature. FAME peaks were identified and quantified by comparison with the retention times and peak areas of 37 FAME standards (Supelco product 47885-U, Sigma Aldrich, Sydney, Australia), which were serially diluted to five concentrations from 10 to 0.625 g /L.

Figure 6 Shimadzu GC2010 gas chromatograph used in this study equipped with a Flame Ionisation Detector.

3.5 Free amino acids

Methanol (1 mL) was used to extract free amino acids (FAA) from freeze dried meat sample according to Mustafa, Åman, Andersson, and Kamal-Eldin (2007), with modifications. Meat sample (0.1 g) was weighed into a centrifuge tube and 1mL of methanol added. The mixture was vortexed and then centrifuged at 2000 xg for 2mins. A commercial free amino acid kit (EZ: faastTM, Phenomenex®, USA) was used to profile amino acids (user manual shown in Appendix). All steps, including the solid phase extraction (SPE) sample clean-up, elution from SPE sorbent, derivatisation, and analysis, were performed, as described in the manual provided. 0.2mM Norvaline in N-propanol solution was used as an internal standard.

AA derivatives were separated and quantified on a Shimadzu GC2010 GC with a Flame Ionisation Detector (GC-FID), a split injector and an AOC-20i auto-injector. The ZB-AAA GC column (10m* 0.25mm*0.25um) included in the kit was used. The instrument setting used were recommended by the user manual with modifications to obtain better separation of individual free amino acid. The derivatised samples recovered were injected (1uL) at a split ratio of 1:15 at 300℃ into the column. Initial oven temperature was 50℃. It was then raised to 120℃ at a rate of 50℃ per minute, and held for 30 seconds. Temperature was again increased to 165℃ at a rate of 5℃ per minute. Once the temperature reached 165℃, it was further increased at a rate of 20℃ per minute to reach 320℃ and held there for 1 minute. Nitrogen/air with a column flow at 1.46 mL/min was used as carrier gas.

Amino acids in samples were identified and quantified by comparison of their retention times with those produced from a mixture of 26 AA standards included in the kit at four calibration levels, ranging from 400 to 50 nmol/mL. All peak areas in the chromatogram were corrected to the area and concentration of the internal standard peaks before any compositional calculations were made. The concentration of each amino acid identified in the samples was presented as mg of amino acid in 1g of HPP lamb sample.

3.6 Statistical analysis

The experimental data in this study was collated by Microsoft Office Excel 2011 and subjected to statistical analysis using the XLSAT MX software release 2010 (Addin soft, USA). Analysis of variance (ANOVA) was carried out at the 0.05 level of significance to analyse the effect of HPP processing (control, P200, P300, P400 and P600) on lipid oxidation, fatty acid, and amino acid content in 8 different lamb cut samples (tenderloin (T), rump (R), knuckle (K), inside (I), heel (H), flat (F), eye of loin (E) and bolar (BL) muscles). A two-way analysis of variance was carried out on the fatty acid profiles for each pressure treatment. When ANOVA was significant (p values less than 0.05), means were separated by pairwise comparison using the Fisher's least significant difference test.

Chapter 4 Result and Discussion

4.1 Lipid oxidation

4.1.1 Lipid oxidation value among different control cuts

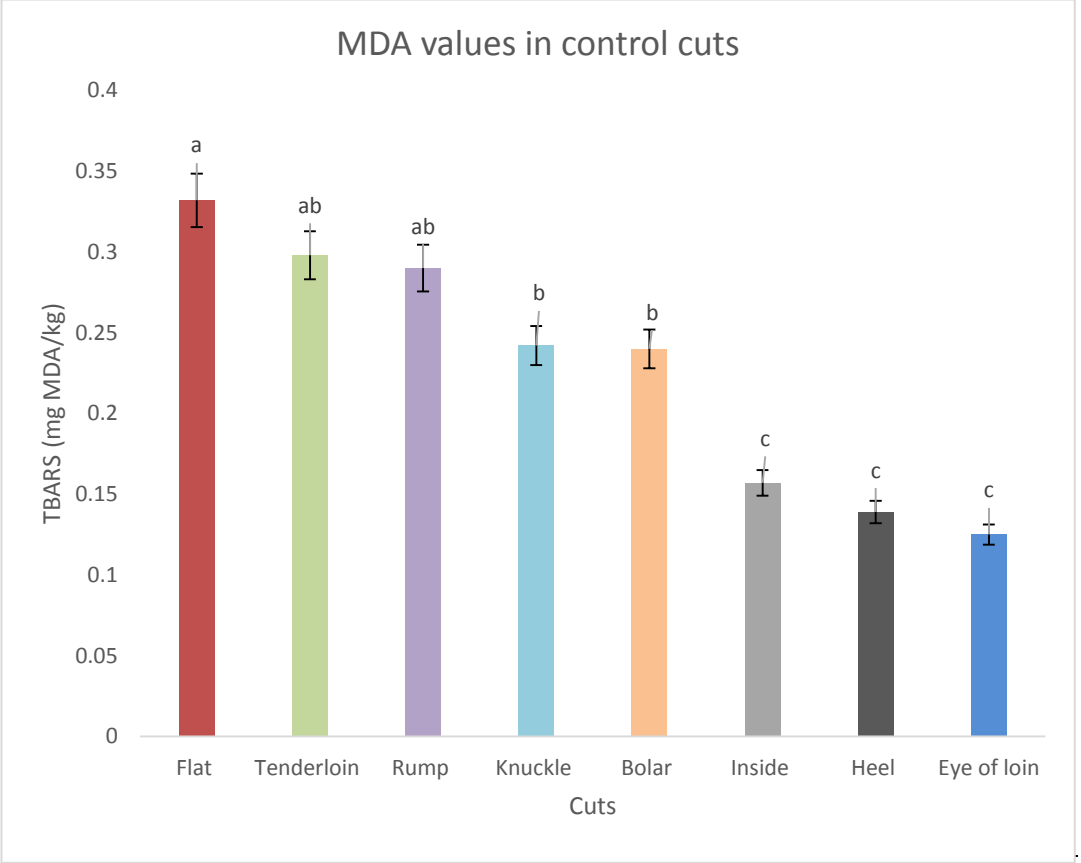
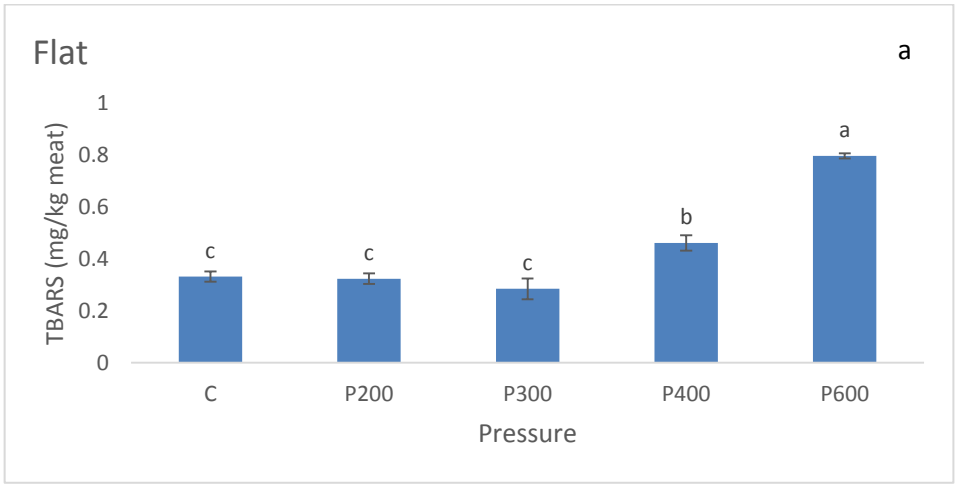


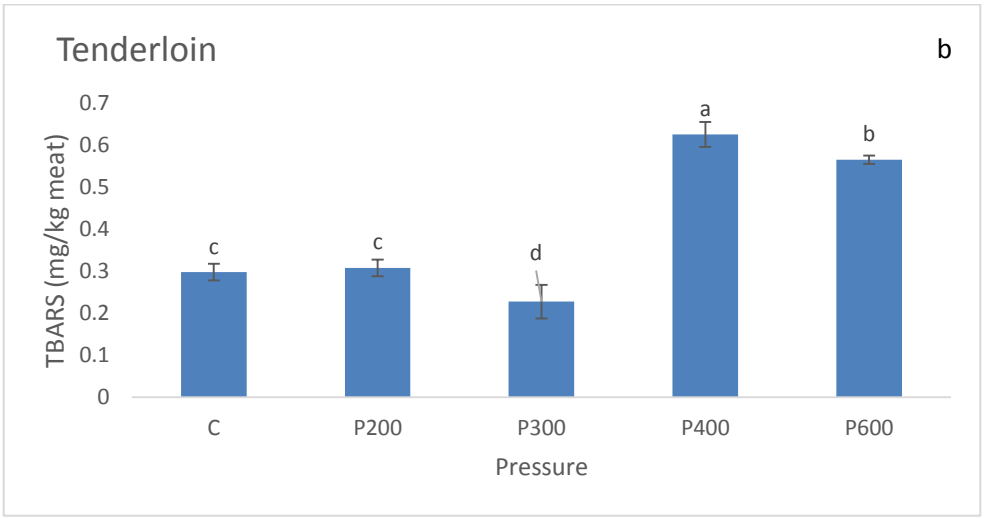
Figure 7 Changes in lipid oxidation marker (TBARS) in different control cut samples. Note: [a, b, c] mean of TBARS value with different cut samples, differs significantly using Fisher's least significant difference ($p < 0.05$).

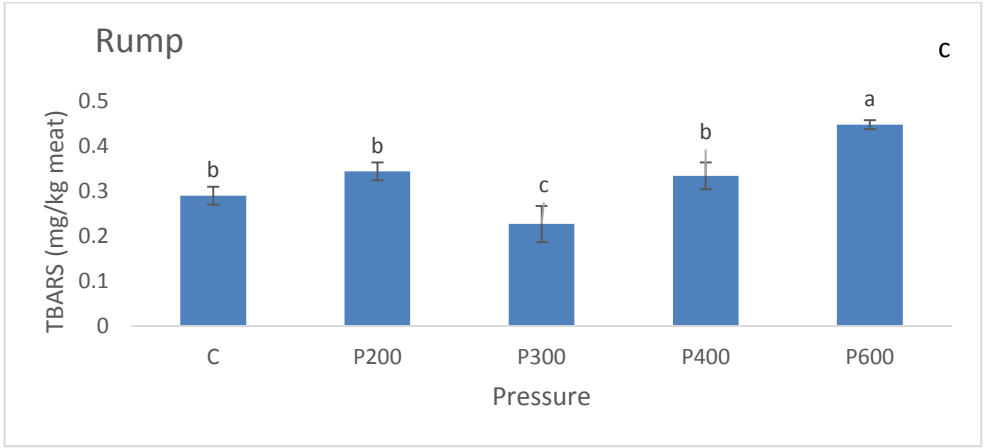
In this study, the lipid oxidation levels of eight different cuts (flat (F), tenderloin (T), rump (R), knuckle (K), bolar (BL), inside (I), heel (H) and eye of loin (E)subjected to high pressure processing (0MPa, 200MPa, 300MPa, 400MPa and 600MPa) were determined. Lipid oxidation is a very important factor that affects lamb meat quality and acceptance, according to many researchers (Frankel, 2014; Linares et al., 2007; Maqsood et al., 2015; Shahidi & Zhong, 2010; Vázquez et al., 2013), As lipid content may vary in different animal muscles, the level of lipid oxidation may similarly vary as well. However different muscles are not the only factor that can influence the level of lipid oxidation. Breed, age, gender and other factors also determine the content of fat in beef muscles and can influence the oxidation of lipids (Rhee, Anderson, & Sams, 1996).

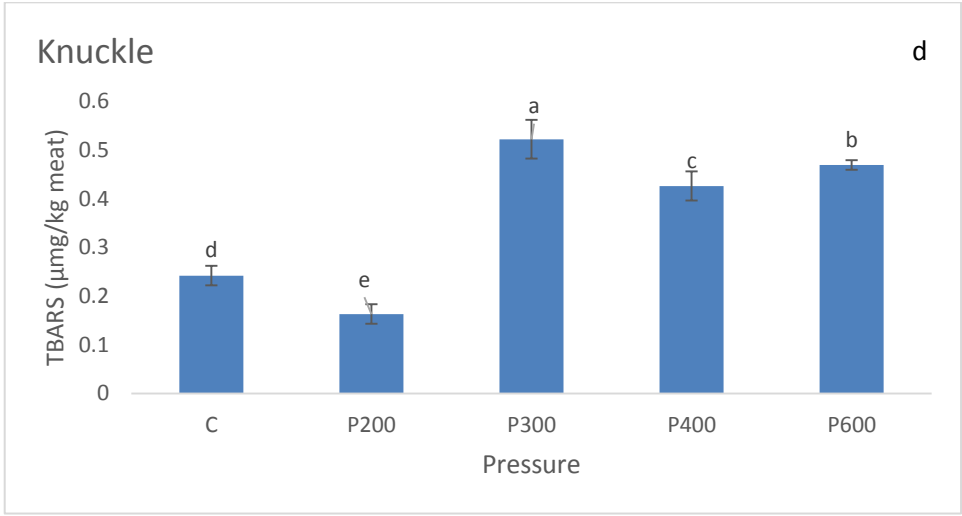
Significant differences were found in overall oxidation level with the eight different types of cut. According to Park et al. (2007), TBARS values were not affected by packaging method or storage time ($P>0.05$), but were affected by pork meat cuts (belly and loin). Oxidation values of belly cut were higher ($P<0.05$) than loin. In contrast, Kannan, Kouakou, and Gelaye (2001) analysed different cuts of goat meat and reported no significant differences ($P>0.05$) in overall oxidation level in leg shoulder (cut heel), arm (cut flat), and loin/rib (cut eye of loin) cuts.

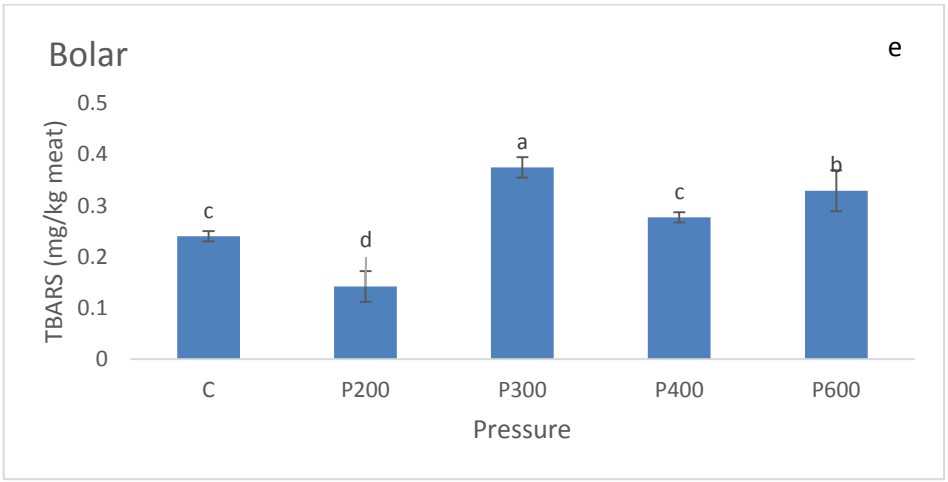
All the values in control cut samples were below 0.5 mg MDA/kg (0.13 to 0.33 mg MDA/kg). However, according to Wood et al. (2008) TBARS values above 0.5 mg MDA/kg will produce a rancid flavour, which can be detected by consumers. Similarly, Bohac, Rhee, Cross, and Ono (1988) analysed beef *longissimus dorsi* (eye of loin), psoas major (tenderloin), *semimembranosus* (inside), and *semitendinosus* (heel) muscles and reported values between 0.24 to 0.35 mg MDA/kg. Figure 7 showed that flat (0.33 mg MDA/kg), tenderloin (0.30mg MDA/kg) and rump cuts (0.29 mg MDA/kg) had significantly higher TBA values ($P<0.05$) than other cuts. Heel and eye of loin cuts had the significantly lowest TBARS value ($P<0.05$). These findings are supported by Badiani et al. (2002) who reported that flat cut had the highest lipid content compared to eye of loin, and heel cuts of raw beef muscles. Rhee, Ziprin, Ordonez, and Bohac (1988) further reported that the potential of porcine muscle lipids to undergo lipid oxidation may vary substantially among the same retail cuts and different animals even if the post-mortem history of the meat is similar. Results from our study on different lamb cuts support this.

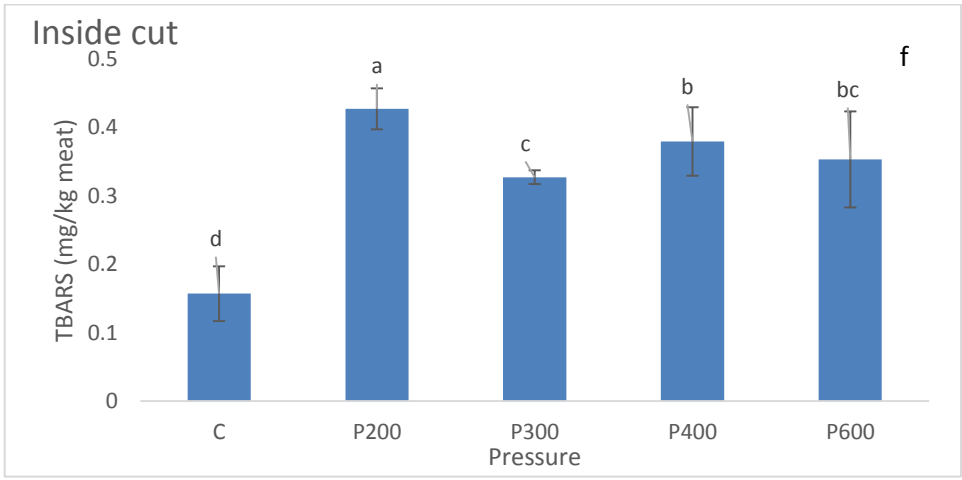

Flat
a
TBARS (mg/kg meat)
1
0.8
0.6
0.4
0.2
0
c
c
c
b
a
C
P200
P300
P400
P600
Pressure

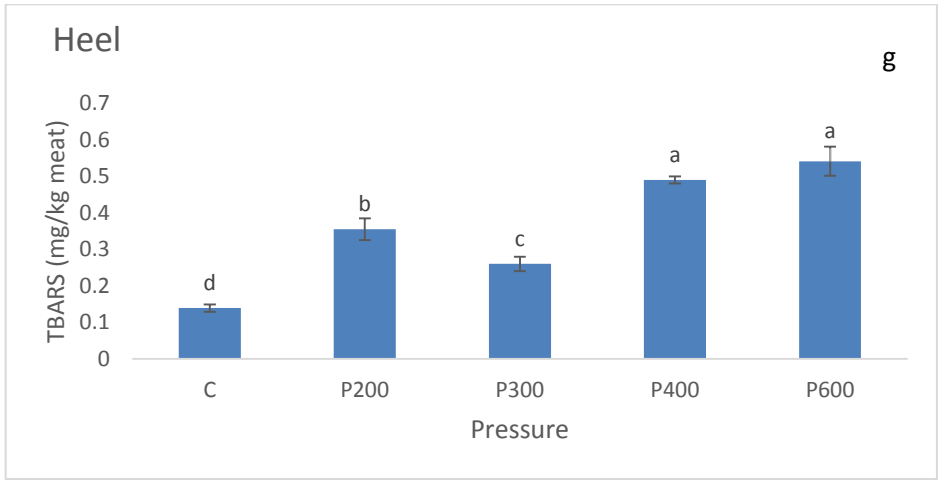

Tenderloin
b
TBARS (mg/kg meat)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
c
c
d
a
b
C
P200
P300
P400
P600
Pressure


Rump
c
TBARS (mg/kg meat)
0.5
0.4
0.3
0.2
0.1
0
b
b
c
b
a
C
P200
P300
P400
P600
Pressure


Knuckle
d
TBARS (µmg/kg meat)
0.6
0.5
0.4
0.3
0.2
0.1
0
d
e
a
c
b
C
P200
P300
P400
P600
Pressure







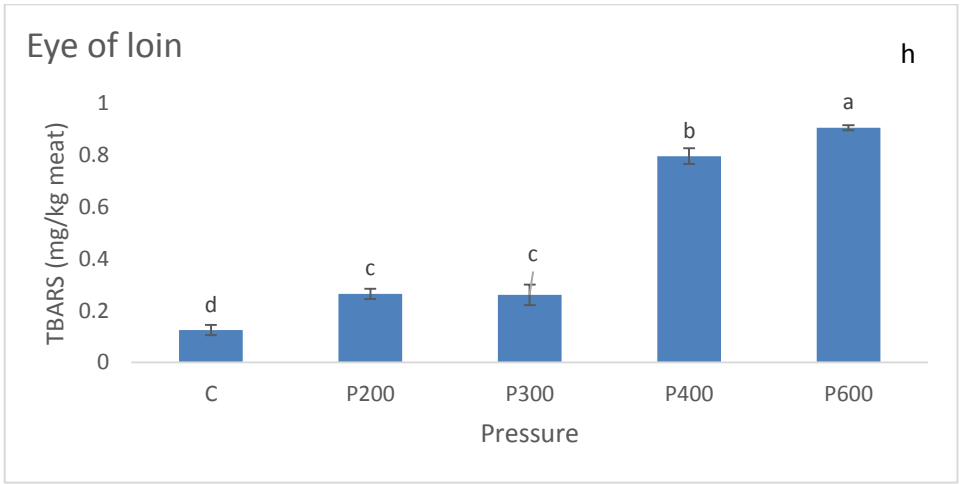


Figure 8 TBARS values of different lamb cuts subjected to high pressure processing.
Note: [a,b,c,e,f] mean of TBARS value with different cut samples, differs significantly using Fisher's least significant difference ($p < 0.05$).

4.1.2 Effect of different HPP treatments on lipid oxidation value

As mentioned above, the TBARS value above 0.5 mg MDA/kg are considered level of lipid oxidation products will produce a rancid flavour. HPP treatment at higher pressures resulted in some samples having high TBARS values exceeding 0.5 mg MDA/kg. Specifically, the eye of loin cuts (Figure 8h) had significantly highest ($P<0.05$) MDA values when treated at 400MPa (0.80mg MDA/kg) and 600MPa (0.9mg MDA/kg), followed by flat cut (Figure 8a) at 600MPa sample (0.8mg MDA/kg), heel cut (Figure 8g) at 600MPa sample (0.54mg MDA/kg), and tenderloin cut (Figure 8b)400MPa (0.63mg MDA/kg) and 600MPa (0.57mg MDA/kg). Similarly, McArdle et al. (2013) reported significant ($P<0.05$) increase in TBARS values of lamb M. *pectoralis profundus* muscle after 30 days' storage with increase in pressure (200MPa, 400MPa and 600MPa at 60°C) during high pressure treatment.

Only knuckle cut (Figure 8d) had a TBARS value above 0.5 mg MDA/kg at 300MPa (0.52mg MDA/kg). Similarly, H. Ma et al. (2007) reported on TBARS value of beef muscle (stored for 7 days at 4°C) and treated at 20 °C. The highest TBARS level ($P<0.05$) was achieved at 400MPa (0.67mg MDA/kg), that then decreased at 600MPa (0.52mg MDA/kg) and 800MPa (0.41mg MDA/kg).

In general, the higher levels of pressure (400MPa and 600MPa) employed, resulted in higher oxidation values exceeding 0.5 mg MDA/kg in eye of loin (Figure 8h), flat (Figure 8a), heel (Figure 8g) and tenderloin (Figure 8b) cuts except knuckle (Figure 8d) that had the highest oxidation value at 300MPa. On the other hand, lower pressures of, 200MPa and 300MPa, resulted in oxidation values less than 0.5 mg MDA/kg for all cuts except the knuckle cut at 300 MPa. In previous studies (Cheah & Ledward, 1996; Ma et al., 2007), elevated pressures at room temperature (24 ^{o}C ±) decreased the oxidative stability of red meat. The pressures required to initiate these changes were lower for beef (200 MPa), compared to pork (300 MPa) and chicken (600 MPa), although the post-slaughter history of the samples varied (Ma et al., 2007). It has been postulated that this phenomenon is due to the release of 'free' iron from the iron complexes present in meat. As the concentration of 'free' iron increased in red meat samples after pressure treatment (Defaye, Ledward, MacDougall, & Tester, 1995; Ma et al., 2007; Wang et al., 2013), chelating agents, such as EDTA, effectively prevented the increased rates of oxidation seen in pressure-treated pork (Cheah & Ledward, 1995). It is also possible

that the effects of pressure may relate to changes in the integrity of the cell membrane (Beltran et al., 2003).

4.2 Fatty acids

4.2.1 Fatty acids value among different control cuts

In this study, the fatty acid composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in selected New Zealand lamb meat cuts is summarised in Table 7. Fatty acid composition also contributes to quality traits of meat such as nutritional value, as well as flavour, and textural properties. It varies widely depending on species, degree of trimming, nature of processing or cooking, and preservation techniques employed (Gerber et al., 2009). Furthermore, Schmid (2010) indicated that most common meats (lamb, beef, pork) have similar proportion of saturated (45% to 50%) and monounsaturated fatty acids (38% to 43%), and provide a small quantity of polyunsaturated fatty acids (12% to 20%).

The results in our study were similar to fatty acid composition of other livestock species reared for meat production (Wood et al., 2004). Major fatty acids in lamb muscle lipids were oleic acid (C18:1), palmitic acid (C16:0) and stearic acid (C18:0) (Kanatt et al., 2006), similar to our results. Saturated fatty acids present in this study included C16:0, C17:0, C18:0, C20:0, C22:0 and C23:0 (Table 7). These fatty acids except for C23:0 were significantly higher in the bolar cut, followed by the eye of loin, knuckle, tenderloin, and flat cuts. In contrast, these fatty acids were the least significant ($P<0.05$) for inside, rump and heel cuts. Similarly, Rhee et al. (1988) analysed the fatty acids composition of beef rump, tenderloin, inside and heel cuts and showed that C18:0 in tenderloin was significantly ($P<0.05$) higher compared to rump, inside and heel cuts. Badiani et al. (2002) analysed fresh and cooked beef bolar, flat and heel cuts and similarly reported that bolar cut contained the highest ($P<0.05$) level of fatty acids compared to flat and heel cuts. In addition, the value of total saturated fatty acids in bolar cut was about two times more than flat and heel cuts, which was again similar to our result.

The monounsaturated fatty acids in this project were C16:1, C17:1 and C18:1n-9. Kelly et al. (2001) reported that oleic C18:1 was the most abundant monounsaturated fatty acids in meat product similar to our study. The eye of loin cut had the significantly highest ($P<0.05$) level of C16:1, C17:1, C18:1n-9, and total monounsaturated fatty acids. Inside and rump cuts had the significantly least ($P>0.05$) C16:1, C17:1, C18:1n-9 fatty acids, and total monounsaturated fatty acids, content compared to other cuts. Similarly,

Badiani et al. (2002) analysed fresh and cooked beef bolar, flat and heel cuts. They found that the monounsaturated fatty acids in bolar cut was significantly higher ($P<0.05$) than flat and heel cuts.

The polyunsaturated fatty acids reported in this study included 18:2n-6, 18:3n-6, 18:3n-3, 20:4n-6, 20:5n-3 and 22:2n-6 fatty acids. Kelly et al. (2001) reported that linoleic C18:2 was the major unsaturated fatty acid in meat product similar to our study for all cuts. Generally, tenderloin cut had the significantly ($P<0.05$) highest level of PUFAs. The least significant ($P<0.05$) levels of PUFAs were present in flat, heel and inside cuts. Similarly, Manner, Maxwell, and Williams (1984) reported significantly higher level of C18:2 in tenderloin cut than the heel cut in steers. Badiani et al. (2002) also reported that total polyunsaturated fatty acids in beef bolar cut were significantly ($P<0.05$) higher than flat and heel cuts. However, Rhee et al. (1988) found no significant ($P>0.05$) differences among inside, heel, rump, and tenderloin cuts in porcine meat. The levels of C18:3n-6, C20:4n-6, C20:5n-3 and C22:2n-6 were very low in all samples, and did not affect the total PUFA value.

According to Wood et al. (2004), the polyunsaturated/saturated fatty acid (PUFA/SFA) ratios for lamb is typically 0.1 but can be higher in some muscles. A higher PUFA to SFA ratio ($\geq$ 0.4) is desirable to decrease the risks of cardiovascular disease and metabolic syndrome (Haffner, 2006). Factors that affect this ratio include animal breed, sex and nutrition (Enser et al., 2000). Our result showed that the PUFA/SFA ratios of heel (0.43), inside (0.53) and rump (0.51) cuts were higher than 0.4.

The ratio of omega 6 to omega 3 PUFAs (n6: n3) ratio shown in Table 7 is also important as it is a risk factor in cancer and coronary heart diseases (Enser et al., 2000). The recommendation is for a ratio of less than 4 (Wood et al., 2004). The n6:n3 ratios in this study ranged from 2.65 to 3.90 for all samples that falls with the recommended ratio.

Table 7 Fatty acid composition (g/100g) of different New Zealand lamb meat cuts.

Fatty acids/Cuts	Flat	Tenderloin	Rump	Knuckle	Bolar	Inside	Heel	Eye of loin	P-value
C16:0	8.02±0.61^{d}	10.84±0.29^{c}	4.5±0.44^{e}	10.53±0.02^{c}	14.96±0.03^{a}	3.8±0.16^{f}	5.14±0.11^{e}	14.08±0.09^{b}	****
C16:1	0.56±0.05^{e}	0.71±0^{c}	0.35±0.02^{fg}	0.62±0.01^{d}	0.77±0.03^{b}	0.31±0.01^{g}	0.39±0.02^{f}	0.9±0.01^{a}	****
C17:0	0.44±0.04^{e}	0.73±0.05^{c}	0.32±0^{f}	0.67±0.01^{d}	1.06±0^{a}	0.27±0^{g}	0.35±0.01^{f}	0.82±0.01^{b}	****
C17:1	0.31±0.01^{cd}	0.41±0.09^{ab}	0.22±0.01^{e}	0.37±0.01^{bc}	0.48±0^{a}	0.21±0.01^{e}	0.25±0de	0.46±0.01^{a}	****
C18:0	5.58±0.59^{d}	11.63±0.18^{b}	4.58±0.17^{e}	10.86±0.05^{c}	15.86±0.21^{a}	3.2±0.23^{f}	4.63±0.15^{e}	11.33±0.06^{bc}	****
18:1n9	12.77±0.69^{d}	16.29±0.5^{b}	6.89±0.72^{f}	14.53±0.05^{c}	16.29±0.03^{b}	6.13±0.26^{f}	8.27±0.3^{e}	20.28±0.12^{a}	****
18:2n6	2.28±0.27^{e}	5.64±0.15^{a}	3.19±0.07^{c}	3.96±0.03^{b}	2.6±0.09^{de}	2.43±0.22^{de}	2.78±0.1^{d}	3.48±0.22^{c}	****
18:3n6	0.21±0.05^{c}	0.4±0.08^{b}	0.24±0.05^{c}	0.39±0.02^{b}	0.68±0.03^{a}	0.15±0.04^{c}	0.23±0^{c}	0.49±0.06^{b}	****
18:3n3	0.72±0.03^{de}	1.38±0.02^{a}	0.76±0.03^{d}	1.12±0.01^{c}	1.29±0.02^{b}	0.64±0.03^{f}	0.71±0^{e}	1.1±0.02^{c}	****
C20:0	0.27±0.03^{c}	0.41±0.01^{b}	0.28±0.02^{c}	0.39±0.01^{b}	0.53±0.02^{a}	0.24±0.01^{c}	0.26±0.03^{c}	0.41±0.02^{b}	****
20:4n6	0.35±0.12	0.44±0.05	0.45±0.01	0.41±0.02	0.25±0	0.41±0.01	0.37±0.09	0.28±0.09	ns
20:5n3	0.32±0.03^{ab}	0.34±0.01^{a}	0.34±0^{ab}	0.31±0.01^{b}	0.18±0^{d}	0.33±0.03^{ab}	0.33±0^{ab}	0.27±0^{c}	****
C22:0	0.19±0.02	0.23±0.05	0.19±0	0.22±0.01	0.24±0.01	0.17±0	0.18±0	0.2±0.01	ns
22:2n6	0.21±0.05^{cde}	0.25±0.01^{cd}	0.19±0.02^{ef}	0.26±0.02^{c}	0.39±0.01^{a}	0.14±0.01^{f}	0.2±0.03^{de}	0.32±0.01^{b}	****
C23:0	0.12±0.12	0.1±0.08	0.23±0	0.24±0.03	0.2±0	0.14±0	0.12±0.02	0.1±0.18	ns
SFA	14.62±1.4^{d}	23.94±0.55^{c}	10.09±0.63^{e}	22.91±0.07^{c}	32.85±0.21^{a}	7.82±0.4^{f}	10.69±0.31^{e}	26.92±0.33^{b}	****
MUFA	13.64±0.75^{d}	17.4±0.59^{b}	7.45±0.75^{f}	15.53±0.05^{c}	17.53±0^{b}	6.65±0.28^{f}	8.91±0.32^{e}	21.65±0.1^{a}	****
PUFA	4.09±0.54^{f}	8.45±0.32^{a}	5.16±0.07^{de}	6.46±0.05^{b}	5.4±0.15^{cd}	4.1±0.26^{f}	4.62±0.22^{ef}	5.93±0.35^{bc}	****
n-3	1.04±0.05^{d}	1.73±0.03^{a}	1.1±0.03^{d}	1.43±0^{b}	1.48±0.02^{b}	0.97±0.01^{e}	1.04±0^{d}	1.36±0.02^{c}	****
n-6	3.04±0.49^{e}	6.73±0.29^{a}	4.06±0.1^{cd}	5.03±0.05^{b}	3.92±0.13^{d}	3.13±0.26^{e}	3.58±0.22^{de}	4.57±0.37^{bc}	****
PUFA/SFA	0.28±0.01^{d}	0.35±0.01^{c}	0.51±0.01^{a}	0.28±0^{d}	0.16±0^{f}	0.53±0.01^{a}	0.43±0.01^{b}	0.22±0.02^{e}	****
n6:n3	2.92±0.21^{cd}	3.9±0.14^{a}	3.7±0.15^{ab}	3.5±0.05^{ab}	2.65±0.02^{d}	3.22±0.23^{bc}	3.43±0.22^{ab}	3.35±0.41^{bc}	***
total	32.34±2.7^{d}	49.79±1.46^{b}	22.71±1.31^{e}	44.9±0.03^{c}	55.77±0.36^{a}	18.57±0.94^{f}	24.21±0.85^{e}	54.5±0.78^{a}	****

Note: Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly within cuts. SFA stands for saturated fatty acids; MUFA stands for monounsaturated fatty acids; PUFA stands for polyunsaturated fatty acids. $P < 0.0001$ presented as ∗∗∗∗for level of significance; $P < 0.001$ presented as ∗∗∗for level of significance; $P < 0.01$ presented as ∗∗level of significance; $P < 0.05$ presented as ∗ for level of significance and ns meaning not statistically significant.

Table 8 Fatty acid composition (g/100g) of New Zealand lamb cuts after HPP treatment (200MPa, 300MPa, 400MPa and 600MPa).

Cuts	Condi -tion	C16:0	C16:1	C17:0	C17:1	C18:0	18:1n9	18:2n6	18:3n6	18:3n3	C20:0	20:4n6	20:5n3	C22:0	22:2n6	C23:0
Flat	C	8.02±0.02^{a}	0.56±0.01^{a}	0.44±0.01^{a}	0.31±0.01^{a}	5.58±0.05^{a}	12.77±0.05^{a}	2.28±0.03^{c}	0.21±0.02	0.72±0.01	0.27±0.01	0.34±0.02	0.32±0.01^{b}	0.19±0.01	0.21±0.02^{a}	0.11±0.03
	200	4.32±0.12^{c}	0.36±0^{bc}	0.33±0^{b}	0.24±0^{b}	4.26±0.07^{b}	7.03±0.15^{c}	3.03±0.11^{a}	0.19±0.03	0.79±0.06	0.26±0.01	0.43±0.07	0.32±0^{b}	0.18±0.01	0.15±0.01^{bc}	0.12±0.02
	300	4.76±0.2^{b}	0.37±0.01^{b}	0.33±0.01^{b}	0.24±0^{bc}	4.43±0.17^{b}	8.11±0.33^{b}	3.08±0.19^{a}	0.22±0.01	0.74±0	0.24±0.01	0.37±0	0.34±0.01^{b}	0.2±0.01	0.18±0.01^{ab}	0.1±0.01
	400	4.16±0.18^{c}	0.33±0.01^{c}	0.3±0.01^{c}	0.22±0^{c}	3.86±0.2^{c}	6.98±0.28^{c}	2.74±0.26^{ab}	0.19±0.01	0.72±0.04	0.24±0	0.37±0	0.34±0.01^{b}	0.18±0.01	0.15±0.03^{bc}	0.1±0
	600	4.26±0.11^{c}	0.33±0.02^{c}	0.31±0^{c}	0.22±0.01^{bc}	4.1±0.14^{bc}	6.66±0.22^{c}	2.57±0.13^{bc}	0.19±0.01	0.71±0.01	0.26±0.03	0.55±0.1	0.36±0^{a}	0.18±0.01	0.11±0.01^{c}	0.12±0.05
	P	****	****	****	****	****	****	*	ns	ns	ns	ns	*	ns	*	ns
Tenderloin	C	10.84±0.03	0.71±0.03^{a}	0.73±0	0.41±0	11.63±0.21	16.29±0.03^{a}	5.64±0.09	0.4±0.03	1.38±0.02	0.41±0.02	0.44±0	0.34±0	0.23±0.01	0.25±0.01^{a}	0.1±0^{c}
	200	5.75±2.68	0.43±0.14^{b}	0.45±0.25	0.3±0.13	6.21±4.02	8.87±3.77^{b}	4.12±1.91	0.31±0.15	0.92±0.32	0.29±0.08	0.44±0.02	0.33±0.01	0.19±0.03	0.14±0.03^{b}	0.2±0.02^{a}
	300	7.59±0.24	0.51±0.03^{b}	0.52±0.01	0.32±0.02	7.75±0.11	11.76±0.26^{b}	3.58±0.11	0.33±0.06	1±0	0.3±0	0.36±0.01	0.33±0.01	0.23±0.01	0.26±0.01^{a}	0.1±0.01^{c}
	400	7.17±0.09	0.48±0.02^{b}	0.49±0	0.29±0	7.25±0.04	11.11±0.05^{b}	3.99±0.22	0.32±0	1.04±0.02	0.34±0.04	0.46±0.09	0.34±0	0.2±0.02	0.16±0.02^{b}	0.14±0.01^{b}
	600	6.85±0.1	0.45±0.02^{b}	0.46±0.01	0.28±0.01	6.89±0.16	10.58±0.2^{b}	3.13±0.01	0.28±0.02	0.89±0.03	0.33±0.05	0.35±0.01	0.32±0	0.18±0	0.13±0^{b}	0.1±0.01^{c}
	P	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	**	**
Rump	C	4.5±0.09^{c}	0.35±0.01^{c}	0.32±0.01^{d}	0.22±0.01^{c}	4.58±0.06^{d}	6.89±0.12^{c}	3.19±0.22^{a}	0.24±0.06	0.76±0.02^{c}	0.28±0.02^{b}	0.45±0.09	0.34±0^{a}	0.19±0.01	0.19±0.01^{a}	0.23±0.18
	200	3.83±0.19^{d}	0.32±0.01^{d}	0.27±0.01^{e}	0.22±0.01^{c}	3.31±0.13^{e}	6.71±0.28^{e}	2.59±0.24^{bc}	0.2±0.03	0.68±0.02^{e}	0.23±0^{c}	0.38±0.02	0.34±0.01^{a}	0.18±0.01	0.13±0^{c}	0.09±0
	300	5.7±0.06^{b}	0.38±0.01^{b}	0.42±0.01^{b}	0.25±0^{b}	6.56±0.03^{b}	8.05±0.08^{b}	2.94±0.15^{ab}	0.23±0.01	0.8±0.01^{b}	0.29±0.02^{b}	0.36±0	0.33±0^{a}	0.2±0.03	0.17±0.01^{ab}	0.1±0.01
	400	5.66±0.05^{b}	0.34±0.01^{c}	0.37±0^{c}	0.22±0^{c}	5.43±0.01^{c}	8.32±0.07^{b}	2.32±0.18^{c}	0.23±0.04	0.72±0^{d}	0.27±0^{b}	0.31±0	0.3±0^{b}	0.17±0.01	0.16±0.01^{abc}	0.14±0.04
	600	8.67±0.02^{a}	0.57±0.01^{a}	0.65±0^{a}	0.38±0^{a}	8.79±0.02^{a}	12.83±0.01^{a}	3.38±0.03^{a}	0.33±0.03	0.99±0^{a}	0.33±0.01^{a}	0.36±0	0.29±0.01^{b}	0.2±0	0.14±0.02^{bc}	0.13±0
	P	****	****	****	****	****	****	**	ns	****	**	ns	**	ns	*	ns
Knuckle	C	10.53±0.08^{a}	0.62±0.08^{a}	0.67±0.08^{a}	0.37±0.08^{a}	10.86±0.08^{a}	14.53±0.08^{a}	3.96±0.08^{a}	0.39±0.08^{a}	1.12±0.08^{a}	0.39±0.08	0.41±0.08	0.31±0.08	0.22±0.08	0.26±0.08	0.24±0.08
	200	9.08±0.16^{b}	0.51±0.02^{b}	0.52±0.01^{b}	0.27±0.02^{b}	8.76±0.26^{b}	13.36±0.43^{b}	3.58±0.32^{ab}	0.24±0.03^{b}	0.9±0.06^{b}	0.27±0.08	0.3±0.29	0.24±0.01	0.15±0.01	0.15±0.03	0.04±0.03
	300	6.51±0.02^{c}	0.5±0.01^{b}	0.43±0^{bc}	0.3±0^{ab}	6±0.02^{c}	10.79±0.04^{c}	3.34±0.08^{b}	0.29±0.02^{ab}	0.9±0^{b}	0.33±0.03	0.56±0.05	0.31±0	0.21±0.01	0.12±0.05	0.12±0.01
	400	3.95±0.19^{d}	0.32±0.01^{c}	0.3±0.01^{d}	0.22±0.01^{b}	3.94±0.2^{d}	6.51±0.34^{d}	3.21±0.17^{bc}	0.18±0.01^{b}	0.73±0.02^{c}	0.23±0.04	0.39±0.1	0.3±0.01	0.18±0	0.2±0.04	0.13±0.03
	600	6.09±0.61^{c}	0.44±0.05^{b}	0.4±0.04^{c}	0.27±0.01^{b}	5.54±0.59^{c}	9.97±0.69^{c}	2.7±0.27^{c}	0.24±0.05^{b}	0.78±0.03^{c}	0.3±0.03	0.41±0.12	0.32±0.03	0.18±0.02	0.14±0.05	0.11±0.12
	P	****	**	**	*	****	****	*	*	**	ns	ns	ns	ns	ns	ns
Bolar	C	14.96±0.29^{a}	0.77±0^{b}	1.06±0.05^{a}	0.48±0.09^{b}	15.86±0.18^{a}	16.29±0.5^{c}	2.6±0.15^{bc}	0.68±0.08^{a}	1.29±0.02^{a}	0.53±0.01^{a}	0.25±0.05	0.18±0.01^{d}	0.24±0.05	0.39±0.01^{a}	0.2±0.08

Cuts	Condi -tion	C16:0	C16:1	C17:0	C17:1	C18:0	18:1n9	18:2n6	18:3n6	18:3n3	C20:0	20:4n6	20:5n3	C22:0	22:2n6	C23:0
	200	14.39±0.24^{a}	0.76±0.05^{b}	0.87±0^{b}	0.45±0.03^{bc}	12.68±0.27^{b}	19.8±0.4^{b}	2.41±0.05^{c}	0.47±0.02^{b}	1.05±0.03^{b}	0.46±0.08^{abc}	0.29±0.02	0.23±0.01^{bc}	0.2±0.02	0.22±0^{c}	0.18±0.1
	300	15.17±0.16^{a}	1.3±0.02^{a}	0.89±0^{b}	0.64±0.02^{a}	10.52±0.16^{c}	22.91±0.02^{a}	3.32±0.69^{ab}	0.7±0.07^{a}	1.29±0.01^{a}	0.47±0.02^{ab}	0.26±0.02	0.24±0^{b}	0.26±0.03	0.28±0.04^{b}	0.13±0.01
	400	12.01±0.39^{b}	0.69±0^{c}	0.75±0.04^{c}	0.42±0.05^{bc}	10.93±0.38^{c}	16.6±0.52^{c}	3.18±0.08^{ab}c	0.45±0.05^{b}	0.97±0.1^{b}	0.41±0.02^{bc}	0.44±0.25	0.21±0.01^{c}	0.23±0.01	0.16±0.04^{c}	0.12±0.03
	600	9.34±0.43^{c}	0.59±0.01^{d}	0.66±0.03^{d}	0.35±0.02^{c}	10.51±0.46^{c}	14.3±0.6^{d}	3.81±0.11^{a}	0.37±0.03^{b}	1.02±0.07^{b}	0.37±0.01^{c}	0.38±0.01	0.29±0^{a}	0.2±0	0.18±0.01^{c}	0.11±0.02
	P	****	****	****	*	****	****	*	**	**	*	ns	****	ns	***	ns
Inside	C	3.8±0.11^{a}	0.31±0.02	0.27±0.01	0.21±0	3.2±0.15^{a}	6.13±0.3^{a}	2.43±0.1	0.15±0^{b}	0.64±0	0.24±0.03	0.41±0.09	0.33±0	0.17±0^{bc}	0.14±0.03	0.14±0.02
	200	3.32±0.07^{b}	0.31±0.03	0.24±0.01	0.2±0.01	2.9±0^{ab}	5.44±0.02^{b}	2.41±0.01	0.16±0.01^{a}	0.62±0.01	0.25±0.04	0.53±0.18	0.36±0.03	0.17±0.01^{bc}	0.1±0.02	0.1±0.02
	300	2.97±0.2^{c}	0.28±0	0.22±0	0.2±0	2.51±0.1^{c}	4.88±0.21^{b}	2.2±0.09	0.12±0^{d}	0.57±0.02	0.2±0.01	0.4±0.06	0.33±0.01	0.18±0^{b}	0.15±0.03	0.1±0.01
	400	3.12±0.13^{bc}	0.28±0	0.23±0	0.2±0	2.72±0.07^{bc}	5.25±0.38^{b}	2.44±0.01	0.13±0^{cd}	0.63±0.06	0.21±0	0.41±0.05	0.37±0.04	0.21±0.01^{a}	0.2±0.03	0.11±0.03
	600	3.33±0.11^{b}	0.3±0	0.25±0.02	0.19±0.01	2.97±0.21^{ab}	5.36±0.23^{b}	2.4±0.18	0.14±0^{c}	0.6±0	0.22±0.02	0.37±0.01	0.33±0.01	0.17±0^{c}	0.12±0.01	0.16±0.01
	P	**	ns	ns	ns	*	*	ns	**	ns	ns	ns	ns	*	ns	ns
Heel	C	5.14±0.16^{e}	0.39±0.01^{e}	0.35±0^{d}	0.25±0.01^{c}	4.63±0.23^{d}	8.27±0.26^{e}	2.78±0.22	0.23±0.04^{c}	0.71±0.03	0.25±0.01^{c}	0.37±0.01	0.33±0.03	0.18±0^{d}	0.2±0.01^{b}	0.12±0^{a}
	200	7.5±0.45^{c}	0.52±0^{c}	0.48±0.02^{c}	0.32±0.06^{b}	7.75±0.42^{b}	11.55±0.21^{c}	3.65±1.33	0.28±0.02^{bc}	0.9±0.14	0.31±0.01^{bc}	0.39±0.13	0.31±0.08	0.19±0^{cd}	0.21±0.03^{ab}	0.09±0^{b}
	300	11.22±0.45^{a}	0.74±0.05^{a}	0.65±0.02^{a}	0.42±0^{a}	9.63±0.47^{a}	17.3±0.54^{a}	3.27±0.12	0.42±0.03^{a}	0.96±0.04	0.38±0.05^{a}	0.32±0.01	0.26±0.01	0.22±0.01^{b}	0.22±0^{ab}	0.1±0^{b}
	400	9±0.09^{b}	0.58±0.01^{b}	0.54±0.01^{b}	0.33±0^{b}	8.34±0.14^{b}	13.9±0.21^{b}	2.96±0.14	0.29±0.01^{b}	0.9±0	0.33±0.02^{ab}	0.32±0.01	0.27±0.01	0.23±0^{a}	0.25±0.03^{a}	0.11±0.01^{ab}
	600	6.03±0.09^{d}	0.45±0.01^{d}	0.38±0.01^{d}	0.27±0.01^{bc}	5.44±0.12^{c}	10.05±0.1^{d}	2.62±0.05	0.22±0.01^{c}	0.74±0.01	0.27±0.01^{c}	0.36±0.02	0.31±0.01	0.19±0^{c}	0.14±0.01^{c}	0.09±0^{b}
	P	****	****	****	**	****	****	ns	**	ns	*	ns	ns	****	*	*
Eye of loin	C	14.08±0.44^{a}	0.9±0.02^{a}	0.82±0^{a}	0.46±0.01^{a}	11.32±0.17^{a}	20.28±0.72^{a}	3.48±0.07^{a}	0.49±0.05^{ab}	1.1±0.03^{b}	0.41±0.02^{a}	0.28±0.01	0.27±0	0.2±0^{b}	0.32±0.02^{a}	0.1±0
	200	10.03±0.01^{d}	0.63±0.01^{c}	0.63±0.01^{c}	0.37±0.03^{b}	8.73±0.08^{c}	13.82±0.05^{c}	3.15±0.1^{b}	0.38±0.01^{c}	1.03±0.01^{bc}	0.38±0.06^{a}	0.43±0.17	0.32±0	0.19±0.01^{b}	0.17±0.01^{bc}	0.1±0.01
	300	5.26±0.01^{e}	0.38±0.02^{d}	0.35±0^{d}	0.24±0.01^{c}	4.43±0.02^{d}	7.7±0.03^{d}	2.44±0.13^{c}	0.22±0.03^{d}	0.73±0.01^{d}	0.27±0.02^{b}	0.39±0.02	0.34±0.01	0.18±0^{b}	0.14±0.01^{c}	0.13±0.02
	400	11.71±0.14^{c}	0.77±0.01^{b}	0.67±0.01^{b}	0.4±0.01^{b}	10.31±0.12^{b}	18.37±0.14^{b}	3.22±0.03^{b}	0.43±0^{bc}	1±0.01^{c}	0.39±0.03^{a}	0.44±0.2	0.3±0.06	0.25±0.03^{a}	0.2±0.04^{bc}	0.12±0.03
	600	12.76±0.59^{b}	0.8±0.04^{b}	0.81±0.03^{a}	0.49±0.04^{a}	10.76±0.52^{ab}	18.26±0.9^{b}	3.65±0.08^{a}	0.55±0.02^{a}	1.2±0.06^{a}	0.41±0^{a}	0.31±0.02	0.28±0.01	0.2±0^{b}	0.2±0.02^{b}	0.16±0.07
	P	****	****	****	***	****	****	****	****	****	*	ns	ns	*	**	ns

Note: Values are means ± standard error (n = 2). Values with different superscripts (^{a,b,c,d,e}) in the same column differ significantly within cuts. P < 0.0001 presented as ∗∗∗∗for level of significance; P < 0.001 presented as ∗∗∗for level of significance; P < 0.01 presented as ∗∗level of significance; P < 0. 05 presented as ∗ for level of significance and ns meaning not statistically significant.

Table 9 Fatty acid composition (g/100g) of New Zealand lamb cuts after HPP treatment (200MPa, 300MPa, 400MPa and 600MPa).

Cuts	Condition	SFA	MUFA	PUFA	n-3	n-6	PUFA/SFA	N-6/N-3	Total
Flat	C	14.62±0.07^{a}	13.64±0.05^{a}	4.08±0.05^{b}	1.04±0	3.04±0.05	0.28±0^{b}	2.92±0.05^{b}	32.34±0.03^{a}
	200	9.46±0.18^{bc}	7.63±0.15^{c}	4.91±0.28^{a}	1.11±0.06	3.8±0.22	0.52±0.02^{a}	3.42±0.02^{ab}	22.01±0.62^{c}
	300	10.06±0.41^{b}	8.71±0.34^{b}	4.93±0.17^{a}	1.08±0	3.85±0.17	0.49±0.04^{a}	3.57±0.16^{a}	23.7±0.58^{b}
	400	8.85±0.38^{c}	7.53±0.29^{c}	4.51±0.25^{ab}	1.06±0.03	3.44±0.28	0.51±0.01^{a}	3.24±0.34^{ab}	20.88±0.92^{c}
	600	9.23±0.25^{c}	7.21±0.23^{c}	4.5±0.24^{ab}	1.08±0.01	3.42±0.26	0.49±0.01^{a}	3.17±0.27^{ab}	20.93±0.73^{c}
	P	****	****	*	ns	ns	***	*	***
Tenderloin	C	23.94±0.21	17.4±0^{a}	8.45±0.15	1.73±0.02	6.73±0.13	0.35±0^{b}	3.9±0.02	49.79±0.36^{a}
	200	13.08±7.09	9.6±4.04^{b}	6.27±2.38	1.25±0.31	5.02±2.07	0.5±0.09^{a}	3.92±0.68	28.95±13.52^{b}
	300	16.49±0.36	12.58±0.31^{b}	5.87±0.2	1.34±0.01	4.53±0.19	0.36±0.02^{b}	3.39±0.11	34.95±0.46^{ab}
	400	15.59±0.14	11.88±0.07^{b}	6.32±0.27	1.38±0.02	4.94±0.29	0.41±0.01^{ab}	3.58±0.26	33.8±0.48^{b}
	600	14.81±0.31	11.3±0.23^{b}	5.11±0.02	1.21±0.03	3.9±0.05	0.34±0.01^{b}	3.22±0.13	31.22±0.51^{b}
	P	ns	*	ns	ns	ns	*	ns	**
Rump	C	10.09±0.33^{d}	7.45±0.1^{c}	5.16±0.35^{ab}	1.1±0.02^{c}	4.06±0.37^{a}	0.51±0.02^{a}	3.7±0.41	22.71±0.78^{d}
	200	7.9±0.31^{e}	7.25±0.28^{c}	4.33±0.29^{cd}	1.03±0^{d}	3.3±0.29^{bc}	0.55±0.06^{a}	3.22±0.28	19.47±0.31^{e}
	300	13.27±0^{b}	8.68±0.07^{b}	4.82±0.15^{bc}	1.13±0.01^{b}	3.69±0.14^{ab}	0.36±0.01^{b}	3.26±0.09	26.77±0.23^{b}
	400	12.04±0.1^{c}	8.89±0.08^{b}	4.03±0.21^{d}	1.01±0^{d}	3.01±0.21^{c}	0.34±0.02^{b}	2.98±0.21	24.95±0.03^{c}
	600	18.77±0.02^{a}	13.78±0.01^{a}	5.48±0.06^{a}	1.28±0.01^{a}	4.2±0.05^{a}	0.29±0^{b}	3.29±0.01	38.03±0.03^{a}
	P	****	****	**	****	*	***	ns	****
Knuckle	C	22.91±0.08^{a}	15.53±0.08^{a}	6.46±0.08	1.43±0.08^{a}	5.03±0.08^{a}	0.28±0^{c}	3.5±0.18	44.9±0.23^{a}
	200	19.09±0.47^{b}	14.25±0.46^{b}	5.68±0.68	1.19±0.07^{b}	4.43±0.61^{ab}	0.3±0.04^{c}	3.72±0.3	39.02±1.61^{b}
	300	13.59±0.05^{c}	11.59±0.05^{c}	5.53±0.16	1.21±0^{b}	4.31±0.16^{ab}	0.41±0.01^{b}	3.55±0.15	30.71±0.26^{c}
	400	8.73±0.4^{d}	7.06±0.36^{d}	5.02±0.36	1.03±0.03^{c}	3.98±0.33^{b}	0.58±0.02^{a}	3.85±0.2	20.81±1.11^{d}
	600	12.62±1.4^{c}	10.67±0.75^{c}	4.6±0.54	1.1±0.05^{bc}	3.5±0.49^{b}	0.36±0.01^{b}	3.18±0.21	27.88±2.7^{c}
	P	****	****	ns	**	*	****	ns	****
Bolar	C	32.85±0.55^{a}	17.53±0.59^{c}	5.4±0.32	1.48±0.03^{a}	3.92±0.29	0.16±0.01^{c}	2.65±0.14^{c}	55.77±1.46^{ab}

Cuts	Condition	SFA	MUFA	PUFA	n-3	n-6	PUFA/SFA	N-6/N-3	Total
	200	28.77±0.47^{b}	21.01±0.43^{b}	4.66±0.06	1.28±0.02^{b}	3.38±0.04	0.16±0^{c}	2.65±0.01^{c}	54.44±0.83^{b}
	300	27.43±0.31^{b}	24.85±0.02^{a}	6.1±0.82	1.53±0.01^{a}	4.57±0.81	0.22±0.03^{b}	2.99±0.51^{bc}	58.38±0.53^{a}
	400	24.44±0.79^{c}	17.7±0.57^{c}	5.42±0.45	1.18±0.11^{b}	4.24±0.35	0.22±0.01^{b}	3.6±0.03^{ab}	47.56±1.82^{c}
	600	21.19±0.92^{d}	15.24±0.63^{d}	6.05±0.23	1.31±0.07^{b}	4.74±0.16	0.29±0^{a}	3.62±0.07^{a}	42.47±1.78^{d}
	P	****	****	ns	**	ns	**	*	****
Inside	C	7.82±0.31^{a}	6.65±0.32^{a}	4.1±0.22	0.97±0	3.13±0.22	0.53±0.01	3.22±0.22	18.57±0.85^{a}
	200	6.99±0.06^{b}	5.95±0^{b}	4.17±0.2	0.98±0.02	3.19±0.18	0.6±0.03	3.26±0.12	17.11±0.14^{ab}
	300	6.19±0.3^{c}	5.37±0.21^{b}	3.77±0.08	0.91±0.03	2.86±0.05	0.61±0.02	3.16±0.05	15.32±0.59^{c}
	400	6.6±0.16^{bc}	5.72±0.37^{b}	4.17±0.19	1±0.1	3.17±0.09	0.63±0.04	3.17±0.21	16.49±0.34^{bc}
	600	7.09±0.36^{b}	5.84±0.24^{b}	3.95±0.19	0.93±0.01	3.02±0.2	0.56±0	3.24±0.24	16.89±0.79^{bc}
	P	*	*	ns	ns	ns	ns	ns	*
Heel	C	10.69±0.4^{e}	8.91±0.28^{e}	4.62±0.26	1.04±0.01	3.58±0.26	0.43±0.01	3.43±0.23	24.21±0.94^{e}
	200	16.32±0.03^{c}	12.39±0.26^{c}	5.74±1.72	1.21±0.22	4.53±1.5	0.35±0.11	3.69±0.56	34.46±1.95^{c}
	300	22.2±1^{a}	18.45±0.58^{a}	5.45±0.15	1.21±0.06	4.24±0.1	0.25±0	3.49±0.09	46.1±1.74^{a}
	400	18.55±0.27^{b}	14.81±0.22^{b}	5±0.2	1.17±0.01	3.83±0.19	0.27±0.01	3.27±0.14	38.37±0.69^{b}
	600	12.41±0.22^{d}	10.76±0.1^{d}	4.38±0.05	1.05±0.01	3.33±0.06	0.35±0.01	3.17±0.08	27.56±0.27^{d}
	P	****	****	ns	ns	ns	ns	ns	****
Eye of loin	C	26.92±0.63^{a}	21.64±0.75^{a}	5.93±0.07^{ab}	1.36±0.03^{b}	4.57±0.1^{ab}	0.22±0.01^{d}	3.35±0.15	54.5±1.31^{a}
	200	20.05±0.14^{d}	14.82±0.09^{c}	5.49±0.25^{b}	1.35±0^{b}	4.13±0.25^{c}	0.27±0.01^{b}	3.06±0.17	40.36±0.48^{c}
	300	10.62±0.01^{e}	8.32±0.05^{d}	4.27±0.07^{c}	1.08±0.02^{c}	3.19±0.09^{d}	0.4±0.01^{a}	2.96±0.12	23.2±0^{d}
	400	23.44±0.31^{c}	19.53±0.17^{b}	5.6±0.26^{b}	1.31±0.07^{b}	4.29±0.19^{bc}	0.24±0.01^{cd}	3.29±0.02	48.58±0.74^{b}
	600	25.1±1.21^{b}	19.56±0.98^{b}	6.19±0.12^{a}	1.48±0.05^{a}	4.71±0.07^{a}	0.25±0.01^{c}	3.18±0.06	50.85±2.3^{b}
	P	****	****	***	***	***	****	ns	****

Note: Values are means ± standard error (n = 2). Values with different superscripts (^{a,b,c,d,e}) in the same column differ significantly within cuts. SFA stands for saturated fatty acids; MUFA stands for monounsaturated fatty acids; PUFA stands for polyunsaturated fatty acids. P < 0.0001 presented as ∗∗∗∗for level of significance; P < 0.001 presented as ∗∗∗for level of significance; P < 0.01 presented as ∗∗level of significance; P < 0. 05 presented as ∗ for level of significance and ns meaning not statistically significant.

4.2.2 Effect of different HPP treatments on fatty acids value

HPP treated rump and heel cuts had higher SFA and MUFA ($P < 0.05$) contents, as well as lower PUFA/SFA ratio ($P < 0.05$) compared to control sample (Table 9). The increase of SFA was related to the increase ($P < 0.05$) of C16:0 and C18:0 while the increase in MUFA was related to the increase ($P < 0.05$) of 18:1n9 and 18:2n6. PUFA content was not significantly different to control for both cuts suggesting that little or no oxidation occurred (Alfaia et al., 2010). This is supported by our oxidation results for rump and heel cuts that was a maximum at 0.448 and 0.541 mg MDA/kg at 600 MPa.

SFA and MUFA content were lower in HPP treated inside ($P<0.1$), bolar ($P<0.0001$), knuckle ($P<0.0001$), eye of loin ($P<0.0001$), tenderloin ($P<0.05$) and flat cuts ($P<0.0001$) at all pressures compared to control samples. PUFA content was also lower in HPP treated inside (all pressures), knuckle (400 and 600 MPa), eye of loin (300 MPa) and tenderloin (600 MPa) cuts compared to control. The decrease in PUFA was due to the decrease of mainly C18:1 n9and C18:3 n3. Long-chain fatty acids are oxidized more slowly and unsaturated fatty acids are oxidized more rapidly than saturated fatty acids (Leyton, Drury, & Crawford, 1987). Lipid oxidation results from this study showed that as pressure increased oxidation level in lipid also increased. Gray (1978) and Shahidi and Zhong (2010) demonstrated that lipid oxidation is a complex process during which polyunsaturated fatty acids (PUFA) of meat react with molecular oxygen via a free radical chain mechanism to form fatty acyl hydroperoxides and other primary products of oxidation. In addition, Wood et al. (2008) indicated that during processing, ageing and retail display, polyunsaturated fatty acids are not stable, and its oxidative stability is affected by the composition of fatty acids. Pereda, Ferragut, Quevedo, Guamis, and Trujillo (2008) further demonstrated that the decrease in fatty acids can be a result of fatty acid oxidation and acidification that supports the decrease in fatty acids in our study.

As mentioned above the PUFA/SFA ratio is recommended to be above 0.4(Wood et al., 2004). Banskalieva et al. (2000) compiled the PUFA/SFA ratios for different muscles that ranged from 0.16 to 0.49 in goat meat. On the other hand, (Tshabalala, Strydom, Webb, & Kock, 2003) reported PUFA/ SFA ratios from 0.62 to 0.79 in goat meat. In our study, HPP treated inside (all pressures), knuckle (300 and 400 MPa), eye of loin (300 MPa), tenderloin (400 MPa) and flat (all pressures) had PUFA/SFA ratios of more

than 0.4 compared to control samples. Hence HPP can have a positive effect on PUFA/SFA ratios of some lamb cuts. Furthermore, the n:6/n:3 PUFA ratios of all samples in this study remained within the recommended level of ≤4 (Enser et al., 2000). Similarly, McArdle et al. (2013) indicated that high pressure had no significant effect on n6: n3 ratios in lamb meat.

Table 10 Free amino acid composition (mg/100g) of different New Zealand lamb meat cuts.

CUTS	Flat	Tenderloin	Rump	Knuckle	Bolar	Inside	Heel	Eye of loin	P value
Nonessential									
Alanine	54.3±2.36^{d}	72.75±3.61^{b}	78.65±2.48^{b}	58.54±0.11^{cd}	62.79±0.67^{c}	92.89±2.81^{a}	78.25±8.27^{b}	54.85±2.14^{cd}	****
Glycine	49.97±2.63^{bc}	43.82±1.59^{c}	54.69±4.37^{ab}	44.58±1.37^{c}	60.45±2.37^{a}	59.44±4.61^{a}	49.7±1.32^{bc}	44.74±3.95^{c}	**
Serine	26.01±1.39^{a}	21.2±0.22^{ab}	18.28±2.8^{bc}	12.41±2.16^{c}	12.91±2.67^{c}	26.26±4.74^{a}	17.92±4.96^{bc}	17.12±4.37^{bc}	*
Threonine	27.05±0.75^{a}	17.6±0.16^{bc}	18.98±0.14^{b}	15.4±0.85b^{c}	11.93±1.49^{c}	28.76±1.06^{a}	15.31±2.49^{bc}	15.54±6.92^{bc}	**
Proline	12.12±2.57	3.1±0.85	8.7±3.24	6.12±1.52b	9.94±1.42	7.44±0.34	5.84±1.1	8.08±1.65	ns
Glutamic acid	82.66±4.75^{a}	48.44±1.71^{cd}	35.35±2.89^{d}	82.16±6.19^{a}	63.57±6.65^{bc}	56.76±3.68^{c}	78.02±18.46^{ab}	54.66±0.13^{c}	**
Aspartic acid	129.87±2.86^{a}	89.32±1.37^{de}	71.06±9.71^{e}	105.54±3.07^{bcd}	122.33±1.89^{ab}	118.98±1.37^{abc}	100.96±19.5^{cd}	114.73±1.5^{abc}	***
Ornithine	6.02±0.76	4.51±0.07	7.35±2.19	5.45±0.1	4.93±0.06	5.33±0.97	4.94±0.05	5.94±1.29	ns
NEAA	387.99±4.6^{a}	300.73±0.24^{de}	293.05±3.16^{e}	330.21±9.24^{bcd}	348.85±6.75^{bc}	395.87±2.88^{a}	350.96±38.51^{b}	315.66±10.63^{cde}	***
Essential									
Valine	26.05±4.4^{b}	16.88±1.13^{c}	19.12±1.21^{bc}	18.67±4.22^{bc}	13.85±0.47^{c}	38.01±1.34^{a}	19.77±0.1^{bc}	19.61±8.64^{bc}	**
Leucine	15.29±0.67^{bc}	14.06±0.5^{bcd}	16.71±0.95^{b}	15.2±3.33^{bc}	12.07±0.58^{cd}	27.67±1.84^{a}	13.68±2.05^{bcd}	10.71±0.97^{d}	****
Isoleucine	16.87±0.66^{b}	12±0.69^{cd}	23±2.65^{a}	15.44±1.87^{bc}	7.8±0.28^{e}	23.36±0.5^{a}	13.15±1.95^{bcd}	10.58±2.46^{de}	****
Methionine	4.11±0.94^{d}	4.74±0.42^{d}	14.78±0.48^{a}	6.96±1.33^{c}	4.67±0.32^{d}	9.38±0.24^{b}	3.53±0.65^{d}	4.95±1.01^{d}	****
Phenylalanine	11.55±0.23^{de}	15.4±1.54^{bc}	14.8±1.09^{bcd}	13.1±2.11^{cde}	13.05±1.49^{cde}	19.91±0.75^{a}	16.61±1.94^{ab}	10.13±1.5^{e}	**
Lysine	9.62±0.66	10.97±0.12	9.29±0.33	8.24±0.59	8.26±0.26	9.51±0.41	8.67±0.97	10.25±1.61	ns
Histidine	13.24±0.42	12.9±1.35	11.87±0.23	12.66±1.13	13.67±0.75	12.57±1.38	11.81±1.27	10.64±0.25	ns
Tyrosine	11.23±0.5^{b}	15.54±0.78^{a}	11.5±2.62^{b}	11.27±2.58^{b}	10.43±1.44^{b}	17.12±0.71^{a}	9.14±1.08^{b}	16.98±2.4^{a}	**
Tryptophan	6.24±0.6^{cd}	5.23±0.54^{d}	7.16±0.07^{bc}	7.51±0.4^{ab}	5.68±0.51^{d}	8.63±0.02^{a}	5.17±0.77^{d}	8.03±0.51^{ab}	***
EAA	114.19±8.09^{bc}	107.71±7.08^{cd}	128.22±6.62^{b}	109.04±16.37^{bc}	89.48±1.19^{d}	166.17±1.41^{a}	101.52±6.87^{cd}	101.88±9.49^{cd}	****
TOTAL	502.18±3.49^{b}	408.44±7.32^{c}	421.28±9.78^{c}	439.25±25.61^{c}	438.33±7.95^{c}	562.03±1.46^{a}	452.48±45.39^{c}	417.54±20.12^{c}	***

Note: Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly within cuts. EAA stands for essential free amino acids; NEAA stands for non-essential free amino acids. P < 0.0001 presented as ∗∗∗∗for level of significance; P < 0.001 presented as ∗∗∗for level of significance; P < 0.01 presented as ∗∗level of significance; P < 0. 05 presented as ∗ for level of significance and ns meaning not statistically significant.

4.3 Free amino acids

4.3.1 Free amino acids content of different control cuts

In this study, seventeen amino acids (alanine, glycine, valine, leucine, threonine, serine, isoleucine, proline, aspartic acid, methionine, glutamic acid, phenylalanine, ornithine, lysine, histidine, tyrosine, tryptophan) were identified and quantified using the EZ:faast^{TM}, Phenomenex®, USA. The results are given as mean values (mg/100g) of the amount of free amino acids shown in Table 10. The presence of cysteine (CYS) and arginine (ARG), has been previously reported in raw goat meat at 0.01 and12.2 mg/100 g respectively (Madruga et al., 2010), which are quite low. However, these free amino acids were not identified in the current study due to the limitation of the amino acids analysis kit used (Phenomenex, 2003), and has been reported in another study (Trani et al., 2010).

Nine essential free amino acids (valine, leucine, isoleucine, methionine, phenylalanine, lysine, histidine, tyrosine and tryptophan) and eight non-essential free amino acids (alanine, glycine, threonine, serine, proline, aspartic acid, glutamic acids and ornithine) were detected. In general, the type of cuts had a significant ($P<0.05$) effect on free amino acid composition. Madruga et al. (2010) reported that the most abundant free amino acids in the rump of goat meat were, glycine, alanine, and glutamine. Mullen et al. (2000) found histidine to be the main FAA in beef eye of loin cut followed by alanine. Similarly, Watanabe, Ueda, and Higuchi (2004) reported alanine, glutamic acid and aspartic acid were the major amino acids in cattle. Hollo et al. (2007) reported that for beef the highest essential amino acid fractions were lysine and leucine and the major non-essential amino acids were glutamic acid and aspartic acid. They also demonstrated that amino acid composition varied with age, animal, sex, muscles and muscle stress levels. Different feed could also influence the concentration of free amino acids in red meat (Koga, Fukunaga, Ohki, & Kawaida, 1985).

Table 10 showed that the total amount of free amino acids in the inside cut had significantly higher ($P<0.05$) levels of almost all individual free amino acids except for proline, glutamic acid and methionine. Bolar, eye of loin, heel, knuckle, rump and tenderloin cuts had the least significant ($P<0.05$) levels of total free amino acids. Generally, our result shown that alanine, glycine, glutamic and aspartic acid are the

major non-essential amino acids, and valine and leucine are the major essential amino acids.

4.3.1.1 Non-Essential free amino acids content in different control cuts

The major non-essential free amino acids were alanine, glycine, glutamic acid and aspartic acid. Eight non-essential free amino acids (alanine, glycine, threonine, serine, proline, aspartic acid, glutamic acids and ornithine) accounted for approximately 70% to 80% of the total amino acids. In inside and flat cuts, the total non-essential free amino acids value were significantly ($P<0.05$) higher compared to other cuts. On the other hand, eye of loin, rump and tenderloin cuts had the least significant ($P<0.05$) level of total non-essential free amino acids content.

Glutamic acid, one of the non-essential amino acids contribute to meat flavour and is associated with the ‘‘umami’’ term, described as slightly sweet and sour tastes that confer ‘‘mouth satisfaction’’ (Maga, 1994). In fact, Aristoy and Toldrá (1998) analysed the free amino acids in porcine skeletal muscle with different oxidative patterns and demonstrated that the content of non-essential free amino acids such as glutamic acid and proline, as well as total free amino acids in the trapezius muscles (flat) were significantly higher than in rump. Similarly, in our study flat cut had a significantly ($P<0.05$) higher level of glutamic acid, and rump cut had the least significant ($P<0.05$) level of glutamic acid content. Franco et al. (2010) studied total amino acids and free amino acids in different beef muscles (heel, bolar, inside, eye of loin, masseter and cardiac muscles), and reported that glutamic acid was the highest ($P<0.05$) in the heel cut compared to other cuts (bolar, inside, eye of loin) and this was similar to our study ($P<0.05$). In fact, our result shown that knuckle, flat and heel cuts had significantly higher glutamic acid content than other muscles (eye of loin, inside, bolar, tenderloin and rump).

Franco et al. (2010) reported that aspartic acid was the major essential amino acid in all the muscles (heel, bolar, inside, and eye of loin cuts). Similarly, aspartic acid was the major non-essential amino acid in this study, with significantly ($P<0.05$) higher amounts in bolar, eye of loin, flat and inside cuts. Meanwhile, rump and tenderloin had the least ($P<0.05$) significant amount of aspartic acid.

4.3.1.2 Essential free amino acids content in different control cuts

In this study, nine essential free amino acids (valine, leucine, isoleucine, methionine, phenylalanine, lysine, histidine, tyrosine and tryptophan) accounted for 20% to 30% of the total amino acids. The major essential amino acids were valine, leucine, phenylalanine, tyrosine and histidine. Madruga et al. (2010) reported that the most abundant essential free amino acids in the rump of goat meat were, leucine and valine. As seen in Table 10, inside cut had significantly ($P<0.05$) higher essential amino acid (EAA) followed by rump cut. Bolar, eye of loin, and heel cuts had the least significant ($P<0.05$) EAA content.

There were highly significant differences ($p<0.05$) in leucine, isoleucine and methionine content between cuts. Leucine content was highest ($P<0.05$) in inside cut (27.67 mg/100g). However, bolar (12.07 mg/100g), eye of loin (10.71 mg/100g), heel (12.68 mg/100g) and tenderloin (14.06 mg/100g) cuts had the least significant ($P<0.05$) leucine level similar to porcine rump cut (19.15 mg/100g) as reported by M Cornet and J Bousset (1999). However, our result had a higher leucine concentration compared to Madruga et al. (2010) (7.9 mg/100g in raw goat rump cut), and Franco et al. (2010) (beef heel (6.00 mg/100g), bolar (2.90 mg/100g), inside (2.40 mg/100g), eye of loin (1.55 mg/100g)).

Isoleucine content was highest ($P<0.05$) in inside (23.36 mg/100g) and rump (23.00 mg/100g) cuts. Similarly, Cornet and Bousset (1999) reported 26.98 mg/100g isoleucine in porcine rump cut. Bolar (7.8 mg/100g) and eye of loin (10.58 mg/100g) cuts had the least significant ($P<0.05$) level. These results were higher than reported by Franco et al. (2010) for beef heel (4.8 mg/100g), bolar (5.02 mg/100g), inside (5.55 mg/100g), and eye of loin (2.61 mg/100g) cuts.

Methionine was only present at a very low concentration in our study. It was highest ($P<0.05$) in the rump cut (14.78 mg/100g). Bolar (4.62 mg/100g), eye of loin (4.95 mg/100g), flat (4.11 mg/100g), heel (3.53 mg/100g) and tenderloin (4.74 mg/100g) cuts were present at the least significant ($P<0.05$) level. These findings were similar to methionine content reported by Cornet and Bousset (1999) who reported 10.06 mg/100g methionine in porcine rump cut, and Franco et al. (2010) who reported 4.0 mg/100g in beef heel cut, 1.55 mg/100g in beef bolar cut, 1.77 mg/100g in inside cut and 0.87mg

/100g in eye of loin cut. In other red meats, such as camel (Elgasim & Alkanhal, 1992), hen (Elgasim & Alkanhal, 1992) and ostrich (Sales & Hayes, 1996), lysine was the major essential free amino acid. In our results, lysine was not significantly ($P>0.05$) different between all cuts.

Table 11 The concentration of non-essential free amino acids composition (mg/100g) of New Zealand lamb cuts subjected to different pressure treatments (200MPa, 300MPa, 400MPa and 600MPa).

Cuts	treatment	alanine	glycine	serine	threonine	proline	glutamic acid	aspartic acid	ornithine	Total NEAA
Flat	C	54.30±2.36^{c}	49.97±2.63^{c}	26.01±1.39^{b}	27.05±0.75^{b}	12.12±2.57	82.66±4.75^{b}	129.87±2.86^{b}	6.02±0.76	387.99±21.33^{c}
	P200	68.76±1.14^{c}	57.51±4.68^{bc}	21.23±0.22^{c}	19.12±1.61^{d}	10.89±1.36	78.01±2.04^{b}	133.92±0.11^{b}	5.20±0.05	394.62±23.16^{c}
	P300	100.74±11.38^{b}	66.61±2.56^{b}	42.31±2.83^{a}	29.29±1.28^{b}	9.59±0.92	91.08±3.25^{ab}	109.94±0.97^{c}	4.68±0.09	454.24±14.81^{b}
	P400	109.23±4.76^{b}	57.58±2.88^{bc}	20.97±2.35^{c}	23.62±0.79^{c}	7.32±0.73	100.34±5.82^{a}	131.79±1.70^{b}	4.62±0.14	455.47±17.23^{b}
	P600	129.61±8.56^{a}	80.17±6.89^{a}	40.66±0.48^{a}	37.89±0.18^{a}	10.85±1.26	89.21±9.03^{ab}	155.65±6.17^{a}	5.32±0.19	549.36±13.34^{a}
	p	***	**	****	****	ns	*	****	ns	****
Tenderloin	C	72.75±3.61^{b}	43.82±1.59^{b}	21.20±0.22^{a}	17.60±0.16^{a}	3.10±0.85^{c}	48.44±1.71^{d}	89.32±1.37^{c}	4.51±0.07^{b}	300.74±16.16^{d}
	P200	74.51±1.78^{b}	40.68±1.55^{c}	16.46±1.50^{b}	17.49±0.55^{a}	5.38a±0.08^{b}	87.99±1.43^{b}	162.01±5.62^{a}	4.99±0.36^{b}	409.51±23.38^{a}
	P300	46.83±0.69^{c}	33.01±0.13^{d}	19.20±1.32^{ab}	15.78±1.24^{ab}	5.03±1.34^{ab}	141.12±6.36^{a}	113.34±7.28^{b}	5.75±0.09^{a}	380.05±19.75^{b}
	P400	96.96±2.51^{a}	45.22±0.48^{b}	16.47±1.42^{b}	14.95±0.47^{b}	4.14±0.34^{bc}	81.15±1.34^{b}	153.09±0.07^{a}	4.70±0.20^{b}	416.69±14.15^{a}
	P600	96.52±0.74^{a}	51.38±0.91^{a}	12.75±0.55^{c}	14.10±0.82^{b}	6.15±0.37^{a}	59.55±3.18^{c}	85.30±1.39^{c}	4.54±0.02^{b}	330.28±12.39^{c}
	p	****	****	**	*	*	****	****	**	****
Rump	C	78.65±2.48^{b}	54.69±4.37	18.28±2.80^{b}	18.98±0.14^{c}	8.70±3.24^{b}	35.35±2.89^{c}	71.06±9.71^{d}	7.35±2.19	293.05±21.83^{d}
	P200	100.62±4.78^{a}	63.02±2.22	29.46±2.42^{a}	23.78±3.37^{b}	7.81±0.55^{b}	133.35±3.29^{a}	141.29±5.04^{c}	5.46±0.80	504.79±16.89^{bc}
	P300	85.74±0.44^{b}	48.90±5.21	19.47±4.58^{b}	17.42±0.44^{c}	6.98±2.04^{b}	99.52±10.10^{b}	158.85±34.48^{c}	4.91±0.30	441.78±18.47^{c}
	P400	80.27±3.97^{b}	61.57±0.79	18.28±3.85^{b}	15.68±2.16^{c}	11.05±1.55^{b}	124.13±17.80^{ab}	220.55±13.03^{b}	5.22±0.06	536.74±24.81^{b}
	P600	85.83±5.79^{b}	64.46±3.16	37.00±1.40^{a}	33.89±0.53^{a}	16.09±0.55^{a}	140.41±7.35^{a}	272.04±11.14^{a}	5.17±0.46	654.89±23.46^{a}
	P	*	ns	**	***	*	***	****	ns	****
Knuckle	C	58.54±0.11^{c}	44.58±1.37^{b}	12.41±2.16^{c}	15.40±0.85^{b}	6.12±1.52	82.16±6.19^{b}	105.54±3.07^{c}	5.45±0.10	330.21±27.81^{d}
	P200	75.33±4.14^{b}	78.03±1.99^{a}	20.33±2.53^{b}	19.34±2.13^{b}	8.15±0.47	50.13±9.67^{c}	132.79±2.24^{b}	5.47±0.60	389.56±19.57^{c}
	P300	116.50±1.14^{a}	73.29±1.36^{a}	31.47±4.36^{a}	26.85±3.08^{a}	7.31±0.12	49.81±0.16^{c}	105.60±4.94^{c}	5.70±0.40	416.53±24.31^{b}
	P400	111.35±11.77^{a}	56.57±9.02^{b}	17.23±3.14^{bc}	17.24±1.86^{b}	6.30±0.83	75.44±13.11^{b}	153.96±0.59^{a}	6.12±0.23	444.20±20.50^{b}
	P600	118.10±1.46^{a}	80.17±4.51^{a}	32.08±2.16^{a}	30.83±0.91^{a}	8.77±1.00	115.65±2.46^{a}	154.15±1.70^{a}	5.13±0.30	544.87±22.47^{a}
	P	****	**	**	**	ns	**	****	ns	****
Bolar	C	62.79±0.67^{c}	60.45±2.37^{b}	12.91±2.67^{b}	11.93±1.49^{d}	9.94±1.42	63.57±6.65^{b}	122.33±1.89^{c}	4.93±0.06	348.85±11.52^{d}

Cuts	treatment	alanine	glycine	serine	threonine	proline	glutamic acid	aspartic acid	ornithine	Total NEAA
	P200	64.90±8.46^{c}	55.16±8.81^{b}	14.20±3.13^{b}	14.56±1.17^{cd}	10.23±4.07	88.65±16.43^{ab}	145.24±3.21^{b}	5.04±0.11	397.97±19.81^{c}
	P300	83.80±12.40^{b}	96.70±14.68^{a}	25.94±2.09^{a}	21.05±1.40^{b}	8.55±2.61	113.23±15.96^{a}	158.25±2.09^{a}	4.82±0.39	512.36±16.09^{a}
	P400	97.39±6.47^{a}	65.70±2.23^{b}	26.22±7.97^{a}	34.09±0.29^{a}	12.25±1.61	62.80±16.06^{b}	101.90±4.57^{d}	4.54±0.14	404.87±21.07^{c}
	P600	101.91±2.99^{a}	64.11±18.53^{b}	25.28±2.26^{a}	16.77±0.52^{c}	12.62±0.05	101.79±2.78^{a}	158.34±4.03^{a}	5.24±0.10	486.05±14.03^{b}
	p	**	*	*	****	ns	*	****	ns	****
Inside	C	92.89±2.81^{b}	59.44±8.86^{a}	26.26±4.74	28.76±1.06^{ab}	7.44±0.34	56.76±3.68^{c}	118.98±1.37^{b}	5.33±0.97	395.87±27.76^{c}
	P200	112.72±6.60^{a}	67.26±2.47^{a}	39.16±5.94	35.71±5.58^{a}	10.60±3.49	78.32±6.40^{b}	138.85±9.37^{ab}	5.32±0.99	487.93±29.88^{a}
	P300	93.95±5.52^{b}	60.99±10.52^{a}	25.06±14.45	23.35±3.58^{bc}	6.07±0.05	80.21±4.24^{b}	124.52±16.02^{b}	4.71±0.07	418.85±20.41^{bc}
	P400	86.20±0.13^{b}	61.72±0.46^{a}	16.25±3.11	18.72±0.84^{cd}	9.18±0.19	97.85±8.75^{a}	165.74±23.17^{a}	5.31±0.18	460.95±23.41^{ab}
	P600	85.13±4.03^{b}	44.40±4.51^{b}	9.36±2.09	12.67±1.61^{d}	6.17±0.31	104.00±4.69^{a}	174.26±9.99^{a}	5.10±0.14	441.09±11.84^{b}
	P	**	*	ns	**	ns	**	*	ns	****
Heel	C	78.25±8.27^{bc}	49.70±1.32^{d}	17.92±4.96^{b}	15.31±2.49^{b}	5.84±1.10^{c}	78.02±13.46	100.96±19.50^{c}	4.94±0.05^{a}	350.96±19.09^{d}
	P200	112.41±10.54^{a}	75.78±6.64^{b}	32.30±2.07^{a}	28.20±5.44^{a}	7.28±1.09^{bc}	77.19±11.44	138.06±12.71^{ab}	5.62±0.17^{a}	476.84±20.04^{a}
	P300	94.64±10.41^{ab}	63.00±3.99^{c}	30.62±4.57^{a}	27.15±8.48^{a}	10.08±4.56^{ab}	95.68±7.90	102.08±12.88^{bc}	5.19±0.09^{a}	428.43±14.76^{c}
	P400	69.29±0.16^{c}	65.75±5.60^{bc}	11.23±0.45^{b}	15.22±1.34^{b}	10.06±1.82^{ab}	108.11±6.63	158.16±20.73^{a}	5.54±0.47^{a}	443.35±16.47^{bc}
	P600	76.40±8.69^{bc}	97.89±1.34^{a}	29.37±1.63^{a}	25.00±2.47^{a}	12.77±1.64^{a}	79.73±13.28	130.77±6.09^{abc}	5.50±0.32^{a}	457.42±21.04^{b}
	P	**	***	**	*	*	ns	*	ns	**
Eye of loin	C	54.85±2.14	44.74±3.95	17.12±4.37^{b}	15.54±6.92	8.08±1.65	54.66±0.13^{c}	114.73±1.50^{b}	5.94±1.29	315.66±14.27^{c}
	P200	68.11±2.72	55.14±5.62	10.97±4.72^{b}	14.41±1.33	4.54±2.62	150.75±4.34^{a}	188.95±8.31^{a}	5.20±0.26	498.06±11.93^{a}
	P300	55.64±2.78	38.**98**±19.94	11.00±9.90^{b}	11.11±7.01	6.25±1.01	95.85±8.10^{b}	129.63±17.52^{b}	6.67±0.92	355.12±21.79^{b}
	P400	73.67±15.69	56.44±1.13	17.63±4.04^{b}	17.07±1.38	5.50±1.43	110.75±21.82^{b}	186.69±21.74^{a}	4.80±0.07	472.55±20.07^{a}
	P600	39.03±22.25	49.13±6.71	36.82±3.58^{a}	8.77±3.35	5.55±2.44	51.57±9.41^{c}	132.85±26.34^{b}	5.04±0.19	328.75±22.44^{bc}
	p	ns	ns	*	ns	ns	***	**	ns	****

Note: Values are means ± standard error (n = 2). Values with different superscripts (^{a,b,c,d,e}) in the same column differ significantly within cuts. EAA stands for essential free amino acids; NEAA stands for non-essential free amino acids. P < 0.0001 presented as ∗∗∗∗for level of significance; P < 0.001 presented as ∗∗∗for level of significance; P < 0.01 presented as ∗∗level of significance; P < 0. 05 presented as ∗ for level of significance and ns meaning not statistically significant.

Table 12 The concentration of non-essential free amino acids composition (mg/100g) of New Zealand lamb cuts subjected to different pressure treatments (200MPa, 300MPa, 400MPa and 600MPa).

Cut	treatment	valine	leucine	isoleucine	methionine	phenylalanine	lysine	histidine	tyrosine	tryptophan	Total EAA	EAA+NEAA
Flat	C	26.05±4.40^{c}	15.29±0.67^{c}	16.87±0.66^{b}	4.11±0.94^{d}	11.55±0.23^{d}	9.62±0.66	13.24±0.42	11.23±0.50^{d}	6.24±0.60	114.19±8.97^{d}	502.18±27.30^{d}
	P200	33.19±5.04^{bc}	21.72±2.32^{b}	32.26±1.56^{a}	8.86±0.03^{c}	19.39±1.77^{c}	11.51±1.73	12.68±0.23	13.28±0.47^{cd}	6.54±0.14	159.42±10.02^{c}	554.04±17.80^{c}
	P300	34.16±0.44^{bc}	28.03±4.43^{a}	28.58±0.31^{a}	11.68±0.49^{a}	25.78±1.11^{ab}	8.38±0.62	16.82±2.13	22.45±2.84^{a}	6.02±0.32	181.89±10.34^{b}	636.13±20.14^{b}
	P400	40.13±0.09^{b}	29.22±1.72^{a}	30.74±1.75^{a}	12.86±0.39^{a}	25.09±0.84^{b}	11.01±0.71	15.50±0.75	15.11±0.01^{bc}	7.06±0.19	186.71±13.67^{b}	642.18±31.77^{b}
	P600	62.61±3.42^{a}	30.36±0.53^{a}	35.38±5.94^{a}	10.24±0.28^{b}	28.57±1.08^{a}	10.65±0.81	16.65±2.18	16.82±0.21^{b}	7.28±0.68	218.58±12.01^{a}	767.94±19.06^{a}
	p	***	**	**	****	****	ns	ns	**	ns	****	****
Tenderloin	C	16.88±1.13^{c}	14.06±0.50^{c}	12.00±0.69^{d}	4.74±0.42^{c}	15.40±1.54^{b}	10.97±0.12	12.90±1.35	15.54±0.78^{a}	5.23±0.54^{c}	107.71±13.69^{ab}	408.44±21.71^{c}
	P200	16.92±1.08^{c}	16.35±0.13^{b}	23.56±1.04^{a}	5.68±0.56b^{c}	15.37±0.53^{b}	8.99±0.08	13.79±0.20	10.98±0.92^{b}	7.96±0.62^{a}	119.60±11.55^{ab}	529.11±25.62^{a}
	P300	11.99±0.77^{d}	13.10±0.08^{c}	15.17±0.41^{c}	8.64±0.55^{a}	13.92±0.91^{b}	8.63±0.10	11.03±1.35	9.75±0.89^{b}	7.20±0.53^{ab}	99.42±10.91^{b}	479.47±33.01^{b}
	P400	24.56±1.90^{b}	17.88±0.13^{a}	18.90±1.00^{b}	6.00±0.48^{b}	18.93±0.47^{a}	9.78±0.18	13.16±0.83	11.42±0.55^{b}	6.34±0.38^{bc}	126.95±9.51^{a}	543.64±21.47^{a}
	P600	29.03±0.78^{a}	18.87±0.78^{a}	22.21±1.39^{a}	6.69±0.31^{b}	19.63±1.19^{a}	9.89±1.55	11.93±0.51	15.38±1.10^{a}	5.43±0.26^{c}	139.04±7.89^{a}	469.32±15.78^{b}
	p	****	****	****	**	**	ns	ns	**	**	**	***
Rump	C	19.12±1.21^{c}	16.71±0.95	23.00±2.65	14.78±0.48^{a}	14.80±1.09^{b}	9.29±0.33^{c}	11.87±0.23^{c}	11.50±2.62^{b}	7.16±0.07	128.22±10.07^{c}	421.28±34.39^{d}
	P200	35.83±0.87^{a}	22.30±1.39	25.75±0.61	9.47±0.17^{b}	20.02±1.19^{ab}	11.38±0.17^{b}	12.73±0.40^{c}	12.01±0.17^{b}	7.79±1.35	157.28±8.64^{ab}	662.06±33.67^{b}
	P300	26.88±2.60^{b}	26.79±4.85	22.73±1.79	11.57±2.54^{ab}	21.39±1.12^{a}	12.04±1.34^{b}	15.88±0.95^{b}	30.06±1.92^{a}	8.11±0.81	175.44±16.54^{a}	617.22±38.61^{c}
	P400	20.02±0.57^{c}	21.43±0.95	16.78±0.36	10.58±0.23^{b}	24.01±2.22^{a}	8.567±0.50^{c}	18.35±1.27^{a}	15.80±0.76^{b}	7.80±0.08	143.35±11.74^{b}	680.08±33.61^{b}
	P600	34.07±0.91^{a}	24.94±3.86	21.85±3.21	10.56±1.54^{b}	21.95±3.87^{a}	16.32±0.71^{a}	15.80±0.28^{b}	29.95±1.75^{a}	8.46±2.83	183.89±12.88^{a}	838.78±40.25^{a}
	P	****	ns	ns	*	*	***	*	****	ns	***	****
Knuckle	C	18.67±4.22^{b}	15.20±3.33	15.44±1.87^{b}	6.96±1.33	1310±2.11^{b}	8.24±0.59^{d}	12.66±1.13^{b}	11.27±2.58	7.51±0.40	109.04±13.96^{c}	439.25±47.84^{d}
	P200	21.34±4.50^{b}	21.72±2.32	17.81±1.52^{b}	9.14±1.46	21.11±1.97^{a}	9.40±1.35^{cd}	16.20±1.01^{a}	14.38±0.46	8.18±0.80	139.28±10.83^{b}	528.84±37.08^{c}
	P300	34.32±4.63^{a}	22.03±2.37	21.12±0.69^{b}	8.25±0.67	23.28±0.74^{a}	18.00±0.15^{a}	15.46±0.18^{a}	17.46±1.41	6.49±1.20	166.39±9.47^{a}	582.92±35.17^{b}
	P400	29.94±3.89^{ab}	22.11±2.47	21.48±4.47^{b}	6.55±0.79	21.19±4.14^{a}	12.08±1.19^{b}	15.63±0.91^{a}	11.89±0.63	6.85±0.76	147.72±16.05^{ab}	591.93±31.16^{b}
	P600	41.25±6.71^{a}	27.51±3.79	29.61±4.57^{a}	8.75±1.59	23.37±2.71^{a}	11.00±0.42^{bc}	15.60±1.44^{a}	14.69±0.92	7.08±0.26	178.85±13.55^{a}	723.72±39.63^{a}
	P	*	ns	*	ns	*	***	*	ns	ns	****	****
Bolar	C	13.85±0.47^{c}	12.07±0.58^{c}	7.80±0.28^{c}	4.67±0.32	13.05±1.49^{b}	8.26±0.26^{b}	13.67±0.75	10.43±1.44^{c}	5.68±0.51	89.48±10.09^{c}	438.33±23.89^{d}

Cut	treatment	valine	leucine	isoleucine	methionine	phenylalanine	lysine	histidine	tyrosine	tryptophan	Total EAA	EAA+NEAA
	P200	17.77±4.62^{bc}	13.50±1.96^{bc}	11.45±0.44^{bc}	4.63±0.80	13.14±2.14^{b}	9.91±0.75^{b}	13.98±0.72	10.91±1.35^{bc}	5.90±0.40	101.19±9.86^{b}	499.16±34.14^{c}
	P300	20.44±4.38^{bc}	17.02±1.30^{ab}	14.67±3.52^{b}	5.36±1.94	19.85±0.91^{a}	12.60±0.36^{a}	13.68±1.89	19.96±6.23^{a}	6.08±0.42	129.67±13.71^{ab}	642.02±21.94^{a}
	P400	35.18±4.63^{a}	20.69±3.52^{a}	21.24±1.62^{a}	6.07±2.64	18.18±2.15^{a}	8.70±1.47^{b}	12.02±1.61	16.10±3.61^{ab}	5.78±1.68	143.96±17.24^{a}	548.83±31.62^{b}
	P600	31.18±8.97^{ab}	18.77±0.24^{a}	16.57±2.78^{ab}	5.58±2.14	19.96±1.31^{a}	9.89±1.56^{b}	16.86±3.99	11.89±0.99^{bc}	8.30±3.73	139.00±11.67^{a}	625.04±17.73^{a}
	p	*	*	**	ns	*	*	ns	*	ns	***	****
Inside	C	38.01±1.34^{b}	27.67±1.84	23.36±0.50	9.38±0.24	19.91±0.75	9.51±0.41^{b}	12.57±1.38^{b}	17.12±0.71	8.63±0.02^{a}	166.17±10.52^{a}	562.03±15.47^{c}
	P200	46.81±6.33^{a}	25.68±2.71	32.38±2.21	7.07±0.02	22.15±1.79	9.62±0.22^{b}	14.76±1.17^{b}	14.32±2.32	6.20±0.18^{c}	178.98±14.75^{a}	666.91±39.87^{a}
	P300	34.10±0.56^{bc}	23.20±4.50	22.13±9.57	10.44±1.15	19.56±5.18	10.51±2.30^{b}	14.60±0.35^{b}	21.29±3.15	6.95±0.04^{bc}	162.77±13.31^{a}	581.62±33.14^{b}
	P400	24.23±1.87^{d}	24.02±1.26	17.75±0.67	9.22±0.67	20.99±1.94	14.23±0.88^{a}	18.02±1.57^{a}	16.00±0.52	7.70±0.68^{ab}	152.16±11.17^{ab}	613.12±26.66^{b}
	P600	27.12±0.23^{cd}	22.69±0.21	20.46±0.34	7.98±2.32	18.75±1.68	11.42±0.09^{ab}	14.46±0.19^{b}	15.14±0.46	7.27±0.41^{b}	145.28±9.71^{b}	586.37±31.02^{b}
	P	**	ns	ns	ns	ns	*	*	ns	**	**	****
Heel	C	19.77±0.10^{bc}	13.68±2.05^{b}	13.15±1.95^{b}	3.53±0.65	16.61±1.94	8.67±0.97	11.81±1.27^{b}	9.14±1.08^{c}	5.17±0.77	101.52±14.71^{b}	452.48±33.47^{c}
	P200	31.74±0.03^{a}	18.11±0.62^{ab}	22.33±3.47^{a}	7.28±0.21	23.28±4.35	12.42±1.75	15.63±0.64^{a}	14.91±1.58^{a}	6.70±1.24	152.38±13.64^{a}	629.22±36.77^{a}
	P300	26.76±4.55^{ab}	16.89±3.11^{b}	21.10±0.34^{a}	5.25±0.97	18.46±3.48	8.91±0.43	12.69±0.51^{b}	12.27±2.97^{b}	6.22±0.88	128.55±8.47^{b}	556.98±37.94^{b}
	P400	15.98±3.65^{c}	15.17±1.09^{b}	11.31±0.81^{b}	4.96±0.33	15.05±1.35	9.11±2.01	16.11±1.23^{a}	11.67±0.63^{b}	6.22±1.04	105.57±17.89^{b}	548.92±29.07^{b}
	P600	34.74±6.63^{a}	21.63±0.42^{a}	21.55±0.32^{a}	8.47±2.51	24.36±0.12	8.54±3.24	16.40±1.15^{a}	15.09±0.10^{a}	6.54±0.93	157.31±9.61^{a}	614.73±31.85^{a}
	P	*	*	**	ns	ns	ns	*	**	ns	***	****
Eye of loin	C	19.61±8.64	10.71±0.97	10.58±2.46	4.95±1.01	10.13±1.50^{b}	10.25±1.61	14.80±0.25	17.15±2.40	8.03±0.51^{a}	106.21±9.11^{a}	421.86±19.40^{c}
	P200	18.69±4.74	13.22±1.01	11.29±3.97	4.74±2.47	13.5±1.53^{a}	12.56±4.04	13.17±2.88	16.98±5.08	6.46±1.33^{ab}	110.64±11.17^{a}	608.70±22.11^{a}
	P300	18.63±2.86	14.57±0.36	14.42±3.33	3.41±0.42	13.58±0.70^{a}	10.72±0.92	12.26±0.53	12.13±4.33	6.40±0.79^{ab}	106.10±12.43^{a}	461.22±26.91^{b}
	P400	21.21±6.28	13.21±0.12	13.39±3.07	3.35±1.05	14.56±0.58^{a}	14.07±1.04	11.55±1.74	10.60±0.43	6.34±0.40^{ab}	108.28±14.36^{a}	580.83±31.58^{a}
	P600	14.36±0.31	10.27±1.81	6.12±3.13	4.75±2.55	11.51±1.51^{ab}	8.59±2.20	10.64±3.49	9.16±2.37	5.45±1.31^{b}	80.84±13.35^{b}	409.59±29.41^{c}
	p	ns	ns	ns	ns	*	ns	ns	ns	*	*	****

Note: Values are means ± standard error (n = 2). Values with different superscripts (^{a,b,c,d,e}) in the same column differ significantly within cuts. EAA stands for essential free amino acids; NEAA stands for non-essential free amino acids. P < 0.0001 presented as ∗∗∗∗for level of significance; P < 0.001 presented as ∗∗∗for level of significance; P < 0.01 presented as ∗∗level of significance; P < 0. 05 presented as ∗ for level of significance and ns meaning not statistically significant.

4.3.2 Effect of different HPP treatments on free amino acids content

As seen in Table 11 and Table 12, high pressure processing increased the total free amino acids composition significantly ($P<0.05$) compared to control at all pressures for almost all cuts except inside and eye of loin cuts. Many researchers (Toldrá & Flores, 1998; Toldrá et al., 1997) suggested that processing meat would increase the presence of certain free amino acids by proteolysis. Suzuki et al. (1994) suggested that free amino acids have an important role in determining brothy and meaty tastes and as precursors of meat flavour. However, the effect of high pressure processing on free amino acid content is lacking.

Most cuts had significantly ($P<0.05$) the highest level of total amino acids at 600 MPa treatment except for inside, eye of loin and tenderloin cuts. Total amino acids were significantly higher in the tenderloin cut at 200 and 400 MPa, and inside cut at 200 MPa compared to control. The increase in free amino acids was similarly reported by Ohmori and Hayashi (1991) who suggested that high pressure between 100 and 300 MPa for 10 min at 25 °C increases the overall autolytic activity of raw beef round (inside cut) meat and leads to a higher concentration of free amino acids. However, their results also showed that with higher pressure treatments at 400 MPa and 500 MPa, the concentration of free amino acids was identical to that of the control sample unlike results from our study. Campus et al. (2008) studied free amino acids in dry-cured loins, and they showed that high pressure (300 to 400 MPa for 10 min at 20 °C) can stabilize the free amino acid content during storage due to reduction in the activity of amino peptidases. Moreover, Suzuki et al. (1994), reported that high-pressure treatments (200 to 400 MPa at ambient temperature) did not influence amount of amino acids in beef shoulder skeletal muscles (heel).

As for the eye of loin cut, a significant decrease in total amino acids was observed at 600 MPa. In fact, there was a highly significant ($P<0.05$) decrease in the essential amino acids leucine and isoleucine. Simonin et al. (2012) suggested that muscle proteins are vulnerable to oxidative reactions that result in loss of EAA and decrease protein digestibility. The mechanisms and reaction pathways for the oxidation of lipids and proteins are different but are directly linked as both processes may be affected by similar prooxidant and antioxidant factors. Indeed, it seems that protein oxidation is observed under the same pressure levels as those found when lipid oxidation (with more

amino acids produced) occurs (>300 MPa). Results of lipid oxidation in this study supports this and in fact eye of loin had the highest level of lipid oxidation at 0.905 mg MDA/kg. In conclusion, our results showed that HPP processing of all cuts except eye of loin generally increased the concentration of free amino acids.

Chapter 5 Conclusion

As one of the non-thermal food processing techniques, high pressure processing has been increasingly applied on meat in recent years. However, studies have mainly centred on the effects of pressure on food preservation and physical characteristics. In the present study, eight New Zealand lamb meat cuts, tenderloin (T), rump (R), knuckle (K), inside (I), heel (H), Flat (F), eye of loin (E) and bolar (BL) subjected to high pressure processing treatments were evaluated in terms of lipid oxidation, as well as fatty acids and free amino acids composition.

Lipid oxidation is one of the most important chemical reaction that affects meat quality and acceptance. In the present study HPP treatment at higher pressures resulted in some lamb cuts having high TBARS values exceeding 0.5 mg MDA/kg. Specifically, the eye of loin cut had significantly highest ($P<0.05$) MDA values when treated at 400MPa (0.80mg MDA/kg) and 600MPa (0.9mg MDA/kg), followed by flat cut at 600MPa sample (0.8mg MDA/kg), heel cut at 600MPa sample (0.54mg MDA/kg), and tenderloin cut 400MPa (0.63mg MDA/kg) and 600MPa (0.57mg MDA/kg).

Fatty acids and free amino acids are of great significance in meat and meat products. They play an important role in flavour (taste and odour) development. Different from other studies we found more significant effects of HPP treatment at varying pressures with different cuts of meat. HPP treated rump and heel cuts had higher SFA and MUFA ($P < 0.05$) contents, as well as lower PUFA/SFA ratio ($P < 0.05$) compared to control sample. The increase of SFA was related to the increase ($P < 0.05$) of C16:0 and C18:0 while the increase in MUFA was related to the increase ($P < 0.05$) of 18:1n9 and 18:2n6. PUFA content was not significantly different to control for both cuts suggesting that little or no oxidation occurred. This is supported by our oxidation results for rump and heel cuts that was a maximum at 0.448 and 0.541 mg MDA/kg at 600 MPa.

High pressure processing increased the total free amino acids composition significantly ($P<0.05$) compared to control at all pressures for almost all cuts except inside and eye of loin cuts, which is good in terms of nutritional value. Most cuts had significantly ($P<0.05$) the highest levels of total amino acids at 600MPa treatment except for inside, eye of loin and tenderloin cuts. Total amino acids were significantly higher in the

tenderloin cut at 200 and 400 MPa, and inside cut at 200 MPa compared to control. In general, the increase of fatty acids and free amino acids suggesting an increasing nutritional value which may exert enhanced health benefits and prevention of diseases.

Further studies looking at the interaction of lipid oxidation and fatty acids composition would be useful to explain the data and it would be important to add anti-oxidant reagent into red meat then get through high pressure processing to reduce the lipid oxidation in red meat. On the other hand, the study of protein oxidation might explain the increase of free amino acids in our result. Finally, because high pressure processing was evaluated to be food industrial technical it would be good to have the sensory test.

Overall, high pressure processing improved the quality of lamb meat in terms of fatty acids composition, free amino acids composition. However, the lipid oxidation was also increased by high pressure processing, which will influence the quality of lamb meat and not acceptable. The result from this study will be useful to the New Zealand meat industry to increase the nutritional value of New Zealand lamb meat by using high pressure processing. With continuous development of the high pressure techniques to preserve meat product to reach the global market.

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List of abbreviations

AA	Amino acid
BHT	Butylated hydroxytoluene
DDW	Double distilled water
EDTA	Ethylenediaminetetraacetic acid
FAA	Free amino acid
FAME	Fatty acid methyl ester
FID	Flame ionisation detector
GC	Gas chromatography
HHP	High hydrostatic processing
HPP	High pressure processing
MDA	Malondialdehyde
MUFA	Mono unsaturated fatty acid
POV	Peroxide value
PPE	Solid phase extraction
PUFA	Poly unsaturated fatty acid
SFA	Saturated fatty acid
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TEP	Tetraethoxypropane

Appendix:

EZ:faast™, Phenomenex®, USA user manual.

1.1 Reagents

Reagent	Ingredients	Volume
Reagent 1 Internal Standard Solution	Norvaline 0. 2 mM N-propanol 10%	50mL
Reagent 2 Washing Solution	N-propanol	90mL
Reagent 3A Eluting Medium Component I	Sodium Hydroxide	60mL
Reagent 3B Eluting Medium Component II	N-propanol	40mL
Regent 4 Organic Solution I	Chloroform	4 vials, 6mL each
Reagent 5 Organic Solution II	Iso-octane	50mL
Reagent 6 Acid Solution	Hydrochloric Acid 1N	50mL
SD 1, 2, & 3 Amino Acid Standard Mixtures	Please refer to section 4.7 in the manual	2 vials of each SD, 2mL each

3.0 Sample Preparation Procedure

3.1 Setup

The EZ:faast kit packaging has been designed as an efficient workstation. It holds a reagent tray, a vial rack, a pipette rack and a section for sorbent tips and vials. To speed up sample preparation it is recommend that the workstation be arranged as shown in figure 1a. By following directions and markings on the reagent box by breaking along perforations it can be transformed into a reagent tray. When the kit is not in use for several days, the reagent tray (figure 1b) may be conveniently removed and placed in the refrigerator.

3.2 Preparing the Eluting Medium

The volume of prepared Eluting Medium depends upon the number of samples to be analyzed during the day (200µL/sample). The eluting medium should be prepared fresh each day:

1. Use capped vials of appropriate size (not included) for preparation of the Eluting Medium.
2. Combine 3 parts Reagent 3A (Eluting Medium Component I) with 2 parts Reagent 3B (Eluting Medium Component II) in an appropriate sized vial (see Table 2, page 5, for reagent volumes based on number of samples). Mix briefly.
3. Store prepared eluting medium during the day at room temperature. Discard any unused mixture at the end of the day.

Workstation Arrangement - (Figure 1)

To speed up sample preparation it is recommended that the workstation be arranged as shown below.

Table 2 - For your convenience check the table below to determine the volume of Eluting Medium components needed depending on your number of samples:

Number of Samples	Reagent 3A Eluting Medium Component I	Reagent 3B Eluting Medium Component II
2	300µL	200µL
4	600µL	400µL
7	900µL	600µL
12	1.5mL	1.0mL
14	1.8mL	1.2mL
19	2.4mL	1.6mL
24	3.0mL	2.0mL
29	3.6mL	2.4mL
34	4.2mL	2.8mL
39	4.8mL	3.2mL
44	5.4mL	3.6mL
49	6.0mL	4.0mL

3.3 Sample Preparation by SPE and Derivatization

Prepare Eluting Medium first; refer to section 3.2 for preparation protocol. The freshly prepared Eluting Medium vial may be placed in one of the empty slots in the reagent tray.

1. For each sample, line up one glass sample preparation vial in the vial rack (Figure 2). Be aware of some variability in vial opening and sorbent tip dimensions, which may prevent the tip from reaching to the bottom of the sample preparation vial.

Note: *Droplets of liquid in SPE tip or spilled sorbent particles will not affect the precision of the assay in any way.*

Glass Vial Line Up - (Figure 2)

For each sample, line up one glass sample preparation vial in the vial rack.

Figure 2

2. Pipette 100µL sample (serum, plasma, urine or other), and 100µL Reagent 1 (Internal Standard Solution) into each sample preparation vial.

Caution: The pH of biological samples is usually around 7. After the addition of Reagent 1 (Internal Standard) the mixture has the correct pH for successful loading onto the SPE tip as described in the next step. With other samples make sure that the sample + Reagent 1 mixture has a pH between pH 1.5 and pH 6.0!

Note: *Samples with amino acid concentrations higher than 10mmol/L (10µmol/mL; e.g. dark colored urine) should be analyzed by pipetting only 50µL (or 25µL) sample in the sample preparation vial instead of 100µL. Concentrations recorded as a result of the GC analysis will be half (one quarter) of the actual concentrations for these samples. Conversely, when low concentrations of amino acids have to be quantified, the volume of sample to be prepared should be 200µL or more.* ***The total amount of amino acids present in the sample to be loaded onto the SPE tip should not exceed 1.2 µmols.***

3. Attach a sorbent tip to a 1.5mL syringe and loosen the syringe piston; immerse the tip and let the solution in the sample preparation vial pass through the sorbent tip by SLOWLY pulling back the syringe piston, in SMALL steps.

Caution: Do not quickly pull back the piston. Try to take at least one minute to pass low viscosity sample (such as urine or standard) through the sorbent tip. For very viscous samples like concentrated plasma, up to 200µL of water can be added to ease the sample transfer through the sorbent. The syringe should be capable of drawing all sample, and subsequent wash reagent into the barrel. Watch as the liquid accumulates inside the syringe barrel and move the piston only as the accumulation slows down. Urine passes relatively fast through the sorbent bed, while serum and plasma pass much slower. If you run out of piston range, detach the sorbent tip, expel the solution from the syringe barrel, then reattach the sorbent tip and proceed with sample preparation.

Note: *the sorbent tip should stay in the sample preparation vial through steps 3-9 (see figure 3) even while dispensing reagents. In case the sorbent tip cannot reach to the bottom of the vial, tilt the vial to about 45°, push the tip into the vial gently, and proceed with the SPE step.*

4. Pipette 200µL Reagent 2 (Washing Solution) into the same sample preparation vial. Pass the solution SLOWLY through the sorbent tip and into the syringe barrel. Drain the liquid from the sorbent bed by pulling air through the sorbent tip. Detach the sorbent tip, and leave it in the sample preparation vial, then discard the liquid accumulated in the syringe.

Note: *save the syringe, as it can be reused with many other samples. For convenience place it into the pipette rack.*

5. Pipette 200µL Eluting Medium (prepared fresh each day, section 3.2) into the same sample preparation vial.

Keep the Sorbent Tip in the Vial - (Figure 3)

Keep the sorbent tip in the sample preparation vial through steps 3-8, even while dispensing reagents.

Figure 3

6. Pull back the piston of a 0.6 mL syringe halfway up the barrel and attach the sorbent tip used in steps 3-6.
7. Wet the sorbent with Eluting Medium; watch as the liquid rises through the sorbent particles and stop when the liquid reaches the filter plug in the sorbent tip.
8. Eject the liquid and sorbent particles out of the tip and into the sample preparation vial. Repeat step 7 and 8 until the sorbent particles in the tip are expelled into the sample preparation vial. Only the filter disk should remain in the empty tip, see figure 4. Keep the syringe as it can be reused with many other samples.
9. Using the adjustable Drummond Dialamatic Microdispenser (included) transfer 50µL Reagent 4 into the sample preparation vial.

Caution: Avoid cross-contamination by not touching the inner wall of the sample vial with the tip of the Microdispenser. The piston will ensure proper transfer of liquids into the vial without the need of touching the vial wall. Use the same Microdispenser with both Reagents 4 and 5. There is no need to change Microdispenser tips or to wash between uses. Change Microdispenser tips only when broken.

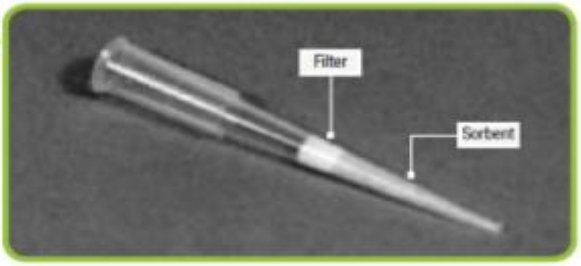
3.4 Optimizing Sample Preparation Time

For experienced users, sample preparation proceeds in 7-8 minutes per sample. This process can be further improved by preparing up to ten samples at a time. For example, at step 2 dispense Reagent 1 (and at later steps all other reagents) in ten vials successively, using the same pipette tip. At step 9, after dispensing Reagent 4, vortex 2-3 vials simultaneously. During each one minute wait at steps 10-12, prepare the next set of samples for SPE.

Sorbent Tip - (Figure 4)

Wet the sorbent with Eluting Medium and stop before it gets to the filter then eject the liquid and sorbent particles out of the tip.

Figure 4



Warning: Do not use regular pipettes and tips with Reagents 4 and 5 as they will contaminate the sample! Use the included Microdispenser for Reagents 4 and 5 ONLY!

Note: *for all subsequent sample preparation steps use a vortex mixer set in the touch (pulse) mode (to about 80% of max speed) for any mixing operations.*

10. Emulsify the liquid in the vial by repeatedly vortexing for about 5-8 seconds. During vortexing hold the sample vial firmly between fingers, and keep it straight as you push it onto the vortex plate. Do not let vial wobble, otherwise liquid may come out of the vial. Allow reactions to proceed 1 minute or more. The emulsion will gradually separate into two layers.

Note: *a longer reaction time than 1 minute at step 10 and at step 11, or later, at step 12, does not affect results.*

11. Re-emulsify the liquids in the vial by vortexing again for about 5 seconds. Allow the reaction to proceed for one additional minute or more.
12. Transfer with the Microdispenser 100µL Reagent 5 (50µL twice, for convenience) and mix for about 5 seconds. Let the reaction proceed for one more minute.
13. Pipette (DO NOT use the Microdispenser for this purpose!) 100µL Reagent 6, and vortex for about 5 seconds. The emulsion will separate into two layers again. The upper, organic layer contains amino acid derivatives to be analyzed by gas-chromatography (see GC set up and calibration in section 4). Sample this layer directly from the sample preparation vial or use a pasteur pipette to transfer part of it into an autosampler vial.

4.0 Gas Chromatographic Analysis

4.1 Column For EZ:faast Free Amino Acid Analysis by GC

The Zebron ZB-AAA GC column comes without a cage. Connect the ends of the column in the usual manner; rest the column coil on the oven bracket. Keep the pieces of thermal thread spaced evenly around the column coil. The maximum column temperature is 320/340°C.

Caution: Always use safety glasses while installing the GC column.

4.2 Instrument Settings:

Constant Flow Mode GC-FID/NPD (recommended)

Injection*	Split 1:15@250°C, 2µL (with hot needle, see section 4.6)
Carrier Gas	Helium 1.5mL/min **constant flow**
Oven Program	32°C/min from 110° to 320°C

Constant Pressure Mode GC-FID/NPD

Injection*	Split 1:15 @ 250°C, 2.0µL
Carrier Gas	Helium, 8 psi (60 kPa) or Hydrogen 30 kPa
Oven Program	35°C/min from 110° to 320°C
Detector	320°C

*When using a Shimadzu GC instrument, please increase the injector temperature to 300°C

For your convenience we have included the GC method for the Agilent 6890 GC system on the reference CD included with the kit. To use the included method: copy the method folder into the appropriate method folder in your software .

4.3 Mode of Operation

For best resolution, a rate of 35°C/min is preferred with instruments operating in constant pressure mode only. Electronic Pressure Control (EPC) or Advanced Flow Controller (AFC) equipped instruments should be operated preferably in constant flow or constant velocity mode. With these instruments a temperature gradient of 30-32°C/min is fast enough to elute the least volatile derivatives (i.e. those of cystine and homocystine) with similar retention times to constant pressure mode. If the instrument is not equipped with the EPC option, you may use a pressure raise of 3kPa/min.

4.4 Liners

Use the best deactivated liners supplied by the instrument manufacturer. Good results were obtained with FocusLiners™ (included; Phenomenex P/N AG0-4680; fits Agilent and Varian 1177 injectors). In general, the liner should carry a plug of silanized quartz or pesticide grade glass wool.

4.5 Injection

- Split injection at a ratio of 1:10 to 1:20 is recommended
- Injection volumes of 1.5-2µL are optimal

Quasi-splitless injection mode will produce a 5 to 10 fold increase in sensitivity with some instruments. In this mode, the split valve should be closed for an initial 5 to 7 seconds. Before selecting this injection mode it should be checked experimentally that no significant discrimination of late eluting amino acid derivatives takes place in comparison with common split injection. Alternatively, instruments equipped with EPC/AFC can be operated with double initial head pressure for 6-10 seconds.

4.6 Sampling

Both autosampler and manual sampling can be performed. If manual sampling is preferred, hot needle injection is recommended to prevent discrimination of components with high boiling temperatures. With this technique the sample plug is completely drawn into the syringe barrel, leaving the needle empty. The needle is inserted and kept in the hot injector for about two seconds before injection.

4.7 Calibration Standards

For quantitation purposes, mixtures of amino acid standards should be prepared following the Sample Preparation by SPE and Derivatization procedure described in this manual in Section 3.3. Standard mixtures should be stored in the freezer as some amino acids are not stable in solution. Three vials of different standard mixtures are included in the kit:

SD1: 23 amino acids, 200 nmoles/mL each, as follows:

AAA	ASP	GLY	LEU	PHE	THR
ABA	BAIB	HIS	LYS	PRO	TYR
alLE	C-C	HYP	MET	SAR	VAL
ALA	GLU	ILE	ORN	SER	

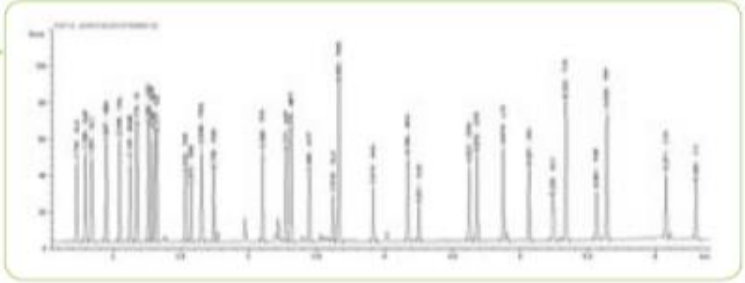
SD2: Complementary amino acids not stable in acidic solution, 200 nmoles/mL each, as follows:

ASN	GLN	TRP

SD3: Complementary urine amino acids, 200 nmoles/ mL each, as follows:

APA	CTH	GPR	HLY	PHP	TPR

Figure 5



A typical chromatogram of a mixture of all three amino acid standard solutions included in this kit. Column and instrumental settings as specified in section 4.1-4.2.