Effect of High Pressure Processing (HPP) and Pulsed Electric Field (PEF) on digestibility of New Zealand chilled lamb

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to be the best of my knowledge and belief, ‘High Pressure Processing (HPP) and effect of pulsed electric field (PEF) on digestibility of New Zealand chilled lamb’, 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.

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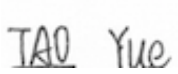
Signed: ……  …….

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List of abbreviations

AA amino acids

ALA alanine

ASN asparagine

ASP aspartic acid

C-0 non-PEF (0 day)

C-7 non-PEF (7 days)

EAAs essential free amino acids

FAAs free amino acids

GC gas Chromatography

GIT human gastrointestinal tract

GLN glutamine

GLU glutamic acid

GLY glycine

HAS heterocyclic aromatic amines

HCl hydrochloric acid

HHP high hydrostatic pressure

HIS histidine

HPP high pressure process

ILE isoleucine

LC-MS liquid chromatography–mass spectrometry

LEU leucine

LYS lysine

MET methionine

NEEAs non-essential free amino acids

NMR nuclear magnetic resonance

ORN ornithine

P-0 PEF (0 day)

P-7 PEF (7 days)

PDCAAS high protein digestibility-corrected amino acid score

PEF pulsed electric filed

PHE phenylalanine

PRO proline

S1 prior to digestion

S2 gastric phase

S3 intestinal phase

SER serine

SGF simulated gastric fluids

SIF simulated intestinal fluids

SSF simulated salivary fluids

TAU taurine

TCA trichloroacetic acid

THR threonine

TRP tryptophan

TYR tyrosine

VAL valine

Abstract

Lamb is one of the widely consumed meats in New Zealand owing to its flavor and high nutritional value such as protein, mineral and vitamins. Recently, High Pressure Processing (HPP) and Pulsed Electric Field (PEF) have been adopted in processing lamb with the advantage of extending the shelf life without reducing the quality. The aim of the project was to examine one of the attributes of nutritional quality of lamb, "digestibility". Digestibility of different lamb cuts at different phases of simulated digestion (undigested, gastric and intestinal, S1, S2 and S3, respectively); the effect of HPP and PEF treatments on digestibility of lamb; storage effect for PEF treated lamb on digestibility have been investigated. The supply of lamb may not be able to meet the demand, using HPP and PEF treatments may be worth investigating. Studies examining the digestibility of meat, practically lamb, and treated (HPP and PEF) lambs, are missing in the literature. This was the first study to investigate the FAAs of HPP and PEF treated lamb cuts after the *in vitro* digestion. The amount of FAAs in the lamb cuts is different. Some lamb cuts are ignored by the consumers though they are still nutritionally valuable to contribute to human health. This was another important part in my research. In this project, examinations of the physical properties of lamb samples such as color, moisture and pH, were also studied. An *in vitro* digestion model was used to simulate the digestion of the processed (HPP and PEF) lamb. Nineteen free amino acids (FAAs), including 9 essential and 10 non-essential amino acids, were identified and quantified in both HPP and PEF lamb samples using EZ-faast kit and GC.

For the HPP treated lamb, the total FAAs in three different digestion phases (S1, S2 and S3) of two lamb cuts (rump and knuckle) were significantly different from one another ($P < 0.05$). The lamb cuts were significantly affected ($P < 0.05$) by the high-pressure treatments, in terms of digestibility. The total FAAs of rump and knuckle increased from 200 MPa to 300 MPa, and decreased from 300 MPa to 600 MPa in S1, S2 and S3. The same pattern was observed with most of the nineteen individual FAAs as well. Different cuts also affected both total FAAs and individual FAAs significantly ($P < 0.05$). The high-pressure condition affected the pH significantly ($P < 0.05$) for both cooked rump and knuckle lamb cuts. Lamb samples processed at higher pressure levels (600 MPa) had higher L* and b * values. Moisture content of the lamb samples differed largely ($P < 0.05$) with high-pressure treatments.

For PEF treated lamb, the total FAAs found in the three digestion phases of the seven lamb cuts (bolar, rump, knuckle, full back, cube roll, shanks and flat) differed significantly ($P < 0.05$) from each other. The storage time (0 day: samples were stored at -20°C immediately after the slaughter. 7 days: samples were stored at 4°C for 7 days and then stored at -20°C until further analysis) had a significant effect ($P < 0.05$) on the amount of FAAs present at all digestion phases (S1, S2 and S3) for both non-PEF and PEF treated lamb. The amount of total FAAs differed depending on the lamb cuts, for all three phases of digestion significantly ($P < 0.05$) as well. For the color, the lightness increased, redness and yellowness decreased from 0 day to 7 days for both non-PEF and PEF treated lamb. Moisture for some cuts showed significant difference ($P < 0.05$) with respect to the storage time (0 day and 7 days).

Chapter 1. Introduction

Lamb, as a type of red meat, is very popular in diet due to the high nutritional value such as high amounts of protein (21.9 %), fat (2 %), essential amino acids, minerals and vitamins, such as niacin, vitamin B6, B12, calcium, sodium and iron (Williamson et al., 2005; Biesalski, 2005; Williams, 2007). New Zealand has the highest density of sheep per unit area in the world. It is one of the main exporters of lamb meat. The value of lamb exports increased from NZD 2.3 to 2.6 billion from 2011 to 2015 (Ngo et al. 2016; Beef and Lamb New Zealand, 2016). With the growing demand for lamb consumption in New Zealand, the supply of lamb may experience shortage in the near future. In order to solve this problem, extending the shelf life by unique processing methods, or applying innovative technology to enhance the digestibility of lamb, or a better utilization of different cuts maybe worth investigating.

The high nutrient content of lamb makes the growth condition favorable for microorganisms and common food borne pathogens, which results in oxidation, flavour loss, nutrition loss and color change prior to consumption (Aymeric, Picouet, & Monfort, 2008). In order to solve this problem, non-thermal treatments such as high pressure processing (HPP) and pulsed electric fields (PEF) had been applied on lamb cuts in this project.

The aim of the project was to examine one of the attributes of nutritional quality of lamb, “digestibility”. Digestibility of different lamb cuts at different phases of simulated digestion (S1, S2 and S3); the effect of HPP and PEF treatments on digestibility of lamb; storage effect for PEF treated lamb on digestibility have been investigated. The supply of lamb may not be able to meet the demand, using HPP and PEF treatments maybe worth investigating. Studies examining the digestibility of meat, practically lamb, and treated (HPP and PEF) lambs, are missing in the literature. This was the first study to investigate the FAAs of HPP and PEF treated lamb cuts after the *in vitro* digestion. The amount of FAAs in the lamb cuts is different (Madruga et al., 2010). Some lamb cuts are ignored by the consumers though they are still nutritionally valuable to contribute to human health. This was another important part in my research. In this project, examination of the physical properties of lamb samples such as color, moisture and pH, were also studied.

This thesis is composed of five chapters. Chapter one is introduction. Chapter two provides a literature review summarizing the nutritional value and consumption of lamb, followed by the processing methods and applications of HPP and PEF, and *in vitro* digestion models used to examine meat and meat products. Chapter three shows materials and methods used in the project, including experimental design, preparation of lamb samples for processing, treatments of the lamb samples with HPP and PEF, sous vide cooking method, analysis of physical properties of cooked lamb meat, extraction and analysis of FAAs in lamb prior to the digestion and during *in vitro* digestion, static *in vitro* digestion protocol and statistical data analysis. In chapter four, individual and total FAAs detected prior to digestion and post to digestion are shown. Physical properties of lamb such as color, pH and moisture are presented and discussed. The conclusion chapter summarizes the main findings of the study, and suggests avenues of future related research.

Chapter 2. Literature review

This chapter is comprised of a critical literature review which is divided into 6 sections. Nutritional value of lamb (Section 2.1), consumption of lamb in New Zealand and other countries (Section 2.2), different muscle cuts of lamb (Section 2.3), analysis of FAAs in meat (Section 2.4), processing methods of meat (Section 2.5) such as sous vide cooking method (Section 2.5.1), high pressure processing (HPP) (Section 2.5.2) and pulsed electric fields (PEF) (Section 2.5.3) and simulation of human digestion *(in vitro)* digestion methods (Section 2.6).

2.1. Nutritional value of lamb

Lean lamb is composed of about 75 % of water, 20 % of protein, 2 % of fat and 3 % of minerals and vitamins (Pedersen et al. 2003). Williams (2007) and Williamson et al. (2005) stated that on average, raw red meat contains about 20 to 25g of protein per 100g. When the meat is cooked, the protein content increases up to 27 to 35 g per 100g, due to the loss of water. Other nutrients of lamb include fat, omega-3 polyunsaturated fatty acids (PUFAs), highly bioavailable minerals and vitamins, such as " iron, zinc, niacin, phosphorus and cobalamin" (Biesalski, 2005; Williams,2007). Iron plays an essential role in producing blood of human (Beard, 2001). Zinc is critical for our immune system to function properly (Bax et al., 2013). Insufficient niacin can lead to the headache, tiredness, skin and mouth lesion (Keene et al., 2014). Phosphorus contribute to the formation of cell membranes and play an important role in children's growth (Kim & Linkswiler, 1979). Cobalamin helps our body to generate red blood cells and are required for DNA and RNA synthesis (Bax et al., 2013). Moreover, meat is low in carbohydrate and therefore it is considered a low glycaemic index food (Biesalski, 2005). Table 1 shows the average nutritional composition (per 100 g) of cooked lean beef, veal, lamb and mutton. Lamb is known to have the highest fat content (4.7 g) and energy (546 kJ). Vitamin A (8.6 mg) and calcium (7.2 mg) in lamb are present in much higher amount than those in beef and veal. Compared with the other four different types of red meat, the mineral contents of lamb, such as iron (2.0 mg), zinc (4.5 mg), magnesium (28 mg), copper (0.12 mg) and selenium (14 mg), are very high. The nutritional composition of meat differs significantly among different animal species

(Pereira and Vicente, 2013), breeds, sex (Arsenos et al., 2002), meat cuts (Pereira & Vicente, 2013) and feeding regimes (Williams, 2007).

Table 1. A table summarizing the average nutrient composition (per 100 g) of four different types of cooked red lean meat (Williams, 2007).

	Beef	Veal	Lamb	Mutton
Energy(kJ)	498	477	546	514
Moisture (g)	73.1	74.8	72.9	73.2
Protein (g)	23.2	24.8	21.9	21.5
Fat (g)	2.8	1.5	4.7	4.0
Cholesterol (mg)	50	51	66	66
Vitamins (mg)				
Thiamine	0.04	0.06	0.12	0.16
Riboflavin	0.18	0.20	0.23	0.25
Niacin	5.0	16.0	5.2	8.0
Vitamin B6	0.52	0.8	0.10	0.8
Vitamin B12	2.5	1.6	0.96	2.8
Pantothenic acid	0.35	1.50	0.74	1.33
Vitamin A	< 5	< 5	8.6	7.8
Beta-carotene	10	< 5	< 5	< 5
Alpha-tocopherol	0.63	0.50	0.44	0.20
Minerals (mg)				
Sodium	51	51	69	71
Potassium	363	362	344	365
Calcium	4.5	6.5	7.2	6.6
Iron	1.8	1.1	2.0	3.3
Zinc	4.6	4.2	4.5	3.9
Magnesium	25	26	28	28
Phosphorus	215	260	194	290
Copper	0.12	0.08	0.12	0.22
Selenium	17	< 10	14	< 10

The averaged values for beef were from diced, stir-fried beef of different cuts (round, rump, topside, silverside, fillet, sirloin, and scotch fillet, T-bone, blade and chuck steak). For the veal, values from diced, stir-fried leg steak and cutlet were averaged. Lamb values were averaged from diced and stir-fried in

different cuts (leg, chump chop, loin chop, cutlet and shoulder). For the mutton, leg roast and casserole were averaged (Williams, 2007).

Lamb is a good source of complete protein that contains amino acids such as alanine, valine, threonine, asparagine, glycine, histidine, lysine, methionine and others. The amino acids are of great importance to our health as they serve as building blocks for proteins, large peptides and small peptides; regulation of gene expression; transport water, glucose, fatty acids, vitamins and minerals; building blocks of skeletal muscle and bone mass; and possess anti-inflammatory properties (Wu, 2013). The amino acids are classified into essential (indispensable) and non-essential (dispensable) amino acids for humans. Essentials amino acids (EAA) are defined as amino acids (AAs) with carbon skeletons being unable to be synthesized or inadequately synthesized by the body. The EAAs must be provided from the diet to meet the requirements of the body. Non-essential AAs (NEAA) are the AAs which can be synthesized in adequate amounts by the body to meet the requirements of the body (Wu, 2009). The nine EAAs found in cooked lamb include histidine (HIS), lysine (LYS), threonine (THR), methionine (MET), isoleucine (ILE), leucine (LEU), valine (VAL), phenylalanine (PHE) and tryptophan (TRY), which play an vital role in people's health. For example, LEU and ILE are branched chain amino acids that support muscle synthesis (Kohlmeier, 2015; Yang et al., 2015). A shortage of HIS and LYS can lead to anemia, mood swing and stunted growth. MET plays an important role in synthesis of cysteine, which protects liver cells from destruction (Wu, 2013). Lacking of PHE, THR and TRP can all lead to memory problem and affect brain functions such as mood swing and mood change (Fernstrom, 2013; Friedman and Levin, 2012). A shortfall of VAL affects the myelin which covers the nerves (Fontana et al., 2016). Glutamic acid (GLU)/glutamine (GLN) is present in the highest amounts (16.5%), followed by arginine (ARG), alanine (ALA) and aspartic acid (ASP) in meat, in general (Williams, 2007). The free amino acids (FAAs), such as leucine, isoleucine, serine (SER), threonine, valine, and phenylalanine are important contributors of aroma formation in meat (Madruga et al., 2010). These FAAs react with sugars and by-products of lipid oxidation in meat to produce heterocyclic compounds that contribute to the aroma and flavor of cooked meat (Penet et al., 1983).

Moreover, the protein in red meat is highly digestible, which is about 94%, compared to the digestibility of beans (78%) and whole wheat (86 %) (Williams, 2007). Red meat

such as beef, was full of essential amino acids and process a high Protein Digestibility-Corrected Amino Acid Score (PDCAAS) of 0.9, where the maximum score is 1.0. And the value of PDCAAS of red meat is known to be higher than the values of most plant proteins (0.5 to 0.7) (Schaafsma, 2000).

2.2. Consumption of Lamb in New Zealand and industrialized countries

New Zealand has the highest density of sheep per unit area in the world. Sheep remains as the predominant type of livestock with an average density of 117/km^2 in New Zealand. From Figure 1, it can be seen that the Manawatu-Wanganui in New Zealand had the densest livestock (303/km^2) and the densest sheep numbers (253/km^2) among all regions (Statistics New Zealand, 2012).

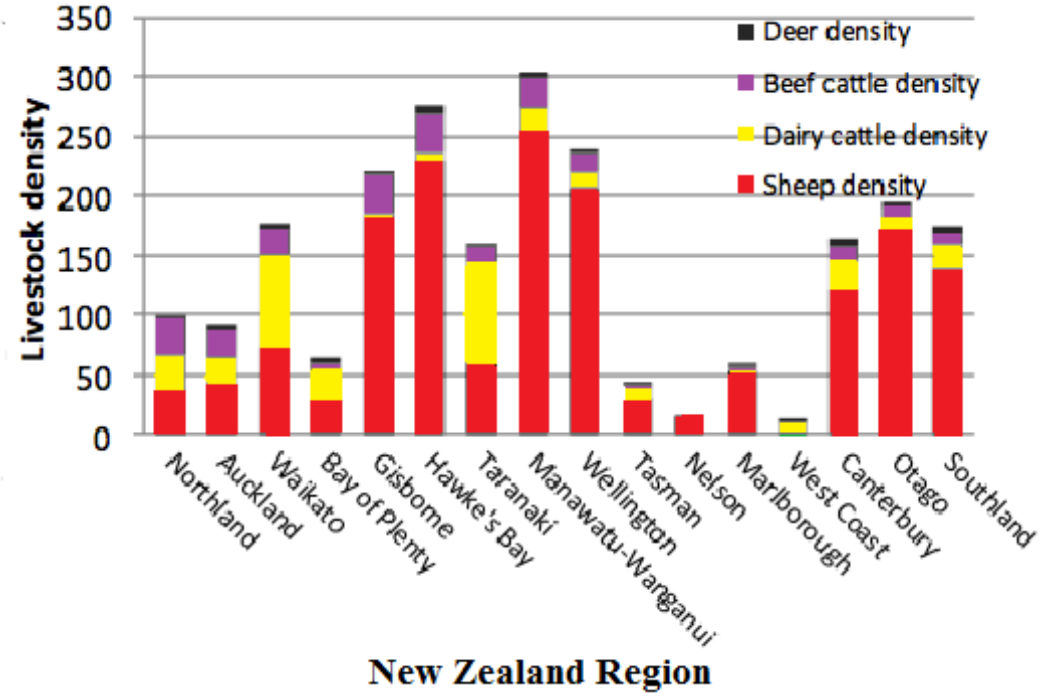

Figure 1. A chart showing livestock per km^2 in different regions of New Zealand (Modified from Statistics New Zealand, 2012).

Moreover, New Zealand is one of the main exporters of lamb meat. The value of lamb exports showed an increasing trend from 2011 (NZD $ 2.3 billion) to 2014 (NZD $ 2.5 billion). In the year of 2014-2015, the total value of lamb exports was NZD $ 2.6 billion, making sheep farming one of the most important agricultural industries (Figure 2) (Ngo et al. 2016; Beef and Lamb New Zealand, 2016).

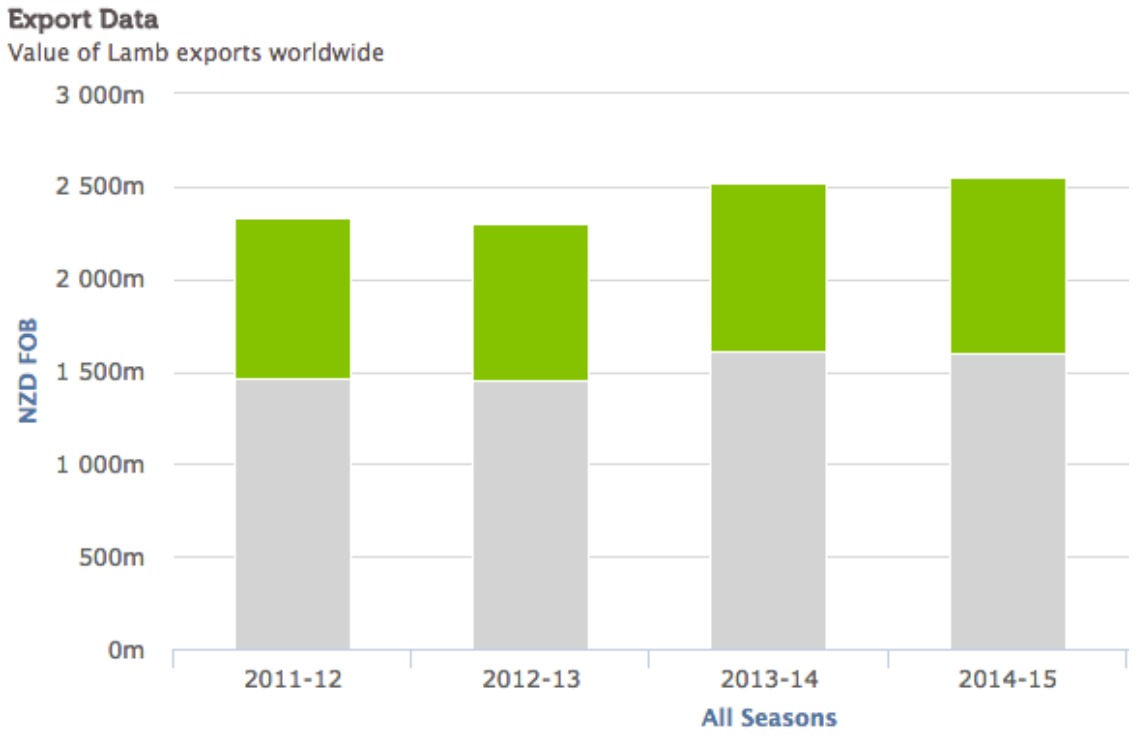

Figure 2. The economic value of lamb exports worldwide from 2011-1015 (data from Beef and Lamb New Zealand, 2016).

Note: Exports refer to all material goods which leave New Zealand for other countries and are valued free on board (FOB). Grey color represent the value of lamb exports from Oct-Apr each year. Green color represents the value of lamb exports whole year

Amongst the six industrialized countries, including USA, Canada, Japan, European Union, Australia and New Zealand, New Zealand had the highest lamb consumption in the year of 2010-2012 (average 8.8 kg), which was much higher than the consumption in USA (about 0.4 kg) (Figure 3). By 2022, the lamb consumption in New Zealand is projected to rise to 10.2 kg per year, which would be a significant increment when compared to beef, pork and poultry. Production of lamb and sheep meat may not be able to meet the rapidly growing demand, particularly for New Zealand. Therefore, a solution for long-term sustainability in supplying of lamb and sheep meat is required which may be solved by improvement in the use of different muscle cuts, enhanced processing technology or increasing of the digestibility to maximize the delivery of nutrients. Researches on these topics are further reviewed in Section 2.3, Section 2.5 and Section 2.6, respectively.

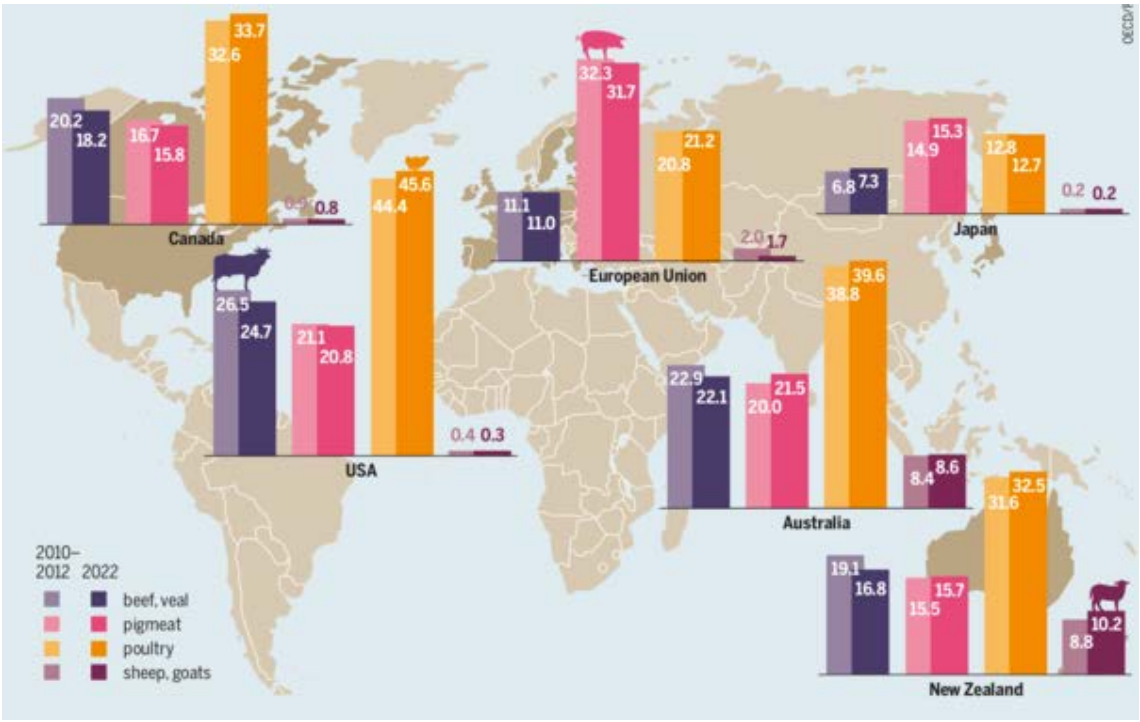

Figure 3. Meat consumption per capital in industrialised countries, average 2010-12 (estimate), and 2022 (forecast). Modified from (Meat Atlas 2014).

2.3. Different muscle cuts of lamb

The meat cuts used in the project are shown in Figure 4. The primal lamb cuts were divided to three main parts: front, whole loin and leg. Front includes bolar. Cube roll and full back are divided from the whole loin. The leg comprised of rump, knuckle, flat and shank. Depending on the type of muscle fibers and what the muscle is primarily used for, the AA content differs in raw meat. Bax et al. (2013) found that the indoor-reared meat contained higher amounts of contractile and structural proteins than the outdoor- reared meat. The difference in the amount of FAAs of different muscle cuts of meat is further summarized in Table 2. The research about the amount of FAAs in lamb cuts is absent.

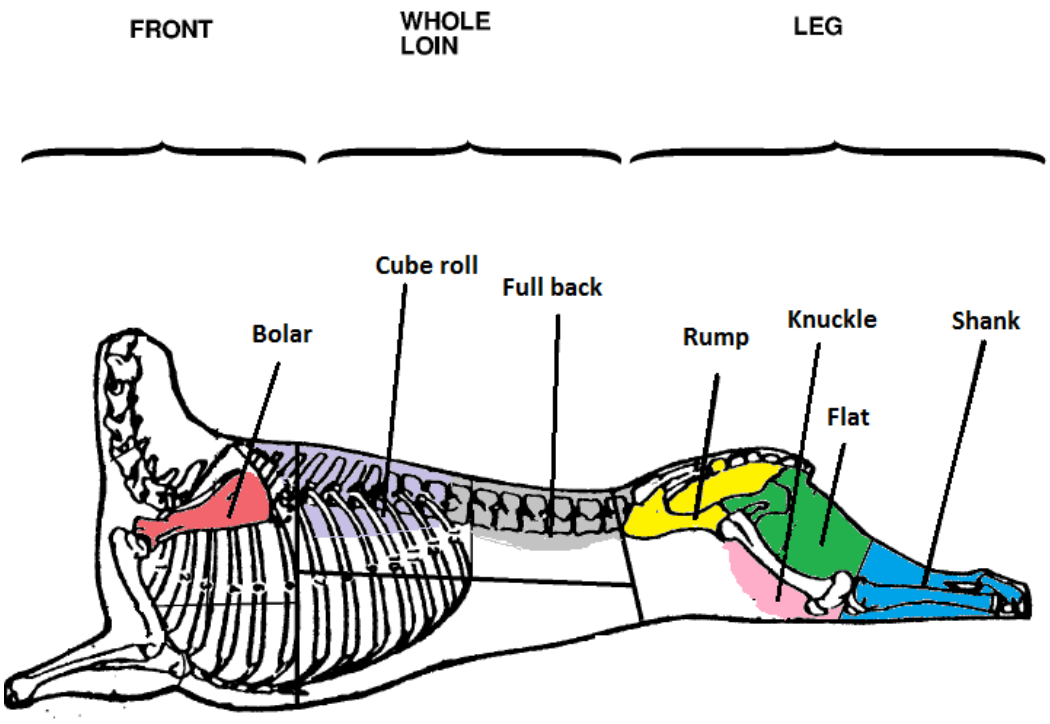

Figure 4. A diagram showing the seven different cuts of lamb used in the project (modified from http://www.aussiermb.org.au/images/lamb.gif)

Table 2. A table summarizing the amount of FAAs present in different cuts of goat meat, pork and beef.

Amino Acid	Raw rump goat meat (mg/100g)	Cooked rump goat meat (mg/100g)	Fresh *masseter* (pork) (µmol/100g)	Fresh *trapezius* (pork) (µmol/100g)	Fresh full back (pork) (µmol/100g)	Fresh heel (beef) (mg/100g)	Fresh knuckle (beef) (mg/100g)	Fresh inside round (beef) (mg/100g)	Fresh full back (beef) (mg/100g)	Fresh *masseter* (beef) (mg/100g)	Fresh *cardiac muscle* (Heart muscle) (beef) (mg/100g)
HIS	4.7	3.4	See ALA	See ALA	See ALA	37.58	41.10	20.89	9.71	49.44	96.24
ILE	4.8	3.2	30.28	31.01	26.98	10.43	5.02	5.55	2.16	6.63	3.32
LEU	7.9	5.1	21.56	17.47	19.15	6.00	2.90	2.40	1.55	3.35	1.79
LYS	5.6	3.7	18.89	17.65	18.23	7.46	4.35	4.52	1.64	5.95	3.53
MET	1.7	1.2	9.14	8.61	10.68	4.00	1.55	1.77	0.87	1.81	1.11
PHE	3.9	2.5	5.57	5.65	5.26	7.46	3.77	4.10	2.46	4.58	3.01
THR	6.4	3.8	15.75	20.05	19.64	11.77	14.01	15.14	5.88	7.64	4.51
VAL	7.6	5.1	67.25	58.82	47.23	8.52	4.10	4.70	1.48	5.54	2.72
ARG	12.2	9.3	15.70	29.94	32.03	10.90	21.81	18.93	n.d	11.35	1.90
ALA	38.7	30.4	359.73 (ALA+HIS)	335.56 (ALA+HIS)	299.15 (ALA+HIS)	21.18	20.83	19.01	5.22	26.39	28.14
ASP	1.2	1.2	74.01	63.67	52.72	2.84	2.61	2.39	2.36	2.95	4.31
CYS	0.04	0.01	n.d	n.d	n.d	8.62	6.05	7.36	2.62	2.47	n.d
GLU	11	6.3	77.82	90.57	82.71	20.10	8.78	11.18	5.00	15.92	28.64
GLY	56.7	38	117.12	153.53	155.09	9.38	6.81	7.77	3.04	5.51	4.34
PRO	4.2	3	35.56	52.80	35.56	5.86	3.60	4.06	2.55	4.31	3.85
SER	8.9	5.1	38.07	35.67	36.79	10.09	5.54	5.75	3.02	9.55	7.30
TYR	3.7	2.3	8.50	8.75	10.01	6.87	30.80	3.54	1.50	4.13	2.02
Total FAAs	220.2	150.9	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
TAU	n.d	n.d	n.d	n.d	n.d	59.81	69.03	74.26	17.82	11.21	18.53
References	a	a	b	b	b	c	c	c	c	c	c

References: (a) Madruga et al., 2010; (b) Cornet and Bousset, 1999; (c) Franco et al., 2010 n.d: not detected

FAAs and dipeptides present in different cuts of meat differ from one another (Cornet and Bousset, 1999). For example, *musculus.masseter* (cheek), *musculus.trapezius* (chuck) and *musculus.longissimus (*full back) in raw pig muscles have the same FAAs but in different amount Cornet and Bousset (1999). GLN differed significantly ($P < 0.001$) between *masseter* (808.57µmol/100g), *trapezius* (583.02 µmol/100g) and *longissimus dorsi* (447.37 µmol/100g). They also found that ASP, GLU and TAU were pleasant to taste while carnosine gave a bitter taste (Cornet and Bousset, 1999). In raw beef, HIS present in the full back was only 9.71 mg/100g, however in cardiac muscle, it was 96.24 mg/100g, showing an enormous difference of a single AA across different cuts. For raw beef, TAU in *masseter* was 111.21 mg/100g, for knuckle and inside round, 69.03mg/100g and 74.26 mg/100g, respectively (Franco et al., 2010). The difference in AA content of different cuts may be caused by a difference in oxidative and glycolytic activities of the muscles, which was seen in lamb by Talmant et al. (1986). Franco et al. (2010) has divided the muscles into oxidative and glycolic muscles. The oxidative ability of the muscle cuts in decreasing order are masseter > cardiac muscle > knuckle > heel > full back > inside cut, where the *masseter* is the most oxidative and the inside cut is the most glycolic. The TAU content was highly correlated to the oxidative ability of the beef muscles (Franco et al., 2010).

Apart from the difference in FAAs arising from different types of muscles in raw meat, cooking method also contribute towards the final composition of FAAs in meat. Madruga et al. (2010) reported that the total FAAs in cooked rump (150.9 mg/100g) was lower than that of the fresh rump (220.2 mg/100g). From cooking, which involves heat, the quantities of LEU, ILE, THR, PHE and VAL have been shown to decrease by 30 to 50%. It is likely that the different cuts and cooking methods of lamb, or meat in general, will result in a difference in digestibility pattern. This is one of the hypotheses for the current project, which had been mentioned in section 1.0. Furthermore, Madruga and others (2010) stated that the total FAAs of cooked goat (150.9 mg/100g) was lower than the raw goat (220.2 mg/100g). Penet et al. (1983) also found that the cooked beef (120.36 mg/ 100 g) contained lower total FAAs than the raw beef (131.08 mg/100g), the cooked pork (70.40 mg/ 100g) lower than the raw pork (100.92 mg/100g). The most abundant FAAs in cooked goat meat are GLY (38 mg/100g), ALA (30.4 mg/100g) and GLN (23.9 mg/100g) (Madruga et al., 2010). However, in fresh heel part of beef, the HIS (37.59 mg/100g), GLU (20.10 mg/100g) and ALA (21.18 mg/100g) were three high amount FAAs (Franco et al., 2010). Madruga et al. (2010) stated that the different

kinds of animals (genetic factors) affect the total FAAs content among beef, lamb and chicken. Heating caused the loss of sugars and protein denaturation that reduced the amount of FAAs up 30 to 50% such as LEU, ILE, THR, PHE and VAL

2.4. Methods of FAAs analysis in meat

Analysis of FAAs in meat requires an extraction step. Most of FAA extraction methods, particularly for meat, involve the use of 5% sulfosalicylic acid (Jurado, García, Timón, & Carrapiso, 2007), 3% perchloric acid (Bauchart et al., 2006) and 7% trichloroacetic acid (TCA) in water (Franco et al., 2010). Penet et al. (1983) used a mixture of methanol, chloroform and water with a ratio of 6:2:2. The use of other solvents, such as ethanol, water/acetonitrile (50: 50, v/v) or 0.1% (v/v) formic acid in 20% (v/v) methanol, can also be found in the literature (Pérez-Palacios et al., 2015). The use of distilled water to extract the FAAs in meat has been trialed by Cornet & Bousset (1999) and Madruga et al. (2010).

Moreover, homogenization step is an important step for extracting the AAs after mixing the sample with the solvent. The use of vortex (Madruga et al., 2010), Omni 500 blender (Jurado et al., 2007), stomacher (Pérez-Palacios et al., 2015) and Omni Mixer (Penet et al.,1983) have been commonly found in the literature. Centrifugation is usually carried out post to the homogenization to separate supernatant which contains the extracted FAA.

GC was the most common detector for testing FAAs (Madruga et al., 2010; Penet et al., 1983; Bauchart et al., 2006; Jurado et al., 2007). HPLC (Cornet & Bousset, 1999; Franco et al., 2010; Suzuki et al., 1994; Campus et al., 2008) and LC-MS (Pérez-Palacios et al.,2015) were also used for measuring FAAs in meat. The use of LC-MS reduced the detection time, sample and solvent amount compared to conventional techniques such as GC. The disadvantage of using the GC-MS for analysis were expensive cost of machine, storage condition (air-conditioning) and requirement of samples purification (Pérez-Palacios et al., 2015).

Table 3 summarizes methods of analyzing FAAs in meat. Pérez-Palacios et al. (2015) found that the use of 0.1 M HCl for extraction and a mixer mill for homogenization had

high extracting ability. The mixer mill reduced the sample size to 0.2 g and solvent volume to 1.5 ml as well as the analysis time to 2 minutes, which seemed advantageous.

The studies of Ohmori et al. (1991), Suzuki et al. (1994) and Campus et al. (2008) analysed the FAAs in the HPP treated meat. Ohmori et al., (1991) used 20 ml $NaHCO_3$ to homogenate beef while Suzuki et al. (1994) used 2.5 vol (v/w) of deionized water with 10 g of minced beef. Campus et al. (2008) used 0.1 M HCl and 2.5 ml acteonitrile for extraction of FAAs in pork. Interestingly, they all found an increase in the total FAAs content after storage. And for the FAAs, Ohomri et al. (1991) found the VAL, ILE, PHE and LYS of beef increased a lot after 7 days storage. A significant increment in FAAs was observed from 100 MPa to 300 MPa, which is larger than the values in 0 MPa and 500 MPa. However apparent increment of ASP, SER, PRO, ALA and LYS were found in beef after 7 days storage (Suzuki et al., 1994). Hence it is important to analysis and quantify FAAs in meat immediately after extraction.

Table 3. A table summarizing different methods of analysing FAAs in meat

Type of meat	Preparation	Solvent for extraction	Separation	Detection	Results	Reference
goat meat	2.5 g raw and cooked	10 ml cold water	Vortex 20 min, 4°C	GC	• FAAs in raw goat meat ≈ FAAs in raw beef and lamb • Fructose and GLY (mg/100g) in goat > beef and lamb	(Madruga et al., 2010)
beef and pork	25 g raw and cooked	75 ml 6:2:2 v/v methanol-chloroform-water	Centrifuged 16200 g 15 min	GC	• Cooking reduced total FAAs for pork (25.23 to 17.60 mg/25g) and beef (32.77 to 30.09 mg/25g) • 16 FAAs found in both beef and pork • Most of individual FAAs decreased post to heating	(Penet et al., 1983)
pork	1 g minced	2 ml purified water	Homogenized, 60s at 4°C Centrifuged 38000 g, 1 h at 4°C	HPLC	• *Masseter*: highest in ASP, GLU and TAU • *Longissimus dorsi*: high in ALA and carnosine • FAAs and dipeptide contents explain the difference in taste of muscles from the same species.	(Cornet & Bousset, 1999)

young bulls	2.5 g	12.5 ml 3% perchloric acid (PCA)	Homogenized for 20s	GC	• 89% of AAs in fresh muscle corresponded to carnosine, anserine and glutathione. • The concentration of FAAs are lower in cooked meat compared to the fresh muscle.	(Bauchart et al., 2006).
pork loin and dry-cured ham	0.2 g ground	0.1M HCl 1.5 ml	Homogenized with 3 stainless steel balls	LC-MS	• A high extracting ability of samples with high FAAs content. • A low limit of detection and quantification for most FAAs.	(Pérez-Palacios et al.,2015)
Galician blonde beef	5g dried	7% TCA	Homogenized Centrifuged at 4000 g for 20 min, 4°C	HPLC	• TAU was the main FAAs in all muscles. • Amount of TAU: highest in *masseter*, *semimenbranous* and *biceps femori:* 40, 35, and 31 %, respectively.	(Franco et al., 2010)
Iberian ham	5 g of ham sample	5% sulfosalicylic acid	Homogenized for 1 min at 2 °C Centrifuged at 8200 g for 10 min	GC	• Total FAAs content increased significantly from 120 to 230 days (drying stage) and then the concentration remained almost steady. • FAAs content plays a major role in amino acid-derived volatile compounds.	(Jurado et al., 2007)

Beef	10 g of minced meat	2.5 vol (v/w) of deionized water	Homogenized for 60s Centrifuged at 15000 g for 15 min	HPLC	• No significant difference was observed in FAAs between each high-pressure treatments. • The content of ASP, SER, PRO, ALA and LYS increased after 7 days of storage.	(Suzuki et al., 1994)
Dry-cured pork loins	5 g of dry-cured samples	0.1 M HCl and 2.5 ml acteonitrile	Homogenized in a stomacher for 8 min at 4 °C	HPLC	• No significant effect of high pressure treatment on the total FAAs after 1 day storage. • The total FAAs of samples treated at 400 MPa showed increase after 45 days storage.	(Campus et al. , 2008)
Fresh beef rounds	5-10g samples	20 ml $NaHCO_3$	Homogenized three times for 30 sec Centrifuged at 10000 g for 15 min	Amino acids analyser (model 835, Hitachi)	• VAL, ILE, PHE and LYS of beef increased a lot after 7 days storage. • A significant increment in FAAs was found from 100 MPa to 300 MPa, which is larger than the values in 0 MPa and 500 MPa.	(Ohomri et al., 1991)

2.5. Processing methods of meat

When an animal is slaughtered and undergoes dressing, a series of unfavourable events may occur. Largely these are related to growth of microorganisms such as *Escherichia Coli, Salmonella typhimurium* and *Listeria monocytogenes* that cause food borne disease. The diverse and high nutrient content of fresh meat makes it highly favorable for the pathogens like *Campylobacter* and *Salmonella spp.* to grow. Often the storage temperature, atmospheric oxygen (O_2), moisture and light have the combined effects on making the meat more susceptible to spoilage. Because of these, loss of flavour and nutritional value, and change in colour may take place prior to consumption (Aymerich, Picouet, & Monfort, 2008).

Minimal processing methods applicable for meat can be divided into thermal and non-thermal methods, as shown in Table 4. The thermal methods included the sous vide processing, infrared heating, microwave heating and ohmic heating. The non-thermal methods include high pressure processing (HPP), pulsed electric fields (PEF) and packaging (Aymerich, Picouet, & Monfort, 2008). The most effective preservation technologies for fresh meat are non-thermal technology. The advantages of these are that they have mild effect on the overall characteristics of the meat, energy saving and environmentally friendly while eliminating pathogens and spoilage micro-organisms (Zhou, Xu, & Liu, 2010). Recent literature of the two processing methods used in the current project, HPP and PEF, are further reviewed in the subsequent sections, 2.5.2 and 2.5.3, respectively.

Table 4. Minimal processing methods used for meat, modified from (Aymerich, Picouet, & Monfort, 2008).

Thermal	**Non-thermal**
Sous vide processing	High pressure processing
Infrared heating	Pulsed electric field
Microwave heating	Ultrasound
Ohmic heating	Packaging

2.5.1. Sous vide processing

Sous vide means “under vacuum” in French (Banerjee & Verma, 2015). This is a method of cooking foods. The raw or par-cooked food is pre-packaged in specialized plastic bags or pouches and vacuum packaged to remove the air surrounding the food. This is followed by cooking the packaged food in a water bath at minimum 70 °C for 2-3 hours (Banerjee, & Verma, 2015).

After cooking, food products are rapid chilled and stored at -18^{0}C. The shelf life is 8 weeks in general without significant changes. Reheating the food products are needed before serving (James and James, 2005). The sous vide method is known to be sufficient to maximize the sensory characteristics of the product and the rapid chilling is possible to avoid the outgrowth of bacteria (Banerjee & Verma, 2015; Juneja et al. 2006).

Many researches using sous-vide processing found an increase in tenderness of meat with high retention of color and nutrients values, reduced oxidative changes and enhanced shelf life (Church and Parsons 2000; Vaudagna et al. 2002). The major concern of using sous vide method is that the juice of meat is left behind the packaging bag (Szerman et al., 2007).

2.5.2. High pressure processing (HPP) of foods

HPP, also known as high hydrostatic pressure (HHP) and ultra-high pressure treatment, is a non-thermal treatment which uses pressure, in the range of 100 to 1000 MPa (Aymerich, Picouet & Monfort, 2008; Zhou, Xu & Liu, 2010; Considine et al., 2008). The pressure is applied in a steel cylinder containing liquid pressure-transmitting medium, such as water (Zhou, Xu & Liu, 2010). The food of interest is vacuum packaged and then treated by the high pressure, where the pressure is rapidly and uniformly transmitted throughout the food. As a consequence of pressure, chemical reactivity, molecular configuration and chemical changes which oppose reactions to increase the volume may take place (Rastogi et al., 2007). Changes in chemical bonds, disruptions in structures, and changes in enzymes, lipids and small molecules also occur with the application of pressure (Rastogi et al., 2007).

The biggest advantage of HPP is that it is highly effective and stable against the microbial spoilage and pathogens in foods (Zhou, Xu & Liu, 2010), therefore extend the shelf life of products. Secondly, HPP enables food processing at ambient or even lower temperature and enables instant transmittance of pressure throughout the system, irrespective of size and geometry, thereby making size reduction optional. And HPP not only causes microbial death that improves the overall quality of foods, but also virtually eliminating heat damage and the use of chemical preservatives (Rastogi et al., 2007). However, the major disadvantage of HPP is the high-pressure condition resulting in the loss of volatile flavor of the meat (Souza et al., 2011)

The HPP was first used for preserving milk in 1899, then the application was extended to fruit and vegetables. In 1990, this method was used for jams, jellies, orange and apple juices, sauces, rice cakes and raw squid (Rastogi et al., 2007). Nowadays, the HPP is mostly used for vegetable products (35%), meat and meat products (29%), followed by the juices and seafood (15% respectively) as shown on Figure 5 (Campus, 2010).

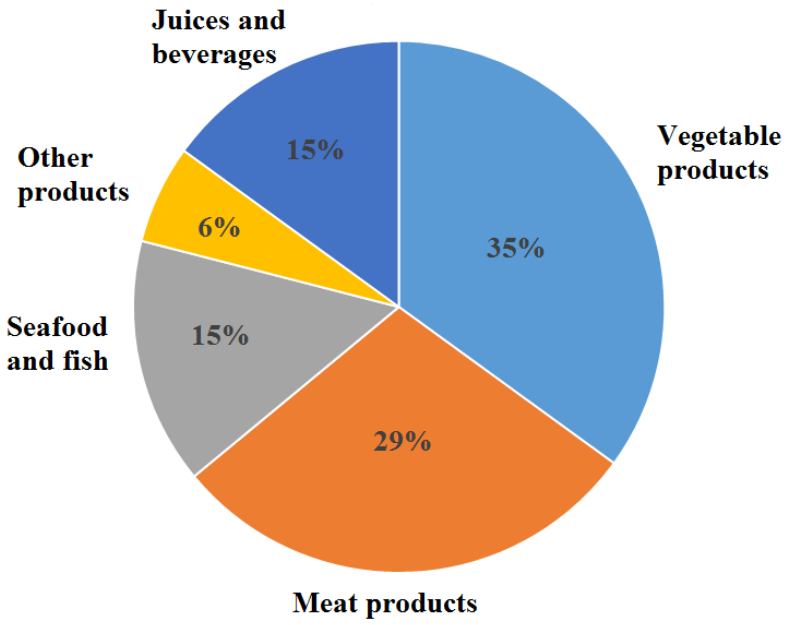

Figure 5. A pie chart showing a distribution of food products that are processed using the HPP method (Campus, 2010).

2.5.2.1. HPP system and its principle

HPP is a batch process that the packaged foods are treated in a chamber surrounded by water as the pressure-transmitting fluid. The primary components of the HPP system include a pressure vessel (a); closure for sealing the vessel (b); a device for holding the closure (c); high pressure intensifier pump (d); a system for controlling and monitoring the pressure and temperature when processing (e) (Figure 6). Usually the pressure of 600 MPa is applied for 3-5 mins and approximately 5 to 6 cycles per hour for compression, which is followed by holding, decompression, loading and unloading. Once the pressure is ceased, the vacuum packaged food is removed from the vessel and stored in a conventional manner (Campus, 2010).

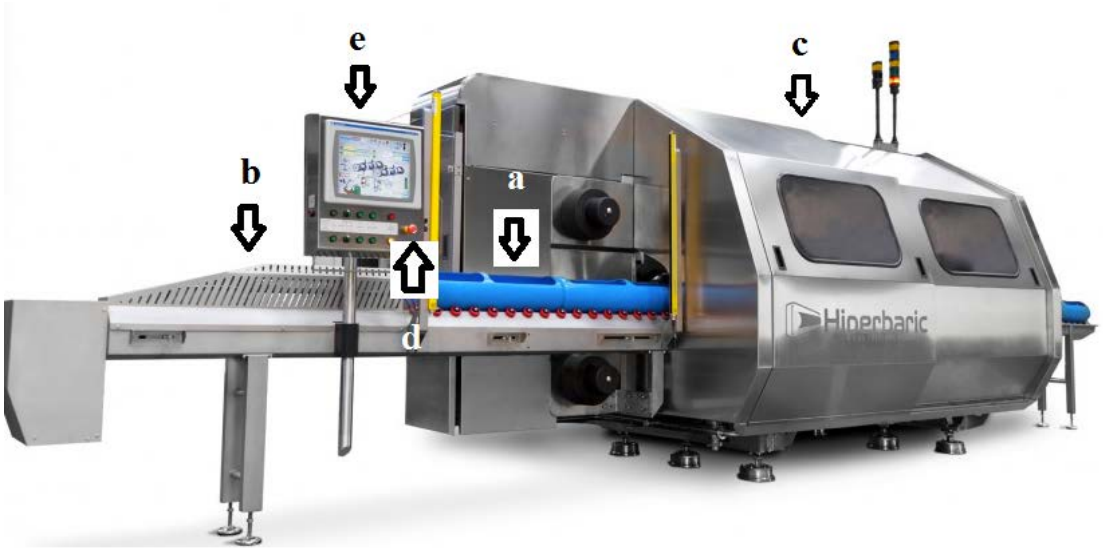

Figure 6. A picture of a High Pressure Processing equipment (Hiperbaric 55) (http://www.hiperbaric.com/en/hiperbaric55)

For lamb, it is first slaughtered and hung within 1 hour of slaughter at 0 °C for 2 days. The muscles are individually vacuum packed in polyamide polyethylene bags. The samples are then treated for 20 minutes at 200, 400 and 600 MPa at 20 °C in a high pressure vessel. The samples are then stored at -20 °C until use (McArdle et al., 2013).

2.5.2.2. Applications of HPP for meat

Table 5 shows the applications of HPP on meat and meat products. The application of high pressure has demonstrated its effectiveness in reducing pathogenic microorganisms, such as *Salmonella typhimurium* (KCTC 1925), *Escherichia coli* (KCTC1682), *Listeria monocytogenes* (KCTC 3569), *Citrobacter.freundii, Pseudomonas.fluorescens and*

Listeria.innocua (Carlez, Veciana-Nogues, & Cheftel, 1995; Bajovic, Bolumar, & Heinz, 2012; Kruk et al., 2011; Hereu et al., 2012). The application of high pressure with increase in the processing temperature has been demonstrated many times as an effective way to minimize the number of microbial colonies (Jung, Ghoul & de Lamballerie-Anton, 2003; Rodríguez-Calleja et al., 2012; Han et al., 2011; Souza et al., 2011). From the past studies, the shelf life was extended to 90 days with 600 Mpa for cooked ham (Han et al., 2011), 7 to 14 days with 600 MPa and 3 to 14 days with 450 MPa for chick breast fillet (Kruk et al., 2011) with the removal of pathogenic microorganisms.

Redness of meat is one of the leading attributes that plays a key role in determining the freshness or the quality of the meat, particularly by consumers. From the literature, it is evident that with the application of pressure through HPP, undesirable change in the color of meat is seen, with a decrease in redness (Souza et al., 2011). Jung et al. (2003) reported that the redness of the fresh beef was maintained with HPP treatments at 130 MPa, however, the redness decreased when the pressure was raised to 520 MPa. Carlez et al. (1995) explained that the meat discolouration in HPP was due to the following: firstly, the whitening effect due to the denaturation of myoglobin, and a haem displacemnet or release (Campus et al., 2008). Secondly, oxidation of ferrous myoglobin to ferric state under high pressure of above 400 MPa. Myoglobin upon the exposure to oxygen forms metmyoglobin in ferric state, with colour turning to brownish red (Young and West, 2001).

Apart from the colour change, the major disadvantage of HPP is the loss of of flavours in meat caused by lipid oxidation (Souza et al., 2011). Lipids and fatty acids play an important role in the meat flavours as certain flavours are hydrophobic such as hexanal and 1-hexanol . The incresed lipid oxidation with the pressure leads to a shortfall of flavours for consumers (Mottram, 1998). In order to minimize the loss of flavour, addition of EDTA (Beltran et al., 2004), rosemary (Bolumar, Andersen, & Orlien, 2011). and tomato extract (Alves et al., 2012) and limited oxygen supply (Bolumar, Skibsted, & Orlien, 2012) during the HPP have been trialled. These works had been observed that offered an improved process that stop and effect the lipid oxidation. Furthermore, lipid oxidation leads to the change of fatty acid content in the product. Wang et al. (2013) found that PUFAs decreased significantly ($p < 0.05$) with 600 MPa. The PUFA: SFA

ratios in the samples were significantly higher with the 20 °C at 200 and 400 MPa (McArdle et al., 2013).

Shigehisa & Hayashi (1991) analyzed the FAAs profile of fresh beef round treated with 100-500 MPa for 10 min at 25 °C. Compared with the control beef, the total FAAs content increased when treated with 100 to 300 MPa, which is larger than that at 0 MPa and 500 MPa, with particularly high increment seen with 100 MPa. The amounts of FAAs, including VAL, LEU, ILE, PHE and LYS, increased when treated with pressures between 100 to 300 MPa. As explained by Shigehisa & Hayashi (1991), this was due to the lysosomes being destroyed by the high pressure (> 200 MPa), resulting in an increase in the activity of the endoproteases and exopeptidases.

Suzuki et al. (1994) used 100, 150, 200, 300 and 400 MPa for 5 min to treat shoulder part of fresh beef. They found increased amounts of SER, GLU, GLN, GLY and ALA with increasing pressure of up to 200 MPa after 7 days of storage at 2 °C compared to that of the 0 day storage (immediate effect). GLU and ALA decreased when the beef was treated at 300 MPa. The contents of ASP, SER, PRO, ALA and LYS increased after 7 days for both untreated and treated muscles.

Campus et al. (2008) stated that there was no significant effect of pressure treatment on the total FAAs of pork after 1 day of vacuum storage. After 45 days of vacuum storage, control and samples treated at 300 and 350 MPa did not differ significantly. However the samples treated at 400 MPa showed a slight increase, the total FAAs increased from 150 mg/100g in 1 day storage to 225 mg/100g in 45 days storage.

High pressure denatures proteins. The degree of denaturation is dependent on the protein (muscle fiber) type, the processing condition and the magnitude of pressure applied (Rastogi et al., 2007). Ohmori et al. (1991) found an increase in total FAAs after one week of storage in beef rounds which were treated with pressure between 100 and 300 MPa. However, an opposite result was seen when pressure between 400 and 500 MPa was applied, although the treatment conditions remained the same, 10 minutes at 25°C, for both cases. The increase in FAAs from HPP treatment in the lower pressure range (100 to 300 MPa) can further be explained by excessive proteolysis taking place, which results in an increase in small peptides and FAAs (Toldrá et al., 1997). And subsequently, these small peptides and oligomeric proteins are dissociated and become

more vulnerable to proteolysis, and degrade to FAAs. The studies of Ohmori et al. (1991) and Toldrá et al. (1997) showed 300 MPa was the most effective pressure in protein denaturation.

Findings from Rastogi et al. (2007) demonstrated that proteins may be able to dissolve and precipitate reversibly in the lower pressure range of 100 to 300 MPa, though with the pressures of greater than 300 MPa, the reversibility is lost. Aminopeptidase and carboxypeptidase become inactive with the application of high pressure, between 400 and 500 MP (Ohmori et al., 1991). With the pressure treatment, the muscle proteins become susceptible to oxidative reactions that result in the loss of essential FAAs and decrease protein digestibility (Simonin, Duranton, & de Lamballerie, 2012).

Table 5. Application of HPP in meat products.

Type of meat	HPP parameters	Microorganisms	Physical properties	Chemical properties	References
Chicken breast fillet	300, 450, 600 MPa 5 min 15°C	• 450 and 600Mpa completely eliminated the *S.typhimurium* (KCTC 1925), *E.coli* (KCTC1682) and *L.monocytogenes* (KCTC 3569).	• 300 MPa reduced juiciness • High pressure increased hardness, cohesiveness, gumminess and chewiness.	• 450 MPa induced lipid oxidation and reduced volatile basic nitrogen values • High pressure resulted in the cooking loss and aroma strength	(Kruk et al., 2011)
Chicken breast fillet	300 MPa 5min 20°C with liquid antimicrobial edible coat and MAP	• Bacterial count was reduced to < 2600 CFU/g. • Combination of antimicrobial coating and HPP in MAP packaging extended shelf life to 28 days.	• Colour and texture were maintained during storage • Minimum hardness, gumminess and chewiness seen at 150 MPa	• Flavour was maintained during the storage	(Rodríguez-Calleja et al., 2012)
Beef	50-600 MPa 20-300 s 10°C	• Microbial growth decreased with increased pressure	• Redness maintained with 130 MPa • Redness decreased gradually with 520 MPa	• N/A	(Jung et al., 2003)

Beef	100, 150, 200, 300, 400 MPa 5 min 2°C	• N/A	• N/A	• SER, GLU, GLN and ALA increased to 200Mpa • GLU and ALA decreased at 300 MPa. • No significant differences in total FAAs and peptides for different pressure • HPP had no effect on brothy and meaty flavours, and cooked flavour of meat.	(Suzuki et al., 1994)
Beef rounds	100 - 500 MPa 25 °C	• N/A	• N/A	• Total FAAs increased after 7 days storage, especially for 100 MPa. • Amounts of VAL, LEU,ILE and PHE increased at 100 to 300 MPa • Aminopeptidases and carboxypeptidase decreased from 100 MPa to 500 MPa.	(Shigehisa & Hayashi, 1991)
Pork	215 MPa 5min 4°C	• HPP inhibited post-mortem metabolism	• Myoglobin solubility was decreased • Improvement in tenderness	• High pressure increased lipid oxidations that causing flavour loss.	(Souza et al., 2011)

Dry cured pork loin	300, 350 and 400 MPa 20°C 10 min	• 350 MPa and 400 MPa reduced the aerobic count at least by 0.7 and 1.6 log_{10}	• High pressure process lead to the increase in the lightness of color	• No significant effect of HPP on the total FAAs after 1 day storage under vacuum • After 45 days of storage under vacuum, control, 300 and 350 MPa did not differ significantly. • 400 MPa showed a slight increase in total FAAs.	(Campus et al., 2008).
Lamb (*M. pectoralis profundus* muscle)	200,400 and 600 MPa, 20 min 20, 40 and 60 °C	• N/A	• High pressure led to the cooking loss. • Increased the Warner Bratzler shear force. • The pH increased with high pressure. • L, a, b values increased with high pressure	• Highest TBARS seen at 400 and 600 MPa at 60 °C • PUFA: SFA ratio increased with treatments at 20 °C at 200 and 400 MPa compared to the control	(McArdle et al., 2013)
Cooked ham	400 and 600 MPa 10 min 22°C	• HPP controlled the bacterial growth in vacuum-packed cooked ham • *Weissella virdescens* and *Leuconstoc mesenteroides* survived HPP treatment and were responsible for the final spoilage.	• 600 MPa extended shelf life to 90 days.	• N/A	(Han et al., 2011)

2.5.3. Pulsed Electric fields (PEF) Processing

PEF is a non-thermal processing method to inactivate microorganisms and enzymes at lower temperatures with short treatment time and reduced adverse effects caused by heat treatment, such as loss of color, flavor and nutrients (Aronsson, Borch, Stenlöf, & Rönner, 2004; Huang, Tian, Gai, & Wang, 2012). PEF is claimed as the "quality friendly" method to meet consumer demands for fresh-like food products (Aronsson, Borch, Stenlöf, & Rönner, 2004). A short and high voltage of PEF is applied through the food product, resulting in instabilities and tension in the cell membrane and facilitates the formation of pores in the membrane (Barbosa-Cánovas and Altunakar, 2006; Huang, Tian, Gai, & Wang, 2012).

In the 1940s, the use of electric field has been applied for food processing (Barbosa-Cánovas & Altunakar, 2006). Nowadays, PEF processing is applied to a large range of food products, such as fruit juices, milk, liquid eggs, dry herbs (Grimi, Mamouni, Lebovka, Vorobiev, & Vaxelaire, 2011; Aronsson, Borch, Stenlöf, & Rönner, 2004;Vazquez-Cabral et al., 2016), meat (Barbosa-Cánovas & Altunakar, 2006; Bekhit et al., 2014) and brine solutions (Mohamed & Eissa, 2012).

The main advantages of PEF application are related to the possibility of non-thermal extraction without losing the product quality (Grimi, Mamouni, Lebovka, Vorobiev, & Vaxelaire, 2011). Aronsson and others (2004) reported that PEF-treated juice retained greater amounts of vitamin C and flavour compounds, and a longer shelf life compared to the heat-treated orange juice.

2.5.3.1. PEF system and its principle

A typical PEF food processing system contains a high voltage pulse generator (a), a treatment chamber (b), a fluid handling system (c) and control and monitoring devices (d), shown in Figure 7. For food application, the parameters are set with pulsed field intensity of 15 to 50 kV cm^{-1}, pulse width of 1 to 5 μs, and pulse frequency of 200 to 400 Hz. Liquid foods are usually treated in low to moderate temperature, of less than 60 °C (Wan et al., 2009). The basic principle of the PEF technology is that food products are applied with short pulses of high electric fields in microseconds to

milliseconds with pulsed field intensity of 10 to 80 kV/cm. The processing time is calculated by multiplying the number of pulses with effective pulse duration. The pulsed electrical currents pass through the food product placed in the PEF chamber between a set of electrodes at room temperature. After the treatment, the food is packaged aseptically and stored under refrigeration (Mohamed & Eissa, 2012).

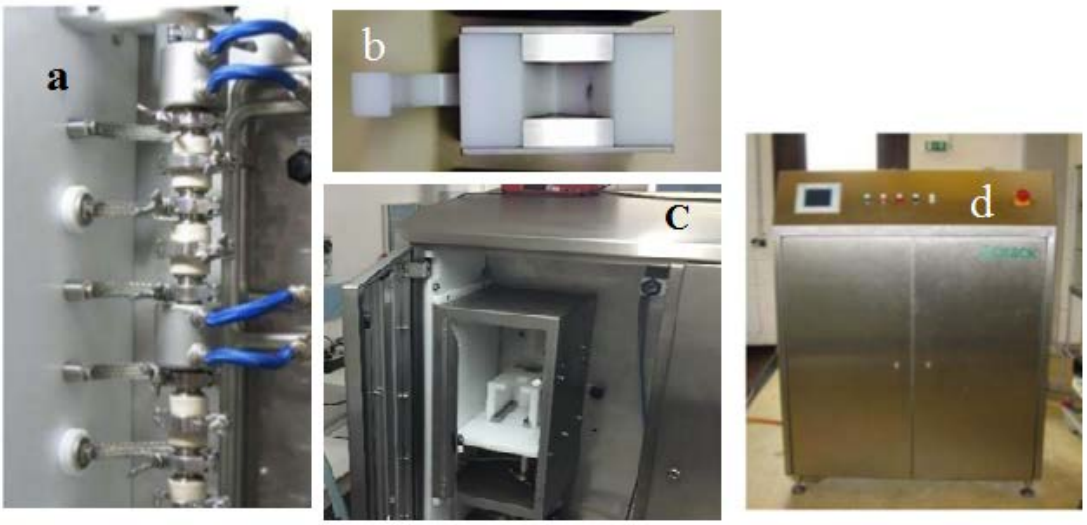

Figure 7. The Pulsed electric field equipment (Elcrack-HVP 5, DIL Quakenbruck, Germany) (b,c are photographs taken in Otago University, a,d were from http://www.dil-ev.de/)

2.5.3.2. Application of PEF on Meat

PEF processing has been adopted to process meat as summarized in Table 6. Research has been focused on beef in the past, but not for lamb. The change in properties of meat, such as texture (Ma et al., 2015), color (Suwandy et al., 2015), volatile compounds (Faridnia et al., 2015 ; Ma et al., 2015), pH (Suwandy et al., 2015) and shelf life (O'Dowd et al., 2013; Suwandy et al., 2015) have been studied after PEF treatment. For meat, the pulsed electric field is normally 1.0-3.0 kV/cm, 5 and 10 kV/cm.

PEF treatment is known to have a major effect on the texture of meat, and the extent of effect differs depending on the type of muscles. Consumers judge the quality of meat based on the appearance of meat, tenderness and toughness. Suwandy et al. (2015)

reported the shear force of PEF treated beef loins decreased from 79 N to 64 N from 3 to 21 ageing days, demonstrating that the shear force was affected by PEF treatment and aging time. The decrease in shear force indicated the increase in tenderness. Faridnia et al. (2015) also found the PEF treatment increased the tenderness of fresh beef. PEF treatment also led to a decrease in toughness of beef (Arroyo et al., 2014). O'Dowd et al. (2013) found that the myofibillar fragmentation increased due to proteolysis in PEF treated beef muscle but with no effect on the shear force. The effect of PEF on the quality of different meat muscles is variable. Beef *longissimus lumborum* muscles were found tougher than beef *semimembranosus* muscle treated by PEF. And beef *semimembranosus* muscle had up to 21.6% reduction in shear force from PEF treatment (Suwandy et al., 2015). Arroyo et al. (2015) who analyzed the fresh beef muscles, reported that the lightness increased, redness and yellowness decreased from 0 day to 7 days. A gradual increase of L values with the ageing period has been also reported in vacuum-packed beef *longissimus thoracis* muscle (Boakye & Mittal, 1996; Joseph & Connolly, 1977).

The amount of volatile compounds in cooked shoulder, rib and loin of lamb, are known to increase due to lipid and protein oxidation after prolonged storage time and freeze–thaw pretreatment with PEF processing (Faridnia et al., 2015; Ma et al., 2015). Temporal dominance of sensations analysis showed that both long storage period and PEF treatment affected the temporal flavor of meaty and oxidized flavor attributes. And PEF treated samples were associated with browned, juicy, livery, and meaty flavor attributes (Ma et al., 2015). Faridnia et al. (2015) also stated that the volatile profile of beef increased due to the lipid oxidation with PEF processing. The treatment led to a significant decrease in shear force of frozen–thawed beef samples after 7 days of storage while no differences were found for the fresh PEF treated samples. These results imply that both applied PEF conditions and sample pre-treatment (fresh or frozen–thawed) should be considered when determining the effect of PEF on meat tenderization (Faridnia et al., 2015), which was also confirmed by Ma et al. (2015).

Faridnia et al. (2015) stated that the PEF treatment significantly increased the purge loss after 7 days of vacuum ageing for both fresh and frozen-thawed samples but did not affect the cooking loss. About 85% of the muscle moisture is located within the myofibrils. With the disruption of myofibrillar structure from PEF, their ability to hold

moisture declines, leading to an increase in purge loss during the storage in freezing condition.

Moreover, the tissue microstructure of beef muscles plays an important role in the tenderness and water holding ability of beef muscles. The activity of proteolytic enzymes in the muscle tissues increased due to ruptures in myofibrils, and this leads to the breakdown of relative microstructure in beef. O'Dowd et al. (2013) found the lack of impact on tenderness was attributed to the pulsed electric field (1.36 $kV.cm^{-1}$) that influenced the microstructure and texture of chicken and fish. To date, nothing has been found on protein denaturation of PEF treated lambs.

Table 6. The application of Pulsed electric field

Meat	Electric field strength (kV/cm)	Specific energy (kJ/kg)	Frequency Pulse width	Microorganisms	Physical properties	Chemical properties	References
Chilled and frozen–thawed lamb: Shoulder, rib, loin	1-1.4	88-109	90 Hz 20 μs	N/A	• PEF treated samples were associated with browned, juicy, livery, and meaty flavor attributes.	• Storage time and frozen–thawed pretreatment led to significant increase in volatile compounds. • Both storage and PEF affected meaty flavor.	(Ma et al., 2015)
Fresh and frozen-thawed Beef: *semitendinosus* (back of thigh)	1.4	250	50 Hz 20 μs	The PEF control the initial viable count (TVC) under 2.69-2.77 log CFU/g.	• PEF caused significant microstructural changes. • PEF increased purge loss.	• No effect of PEF on ratios of PUFAs to saturated fatty acids, ω-6: ω-3 and free FA profiles • Freezing with and without PEF greatly affected the volatile profile of meat.	(Faridnia et al., 2015)
Beef outside flat	1.7 - 2.0	185	50 Hz	N/A	• Temperature, electrical conductivity, and pH were significantly ($P<0.05$) affected by PEF.	• PEF treated beef loins showed an increased proteolysis.	(Suwandy et al., 2015)

					• Tenderness and purge loss significantly ($P<0.05$) increased after PEF compared to the untreated. • Myofibrils increased compared to the untreated during ageing. • PEF induced changes in microstructure and texture of meat.		
beef loins and topsides	5 and 10	Not mentioned	20, 50, and 90 Hz	N/A	• PEF treatment decreased the shear force by up to 19%.	• PEF treated beef loins showed increased proteolysis • The degradation of desmin in PEF treated beef loins increased with ageing time.	(Arroyo et al., 2014)
beef *Semitendinosus* (back of thigh)	1.1–2.8	12.7–226	5–200 Hz	N/A	• PEF treated samples lost more weight than the water bath treated samples at 22 ^{0}C. • Texture was unaffected by the PEF.	• N/A	(O'Dowd, Arimi, Noci, Cronin, & Lyng, 2013)

2.6. Simulation of human digestion using *in vitro* digestion models

Simulation of the human digestion process is technically difficult, costly, and limited by ethical constraints when potentially harmful substances are involved, such as xenobiotics or pathogenic microorganisms (Yoo, 2009). The simulated digestion methods typically include the oral, gastric and small intestinal phase, and occasionally large intestinal phase in the form of fermentation (Hur et al., 2011). *In vitro* digestion systems are used in various fields such as nutrition, toxicology, pharmacology, and microbiology.The *in vitro* methods have the advantage of being less expensive, more rapid, generate reproducible data, less labor intensive and do not have ethical restrictions (Bhat, Kumar & Fayaz, 2015; Minekus et al., 2014).

2.6.1. The human digestion system

Human digestion is a complex process that breaks foods into nutrients that can be absorbed by the body for growth, maintenance and reproduction (Butts, Monro, & Moughan, 2012). The human gastrointestinal tract (GIT) system is composed of mouth, esophagus, stomach, small intestine and large intestine (Minekus et al., 2014). During the digestion process, mechanical transformations to reduce the size of the food particles and enzymatic transformations hydrolyzed macromolecules into smaller constituents that are absorbed into the bloodstream (Guerra et al., 2012). For meat, the proteins are broken down into amino acids or small peptides by gastric pepsin and proteases from small intestine and absorbed through the wall of the small intestine wall into the bloodstream (Santé-Lhoutellier, Engel, Aubry, & Gatellier, 2008). Food was disintegrated in the mouth and stomach, however the enzymatic digestion and absorption of nutrients mainly occur in the small and large intestine (Guerra et al., 2012).

Mastication is a short but important step with a significant influence on the overall digestive process and particularly on gastric emptying rate. The food is manipulated by the tongue, ground by the teeth and mixed with bolus resulting from mechanical and enzymatic degradations in the mouth. Then transported through the esophagus to the stomach by the mechanism of peristalsis (Yoo, 2009) .

In the proximal part of the stomach, fundus and body act as a reservoir for food and initiate the contact between bolus and gastric juice. The gastric juice is mostly composed of pepsin and lipases responsible for protein and lipid digestion, respectively. Hydrochloric acid (HCl) in the gastric juice promotes hydrolysis of proteins and keeps acidic pH of approximately 1.5 at rest (Guerra et al., 2012). Peptic digestion takes place in an acidic environment of pH range, 1.8 to 3.5 at 37 °C. Pepsin is responsible for breaking proteins into mainly polypeptides, and a small amount of oligopeptides and amino acids (Restani, Restelli, Capuano, & Galli, 1992). Many amino acids can be extracted by pepsin such as PHE, LEU, GLU, VAL and TYR (Krehbiel & Matthews, 2003; Monogioudi et al., 2011). Furthermore, PRO and SER residues can also be cleaved by gastric pepsin (Fujioka & Scheraga, 1965; Schmelzer et al., 2007; Vance, LeBlanc, & London, 1997). Gastric emptying is a crucial parameter of digestion which is influenced by many factors such as food composition or structure and biological factors (Guerra et al., 2012).

The acidic chyme from the stomach is then transferred to the small intestine. The small intestine further breaks down the macromolecules and absorb the nutrients through the intestinal lining. The pH is around 6 to 7.5. The intestinal secretion is composed of pancreatic juice, bile, lipase and $NaHCO_3$. Protein digested undergoes a complex digestion process by pancreatic enzymes, which include trypsin, chymotrypsin, elastase, and carboxypeptidases A and B (Krehbiel & Matthews, 2003; Guerra et al., 2012). Restani et al. (1992) stated that the enzymes in intestinal phase breakdown proteins and polypeptides, and liberate small peptide fragments with two to six amino acid residues, which is approximately 60% of total proteolytic products. Trypsin is able to breakdown the peptide bond linked with either or both of LYS and ARG. The linkage between aromatic amino acids is cleaved with the catalysis of chymotrypsin (Sitrin, 2014). FAAs that are released from proteins are absorbed to the blood stream and supply energy and nutrients to human (Krehbiel & Matthews, 2003).

2.6.2. Application of *in vitro* digestion models to meat

Table 7 illustrates the application of the *in vitro* digestion of meat and meat products. Hur and others (2011) classified the foods tested using *in vitro* digestion models. 45% were plant-based foods (starch, tea, rice, or bread), 18% were meats, 9% of dairy foods, 9% of marine foods and 9% of emulsions. The simulation of digestion is often

mimicked sequentially from mouth, stomach to small intestine. The composition of the digestive fluids used contained digestive enzymes, salts, buffers, biological polymers and surface-active components. The most frequently utilized enzymes in *in vitro* digestion models were pepsin, pancreatin, trypsin, α-amylase, lipase and bile salt. The enzymes sourced from human (Almaas et al., 2006), animals (Kitabatake and Kinekawa, 1998) or plants have been used. Stocksdieck, Bonsmann, & Hurrell (2007) and Diaz, Vattem, & Mahoney (2002) utilised pepsin and pancreatic lipase only to evaluate proteins in fresh chicken breast. However, other studies used a wider range of digestive enzymes, such as α-amylase, pepsin, pancreatin, bile and lipase (Santé-Lhoutellier et al., 2008; Hur et al., 2009; Purchas, Busboom, & Wikinson, 2006; Kulp et al., 2003).

The temperature (37°C) and pH were another important contributor for the digestion. Optimal pH for pepsin is pH 3 and for pancreatin, lipase and bile, it is pH 7. Other factors such as sample size and characteristics, ionic composition, mechanical shear and transit time also affect the digestion (Hur et al., 2011).

Meat contains a large of amount of proteins. During the digestion, meat is broken down into peptides, FAAs, free fatty acids and lipids. Hur et al. (2015) showed that there was no significant difference ($p < 0.05$) in the total lipid digestibility in all of the beef patty samples tested post to the simulation in mouth and stomach. However in the small intestinal phase, the total lipid digestibility increased ($p < 0.05$) dramatically in all samples. And samples encapsulated with biopolymers exhibited a significantly lower ($p < 0.05$) free fatty acid content than the control after digestion in the small intestine (Hur et al., 2015). Hur et al. (2009) reported the amount of free fatty acid increased significantly after *in vitro* digestion in the intestinal phase compared to the gastric phase. Kulp, Fortson, Knize, and Felton (2003) stated high amounts of pancreatin increased the accessibility up to 6.4-fold of chicken muscles in the intestinal phase. Bax et al. (2016) reported that cooked bull proteins in muscle for contraction and structure were enzymatically hydrolyzed in the small intestine. Kaur et al. (2016) who analyzed the digestibility of HPP-treated of beef found that HPP caused myofibrillar disorganization to affect the protein digestion. More breakdown of protein in HPP treated beef compared to the untreated meat was seen. And HPP treatment at 600 MPa resulted in the better digestibility in terms of free amino N release compared to 175 MPa. Bax et al. (2013) stated that outdoor-reared pig muscles showed more oxidation and protein denaturation than indoor-reared pig muscles during *in vitro* digestion.

The reason why the digestibility and FAAs content in the small intestinal phase were higher than that of the mouth and stomach was that, amylase used in the oral phase is mainly responsible for converting starches to oligosaccharides and monosaccharaides (glucose), not for proteins in meat (Hur et al., 2011). Pepsin plays an important role in breaking peptide bonds between hydrophobic and aromatic amino acids such as PHE, TYP and TYR (Dunn, 2001). In the intestinal phase, pancreatin breaks down the proteins and protein fractions into smaller peptides and FAAs such as LYS and ARG (Krehbiel & Matthews, 2003) and PHE, TYR and HIS (Sitrin, 2014). Sitrin (2014) stated that pancreatin can easily breakdown the linkage between AAs compared to the pepsin. In the small intestine, partially hydrolyzed cholesterol is mixed with digestive juices (e.g., cholesterol esterase), and the pH increases close to neutral due to the mixing of the chyme with alkali digestive juices (such as bile salts or sodium bicarbonate); this contributes to the oxidation of cholesterol and makes it easy to digest (Guerra et al., 2012; Minekus et al., 2014).

Table 7. Applications of *in vitro* digestion models for meat and meat products

Note: O: oral phase; G: gastric phase; I: intestinal phase

Samples	Enzymes	Digestion times (sampling times)	Results	References
lamb (*M. longissimus dorsi)*(full back)	Pepsin trypsin a-chymotrypsin	G : 1 h G:(take samples at):0,10,20,30,40,60 min, G: (take samples at):0,5,10,20,30 min	• Myofibrillar protein digestibility was not influenced by the diet of lamb. • Increase in digestibility by trypsin and a-chymotrypsin.	(Santé-Lhoutellier, Engel, Aubry, & Gatellier , 2008)
Cooked pigs (full back muscle)	Trypsin a-chymotrypsin Pepsin	G: take samples at 0, 5, 12, 21, 30, 45, 60, 90, 120,180 and 240 min	• Indoor muscles contained higher structural proteins, which changed the solubility. • Indoor-reared meat had less oxidation and protein denaturation than outdoor-reared meat.	(Bax et al., 2013)
Cooked chicken breast	Amylase pepsin pancreatin	O: 10 min G: 30 min I: 3.5 h	• Incubating the meat with amylase and pepsin did not change the accessibility of heterocyclic aromatic amines (HAs). • High amounts of pancreatin increased the accessibility of HAs up to 6.4-fold.	(Kulp, Fortson, Knize, and Felton, 2003)
Raw beef, chicken, lamb, and pork	Pepsin pepsin/pancreatin	G: 1h I: 1h	• LMW (<10kDa) peptides in enzyme digests of myofibrillar proteins were the major facilitators of iron solubility. • Unlike with casein, egg albumin, and most sarcoplasmic proteins, these LMW peptides were generated on pepsin digestion.	(Storcksdieck et al., 2007)

Cooked beef loins	a-amylase mucin BSA pepsin mucin pancreatin lipase bile salt	O: 5 min G: 2 h I: 2 h	• The cholesterol content decreased after *in vitro* digestion in all beef patties but no significant among the beef patties before and after in vitro digestion. • The amount of free fatty acid increased significantly after *in vitro* digestion.	(Hur et al., 2009)
175 MPa and 600 MPa treated bovine steaks	Pepsin Pancreatin	G: took samples at 30 min and 60min I: took samples at 60 min and 120 min	• HPP caused myofibrillar disorganization that effect the protein digestion • HPP treated at 600 MPa resulted in the better digestibility in terms of free amino N release compared to 175 MPa.	(Kaur et al., 2016)
Fresh chicken breast	Pepsin pancreatin bile salt	G: 2 h I: 2h	• Both soluble and insoluble proteins increased ferrous iron by 8–9-fold and dialyzable ferrous iron by 10–13-fold. • Soluble and insoluble proteins in chicken muscle are equally effective at producing dialyzable and reduced iron.	(Diaz, Vattem, and Mahoney, 2002)
Longissimus dorsi muscles (full back) from beef	Pepsin Pancreatin	G: 90 min I: 210 min	• The digestibility of meat samples increased with increasing pH_u ($P<0.001$). • Beef meat was highly digestible.	(Farouk et al., 2014)

longissimus dorisi muscles (full back) from cooked beef	Pepsin Pancreatin bile salt	G: 2 h I: 1 h	• Significant changes were found to take place in the levels of taurine, carnosine, coenzyme Q10 , creatine, and creatinine with cooking and digestion.	(Purchas et al., 2006)
The cooked beef chunk roll	α-amylase mucin BSA pepsin pancreatin lipase bile salt	O: 5 min G: 2 h I: 2 h	• Lipid digestibility and cholesterol oxidation product (COP) production were increased by *in vitro* human digestion and that biopolymer encapsulation could reduce lipid oxidation and COP production.	(Hur et al., 2015)
Cooked *semimembranosus* muscle (back of thigh) of bull	Pepsin Pancreatin	G: took samples at 5 30, 60 and 120min I: 120 min	• The proteins in muscle contraction and structure were enzymatically hydrolyzed in the small intestine.	(Sayd, Chambon, & Santé-Lhoutellier, 2016)
Pork *rectus femoris* muscle (middle of thigh)	Mucin Porcine bile Pancreatin	Took samples at 30 60, 120, 150, 180 and 240 min	• Myofibrillar lipid and protein oxidation increased with time and oxidant concentration, with a negative impact on the digestibility of myofibrillar proteins.	(Oueslati et al., 2016)

Chapter 3. Materials and Methods

In this chapter, preparation of lamb samples (section 3.1), processing methods: HPP (section 3.2) and PEF (section 3.3), cooking method (section 3.4), physical properties of lamb samples (section 3.5), extraction of FAAs from meat (section 3.6), *in vitro* digestion protocol and methods of analysis for FAAs from the digested lamb samples (section 3.7) are described.

3.1. Preparation of lamb samples for processing

Seven different muscles (meat cuts) of lamb, which include bolar, cube roll, full back, rump, knuckle, flat and shank (Section 2.3. Figure 4), were used in this research. All of the lamb samples were obtained from AgResearch Ltd., (Hamilton, New Zealand), with the average cold carcass weight of 145.5 ± 5 kg. All samples were stored at 4 °C for 48 hours post to slaughter for rigor mortis to take place. Each cut was divided into three lots of approximately 100 g and then hermetically vacuum packed in high density polyethylene bags (175mm*200mm, Contour sales) by a vacuum packer (Model DZ400/2D) and stored at 4 °C until further processing.

3.2. Treatment of the lamb samples with High Pressure Processing (HPP)

Two of the lamb cuts, rump and knuckle, were processed using an industrial scale High Pressure Processing (HPP) equipment (HPP 055, Multivac, Multivac Sepp Haggen Müller GmbH & Co., Wolferschwenden, Germany) in FoodBowl Ltd., Auckland in New Zealand. Four different pressures used for this study were 200, 300, 400 and 600 MPa (Table 8).

100 g of vacuum packaged samples were put in a cylindrical loading container of HPP equipment. The samples were then placed into a pressure chamber that was filled with water from a water tank to induce pressure. Water acted as a pressure-transmitting medium, with the initial temperature of around 7 to 8 ^{o}C. The temperature of the water gradually increased with pressure built up during HPP, and it stayed below 25 ^{o}C

throughout the processing. The pressure was increased at a rate of 125 MPa.min^{-1}. The pressure was held for one minute once a set temperature was achieved. The samples were exposed to a high level of isostatic high pressure at 200 MPa, 300 MPa, 400 MPa and 600 MPa, respectively. The whole HPP treatment process took about 15 minutes, from setting of the temperature and to the end of the pressure treatment. Once the pressure treatment has finished, the equipment was depressurized and the samples were taken out and stored frozen at -20 °C in a domestic freezer for analysis. All samples were treated on the same day to minimize day-to-day variation. Control samples of the same cuts, rump and knuckle, were reserved without HPP at -20 °C for analysis.

Table 8. A table showing the HPP treated lamb cuts at different pressures.

Cuts	Control	200 MPa	300 MPa	400 MPa	600 MPa
Rump	100g	100g	100g	100g	100g
Knuckle	100g	100g	100g	100g	100g

3.3. Treatment of the lamb samples with Pulsed Electric Field (PEF)

Seven different cuts of lamb, shanks, flat, knuckle, rump, full back, cube roll and bolar, were treated with Pulsed Electric Field (PEF). The raw and vacuum packaged lamb samples (100g) were cut parallel to the fibre direction into triangular pieces to fill a PEF batch treatment chamber (4cm*3cm*3cm) (Figure 8b). The weight of each triangular piece was approximately 15 g. The fibre direction was arranged in the chamber so that the electric current passed through the fibre perpendicularly.

The lamb samples were processed in a pilot plant scale PEF system (Elcrack-HVP 5, DIL Quakenbruck, Germany) (Figure 8) using batch mode configuration. A typical PEF food processing system contains a high voltage pulse generator (a), a treatment chamber (b), a fluid handling system (c) and control and monitoring devices (d). The electric field strength was set at 0.8-1.1 kV.cm^{-1}, with pulse width of 20 μs and frequency of 50 Hz, to maximize stability and efficiency of the electric field. The PEF treatment took about 3 seconds at room temperature.

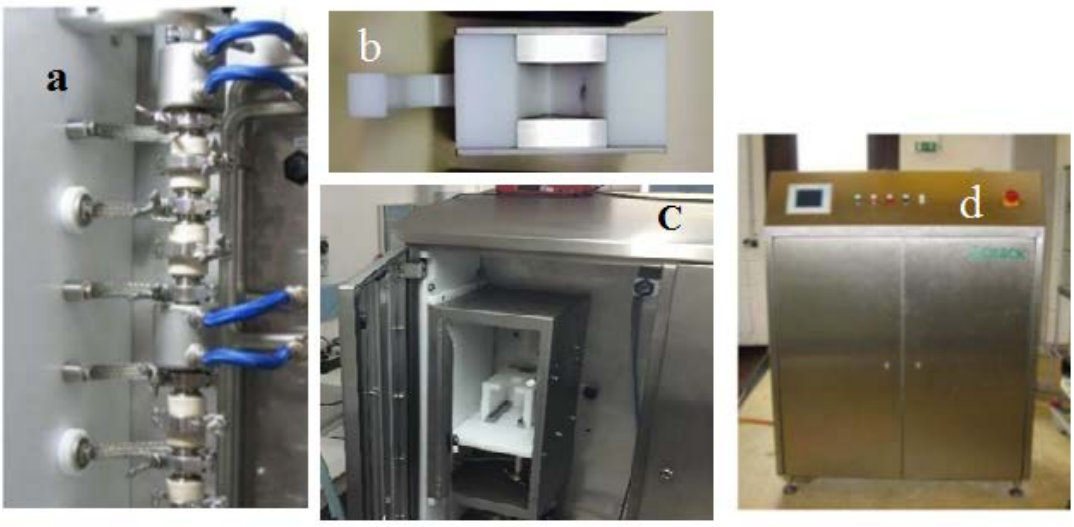

Figure 8. The Pulsed electric field equipment (Elcrack-HVP 5, DIL Quakenbruck, Germany) (b,c are photographs taken in Otago University, a,d were from http://www.dil-ev.de/) (same to Figure 7)

The PEF treated samples were vacuum packed in polyamide polyethylene bags separately (approximate 25 g each sample). PEF (0 day) samples refer to those that were processed by PEF first and then stored at -20 °C immediately. PEF (7 days) samples refer to those that were treated by PEF and stored under chilled condition at 4 °C for 7 days and then stored at -20 °C until further analysis. Similarly, Non-PEF treated samples of the same cuts were prepared for further analysis. Non-PEF (0 day) samples were stored at -20 °C immediately after the slaughter. Non-PEF (7 days) samples were stored at 4 °C for 7 days and then stored at -20 °C until further analysis. This is further summarized in Table 9.

Table 9. A table showing the non-PEF and PEF samples, stored chilled for 0 day and 7 days prior to freezing.

	non-PEF		PEF	
	0 day	7 days	0 day	7 days
Knuckle	25g	25g	25g	25g
Rump	25g	25g	25g	25g
Flat	25g	25g	25g	25g
Shanks	25g	25g	25g	25g
Bolar	25g	25g	25g	25g
Full Back	25g	25g	25g	25g
Cube Roll	25g	25g	25g	25g

3.4. Sous vide for cooking of lamb

In order to cook the samples for digestibility study, sous vide method by García-Segovia and others (2007) was used. Vacuum packaged frozen samples (Figure 9a) were taken out of the freezer (- 20 °C) and they were placed in a water bath (Model 360, Conthern, New Zealand) at 60 °C for two hours. The cooked and packed samples were removed from the water bath and cooled to room temperature (Figure 9b). A coffee grinder (Breville model BCG200) was used to mince and ground the samples for 2 minutes at room temperature. All of the samples were cooked and ground on the same day to minimize batch-to-batch variation. The ground samples were then vacuum packaged (Figure 9c) and stored at -20 °C until use.

Figure 9. Photographs of PEF lamb cuts in frozen (a), sous vide cooked (b) and minced (c) states.

3.5. Physical properties of cooked lamb meat

After cooking the lamb meat, physical properties of HPP and PEF samples were tested, and these included examining of moisture (Section 3.5.1), color (Section 3.5.2) and pH (Section 3.5.3).

3.5.1. Moisture

Moisture content of minced and cooked HPP, PEF and control samples was measured using oven drying method according to AOAC 950.46B (AOAC, 2003). All of the samples were vacuum packed until the moisture analysis to avoid any water loss during the storage. Crucibles used for measurement were cleaned and pre-dried at 105°C to maintain constant weight. The weight of each crucible was recorded and it was stored in a desiccator until use. About 2 g of each sample was spread out in the crucibles to facilitate the evaporation process. All samples were dried in a convection oven (SANYO Electric Biomedical Co. Ltd, Japan, model: MOV-112F) at 105 °C for 12 hours until constant weight was achieved. Crucibles were placed in a desiccator until they were completely cooled down to room temperature. Then the weight of crucibles with dried samples were measured. All samples were measured in triplicate (n=3).

Moisture content was calculated using the formula below:

$$Moisture\ (g/100\ g) = \frac{(W_1 - W_2)}{W_1} \times 100$$

W1= Weight of sample before drying

W2= Weight of sample after drying

3.5.2. Color

A Hunter lab Chroma metre (45/0, Colourflex) was used to measure the colour of the cooked HPP, PEF and control samples. Each sample was cut into a small slice (1 cm*1 cm*0.5 cm) and placed on a petri dish, and then placed on the center of the lens and covered with a lid in order to ensure no light from the surrounding emits to the lens. The Chroma meter was calibrated before taking measurements. L* for lightness (0 = black, 100 = white), a* for redness (-a* = greenness and +a* = redness), and yellowness for b* (-b* = blueness, +b* = yellowness) were determined at 3 different position of each sample (Braghieri et al., 2009). All samples were measured in triplicate (n=3).

3.5.3. pH

A pH meter (HANNA instruments, HI4221-02) was used to measure the acidity and alkalinity of minced and cooked lamb samples according to AOAC 950.46B (AOAC, 2003). Before measurement, the pH meter was calibrated with buffers of pH 4, 7 and 10. 4 g of cooked and minced lamb samples were homogenized with 4 g of distilled water in a coffee blender (model BCG200, Breville) for 30 seconds. A glass pH probe was placed in the mixture. Each sample was measured in triplicate.

3.6. Extraction and analysis of FAAs from HPP and PEF samples prior to the digestibility study

In order to compare the FAA contents between pre- and post to digestion, extraction method modified from Penet et al. (1983) was used. Before the experiment, the HCl, methanol and water were used individually to extract the FAAs in the cooked meat. The FAAs peaks of HPP and PEF lamb samples which were extracted by methanol were detected in GC in higher amounts than that of the water and HCl. Therefore, extraction with methanol was used for extracting the FAAs in the cooked HPP and PEF lamb samples prior to digestion. This is noted as S1 phase in the digestibility study and

throughout the thesis, referring to the FAAs present in cooked lamb samples prior to digestion simulation.

Figure 10 shows a flow diagram of FAAs extraction from the cooked lamb samples prior to digestion in a chronological order. 1 g of cooked and minced lamb sample with 7.5 ml methanol (> 99.9 % for HPLC, Sigma, WXBC1456V) and 5.4 g glass beads of 0.2 cm in diameter were added into a 15 mL polypropylene centrifuge tube (Labcon, 3136-345-008). The mixture was then homogenized by a vortex genie (Model: G560E, Scientific Industries, INC) for 30 minutes at room temperature with the highest speed of 10 (range 0 to 10). The pH of the mixture was adjusted to suit the workable pH range recommended by a commercial kit called, EZ Faast amino Acid Analysis kit (Phenomenex, KG0-7166), which was between pH 2.0 and 5.0. When the pH fell outside this range, it was adjusted using 1 M NaOH (prepared from 98% NaOH, Sigma, S8045), 6 M HCl and 1 M HCl (prepared from 37% HCl, Sigma, H1758). The pH adjusted solution was then centrifuged at 4000 rpm (Gyrozen, model: 1580R) for 8 minutes, at room temperature. The supernatant was collected and used for analysis of FAAs using the EZ Faast kit (Appendice 5.) and by Gas chromatography (GC) (Shimadzu, GC-2010).

The ZB-AAA GC column (10m* 0.25mm*0.25um) included in the EZ Faast kit was used for GC analysis. The initial column temperature was set at 50 ℃. The reaction temperature was set at 300 ℃. The reaction time was set at 20 minutes for each sample. The cooling period was set at 10 minutes. Nitrogen/air were used as carrier gas with a column flow at 1.46 mL/min. The same settings were used for analyzing the FAAs found in the digested lamb samples (section 3.8.3.).

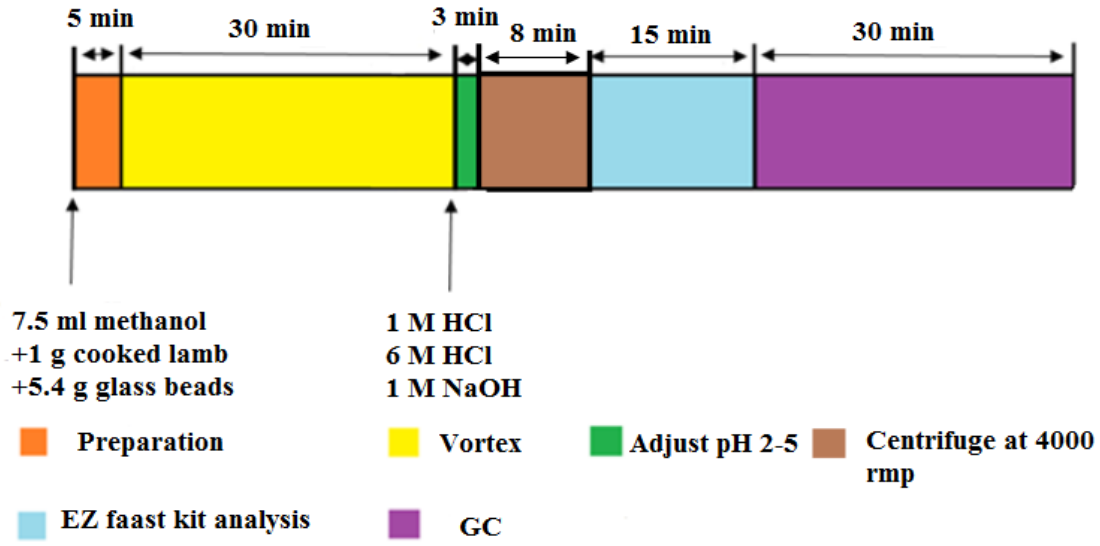

Figure 10. A flow diagram summarising the extraction process of FAAs from the cooked lamb samples prior to digestion, in a chronological order.

3.7. *In vitro* digestion of high pressure processing (HPP) and pulsed electric field (PEF) lamb

In vitro enzymatic digestion method used to simulate digestibility of HPP and PEF treated lamb followed the protocol published by Ferranti and others (2014) and Minekus et al (2014).

A screw-capped glass bottle was used as a reactor to simulate the consecutive three-stage digestion, including oral phase, gastric phase and intestinal phase. A multi-enzyme system composed of salivary α-amylase from *Aspergillus oryzae* (oral phase) (Sigma), pepsin from porcine gastric mucosa (gastric phase) (Sigma), pancreatin from porcine pancreas (intestinal phase) (3X U.S.P., MP biomedicals, LLC), bile extract from porcine (intestinal phase) (Sigma) and lipase (Lipozyme 100L, Novozymes) (intestinal phase) were used in the digestion.

Activities of the three major enzymes for the digestibility, α-amylase, pepsin and pancreatin, were tested before use, according to the standard protocols detailed in Appendix 1-5. All of the digestibility trials were carried out in triplicate for each of the HPP and PEF treated lamb samples.

3.7.1. Enzyme activity assay

The activity of α-amylase (EC 3.2.1.1) (Appendix 1) from the *Aspergillus oryzae* (≥ 150 units/mg protein biuret, Sigma) was determined based on Bernfeld (1955) published by Sigma®. Briefly, the assay was based on soluble potato starch: one unit liberates 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20 °C.

The activity of pepsin (EC 3.4.23.1) (Appendix 2) from porcine gastric mucosa (Sigma-Aldrich P-7000), was determined using a method based on the stop-point assay of haemoglobin degradation developed by (Anson, 1938) and published by Sigma®. The assay was based on using bovine blood haemoglobin as a substrate: one unit produces a ΔA280 of 0.001 per minute at pH 2.0 and 37 °C, and measured as TCA-soluble products.

Activity of pancreatin from porcine pancreas (Pancreatin 3X U.S.P., MP Biomedicals, LLC) was tested based on the proteolytic activity of trypsin and chymotrypsin. Assay of Trypsin (EC 3.4.21.4) activity (Appendix 3) was based on N_{α}-Benzoyl-arginine ethyl ester (BAEE) -one BAEE unit of trypsin activity producing a ΔA253 of 0.001 per minute with BAEE as substrate at pH 7.6 at 25 °C in a reaction volume of 3.20 mL As for chymotrypsin (EC 3.4.21.1), enzymatic activity was determined based on N-Benzoyl-L-tyrosine ethyl ester (BTEE) (Appendix 4): one unit hydrolysing 1.0 nmol of BTEE per minute at pH 7.8 at 25 °C.

3.8. Static *in vitro* digestion protocol

3.8.1. Preparation of simulated digestion fluids

Simulated digestion fluids including simulated salivary fluids (SSF), simulated gastric fluids (SGF) and simulated intestinal fluids (SIF) were prepared according to Minekus et al. (2014).

Simulated electrolyte stock solution was made by dissolving chemical constituents in deionized water (Table 10). Each of the simulated electrolyte stock solution, referred to as SSF, SGF and SIF, was made up to x 1.25 concentrate, stored at -20°C. $CaCl_2(H_2O)_2$

was prepared alone as precipitation may occur when added to the electrolyte stock solutions. It was added to the final digestion fluids at the end with the digestive enzymes. 1 M NaOH, 6 M HCl and 1 M HCl were used to adjust the pH during the digestion simulation inside a reactor. SSF, SGF, SIF and $CaCl_2$ $(H_2O)_2$ were prepared separately every 5 days and stored in the freezer until use. Digestive enzymes were added to the SSF, SGF and SIF immediately before commencing the digestibility experiment.

Table 10. Preparation of stock solutions for simulated digestion fluids (Modified from Minekus et al., 2014).

		SSF pH=7	SGF pH=3	SIF pH=7
Constituent	Stock conc. g/L	Conc. in SSF mmol/L	Conc. in SGF mmol/L	Conc. in SIF mmol/L
KCl	37.3	15.1	6.9	6.8
KH_2PO_4	68	3.7	0.9	0.8
$NaHCO_3$	84	13.6	25	85
NaCl	117	0	47.2	38.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.1	0.33
$(NH_4)_2CO_3$	48	0.06	0.5	0
$CaCl_2(H_2O)_2$	44.1	1.5	0.15	0.6

3.8.2. Procedure of static *in vitro* digestion

The static *in vitro* digestion protocol is outlined in Figure 11. The electrolyte stock solution, digestive enzyme solution and deionized water used in the digestion process were incubated at 37°C in a conventional oven until use, in order to keep the enzymes active in their optimal temperature. Three stages of digestion were carried out in a

shaking water bath (Model G76, New Brunswick Scientific co., INC, EDISON, N.J., U.S.A) at 37 °C at a speed of 5 rpm.

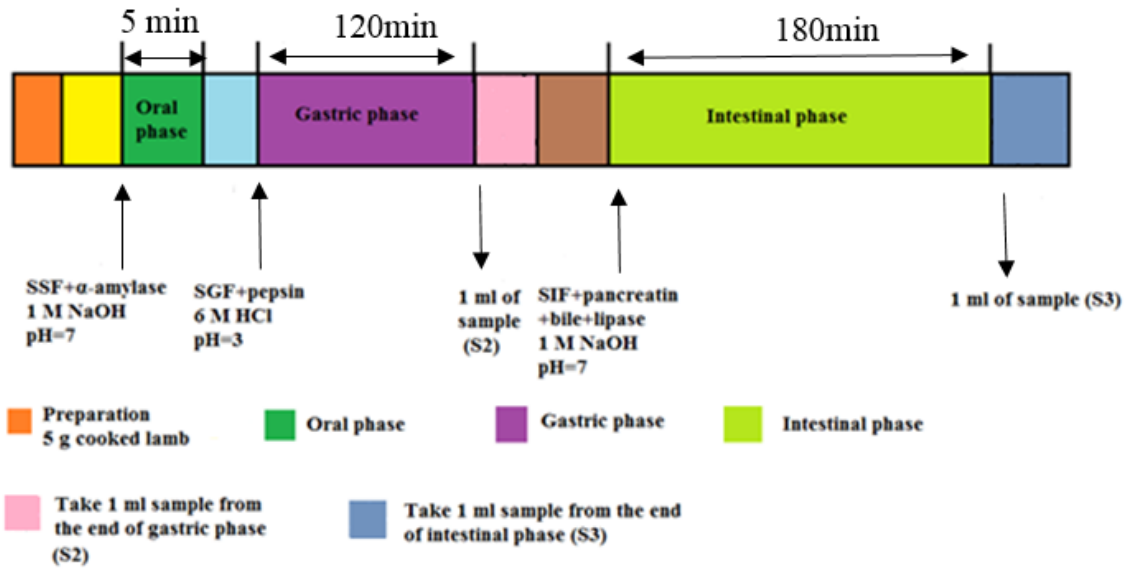

Figure 11. A flow diagram of a simulated static *in vitro* digestion method. SSF, SGF, SIG refer to Simulated Salivary Fluid, Simulated Gastric Fluid and Simulated Intestinal Fluid, respectively.

3.8.2.1. *Oral phase*

5 g of each minced sample with 3.5 mL SSF electrolyte stock solution, 25 µL of 0.3 M $CaCl_2$ solution and 975 µL of water were added into a 50 mL screw-capped glass bottle. The pH was adjusted to 7.0 with 1 M NaOH and the reactor was put in the shaking water bath at 37 °C for 10 minutes to make sure the bottle and the mixture of meat and digestive solution reached 37 °C. Then 0.5 mL of salivary α-amylase solution (1500 U mL^{-1}) was added to the SSF electrolyte stock solution to achieve 75 $U.mL^{-1}$ in the final mixture. The reactor was incubated in a shaking water bath for 5 minutes at 37 °C. No sample was taken at the end of the oral phase.

3.8.2.2. Gastric phase

After the oral phase, 7.5 mL of SGF electrolyte stock solution, 5 µL of 0.3 M $CaCl_2$ solution and 695 µL of deionized water were added to the reactor. The pH was adjusted to 3.0 with 6 M HCl. The sample mixture was incubated in the shaking water bath until

solution temperature reached 37 °C. 1.6 mL of pepsin solution (2500 U mL^{-1}) which was prepared by dissolving the pepsin powder in SGF stock solution, was added. Then the digestion reactor was further incubated in the shaking water bath for 120 minutes at 37 °C. At the end of the gastric phase, 1 mL of sample (S2) was taken from the reactor and snap frozen with liquid nitrogen immediately to completely inactivate the digestive enzymes. The collected samples were stored frozen at -20 ° C until further analysis.

3.8.2.3. Intestinal phase

The final stage of digestion was to simulate the upper intestinal digestion. 11 mL of SIF electrolyte stock solution, 40 µL of 0.3 M $CaCl_2$ solution and 1.31 mL of deionized water were added to the reactor. The pH of the reactor was adjusted to 7.5 with 1 M NaOH. Then 5 mL of pancreatin solution (80 $mg.mL^{-1}$) made up in SIF electrolyte solution, 2.5 ml of bile solution (38.4 $mg.mL^{-1}$) made in distilled water and 0.8 g of Lipozyme lipase (TL100) were added to the digestion reactor. Intestinal digestion was performed in the shaking water bath at 37 °C for 180 min. 1 mL of sample (S3) was taken at the end of the intestinal phase and snap frozen with liquid nitrogen immediately to completely inactivate the digestive enzymes. The collected samples were stored frozen at -20 ° C until further analysis.

3.8.3. Analysis of the digested lamb samples using GC

All of the frozen S2 and S3 samples were thawed in room temperature.The pH of each sample was adjusted to suit the workable pH range recommended by the EZ Faast kit (Phenomenex, KG0-7166), which was between pH 2.0 and 5.0. When the pH fell outside this range, it was adjusted using 1 M NaOH (prepared from 98% NaOH, Sigma, S8045), 6 M HCl and 1 M HCl (prepared from 37% HCl, Sigma, H1758). The pH adjusted solution was then centrifuged at 4000 rpm (Gyrozen, model: 1580R) for 8 minutes, at room temperature. The supernatant was collected and used for analysis of FAAs using the EZ Faast kit and by Gas chromatography (GC) (Shimadzu, GC-2010). The GC settings remained the same from section 3.6, for all of the S1, S2 and S3, for all of the lamb samples.

Digestibility (%) of lamb in S2 =(FAAs in S2-FAAs in S1)/FAAs in S2

Digestibility (%) of lamb in S3 =(FAAs in S3-FAAs in S2)/FAAs in S3

Chapter 4. Results and discussion

4.1. High-pressure process (HPP)

The amounts of FAAs present in rump and knuckle cuts treated with various pressures, ranging from 200 to 600 MPa, are presented as mean ± standard deviation in mg/100g, in Tables 11, 12 and 13. In undigested phase (S1) and digested phases (S2 and S3), nine EAAs and ten NEAAs have been identified and quantified with the use of the EZ:Faast amino acid analysis kit (Phenomenex®, USA) and GC. The nine essential amino acids were valine (VAL), leucine (LEU), isoleucine (ILE), methionine (MET), phenylalanine (PHE), lysine (LYS), threonine (THR), histidine (HIS), and tryptophan (TRP). The ten non-essentials amino acids were alanine (ALA), tyrosine (TYR), glycine (GLY), serine (SER), proline (PRO), aspartic acid (ASP), glutamic acid (GLU), ornithine (ORN), asparagine (ASN) and glutamine (GLN). The results and discussion of the HPP data are separated into three sections:

1. Extent of digestibility of lamb protein in different phases of simulated digestion
2. Digestibility of different muscles (meat cuts) of control and HPP treated lamb
3. Effect of pressure treatment (HPP) on digestibility of lamb

4.1.1 Extent of digestibility of lamb protein at different phases of simulated digestion: undigested phase (S1) and digested (S2 and S3)

Meat proteins are a major source of EAAs for humans. These are broken down by enzymes such as pepsin, pancreatin and bile, into FAAs and small peptides during digestion (Santé-Lhoutellier et al., 2008). Digestibility of protein in lamb can be interpreted as a change in total FAAs over the course of simulated digestion. For the control rump and knuckle, the digestibility was 12.14 % for rump and 13.00% for knuckle in gastric phase. In the intestinal phase, the digestibility was 69.31% and 67.42 % for the control rump and knuckle, respectively.

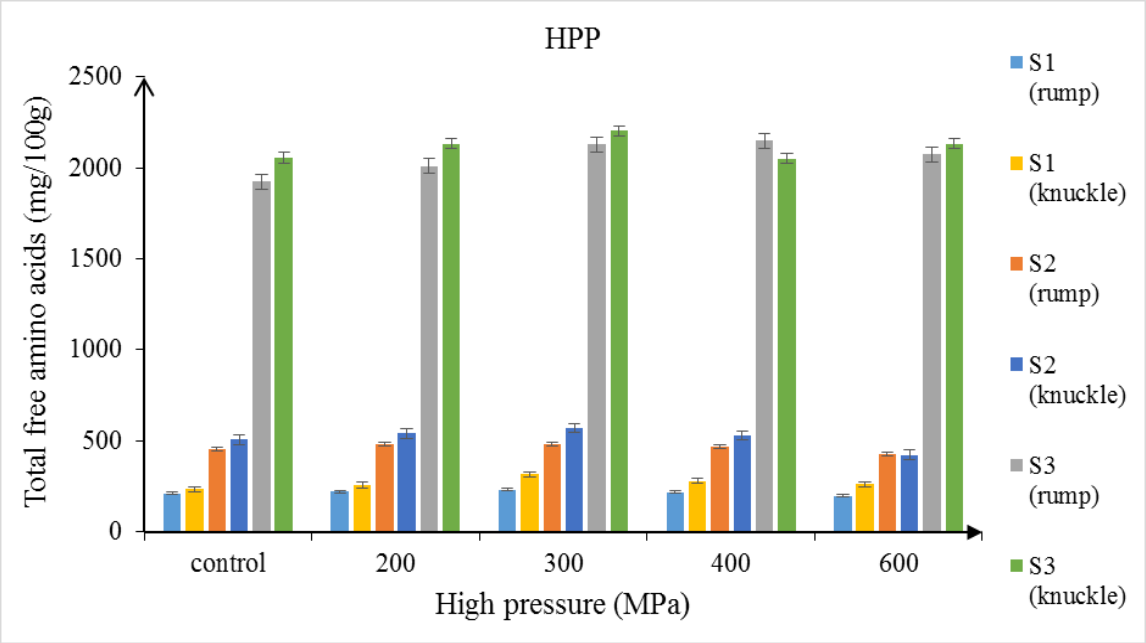

Figure 12. Total FAAs of high-pressure treated rump and knuckle cuts found at different stages of digestion (S1: Before digestion. S2: Gastric phase. S3: Intestinal phase). Mean values are plotted with error bars representing S.D.

The amounts of total FAAs found at different digestion phases for high-pressure treated lamb are shown in Figure 12. For both rump and knuckle, there were significant increases in the total FAAs from prior to digestion, to intestinal digestion, for every pressure condition examined. As shown in Tables 11, 12 and 13, the amounts of total FAAs of the rump (control) increased from 211.45 mg/100g prior to digestion (S1) to 454.12 mg/100g in gastric phase (S2) and reached 1925.31 mg/100g in intestinal phase (S3). The total FAAs of knuckle (control) increased from 235.14mg/100g prior to digestion to 505.42 mg/100g in gastric phase and 2056.80 mg/100g in intestinal phase.

The total FAAs of knuckle were all higher than that of the rump. For example, for the control samples prior to digestion, the knuckle had higher total FAAs than the rump, 235.14 mg/100g and 211.45 mg/100g, respectively. In the gastric phase of 300 MPa treated samples, the knuckle (567.46 mg/100g) was much higher than the rump (481.69 mg/100g) in total FAAs. However, the knuckle treated with 600 MPa in gastric phase and 400 MPa in intestinal phase were lower in total FAAs compared to values of rump. For instance, the 400 MPa treated rump in intestinal phase was 2148.97 mg/100g, which was higher than the knuckle in the same condition, 2053.18 mg/100g.

In general, the total FAAs values of the prior to digestion were the lowest, which was about a half of that found in gastric phase. Intestinal phase was approximately four times and nine times higher than S2 and S1 respectively. FAAs of control and HPP treated lamb (rump and knuckle) before digestion and *in vitro* digestion are discussed in detail in sections 4.1.1.1. and 4.1.1.2.

4.1.1.1 FAAs present in rump and knuckle cuts prior to digestion (S1)

Methanol was used to extract the FAAs present in cooked rump and knuckle cuts prior to digestion simulation for analysis. The total FAAs of the rump and knuckle cuts of the control samples were 211.45 and 235.14 mg/100g, respectively. The values obtained from the current study were higher than the reported values from Madruga et al. (2010), who used water to extract the FAAs in cooked rump cuts of goat meat, and obtained 150.9 mg/100g. Apart from the use of different chemicals for extraction, the same protocol was used, using the EZ faast kit. This may be owing to the use of water for extraction and a different type of meat. In a preliminary study (section 3.6.), extraction of FAAs with the use of water has been trialed and this has resulted in lower amounts of total FAAs than those extracted with methanol (data not shown). In the study by Madruga et al. (2010), the most abundant FAAs were GLY (38 mg/100g), ALA (30.4 mg/100g) and GLU (23.9 mg/100g). Compared with the cooked rump in my research, the GLN (37.00 mg/100g), ALA (36.87 mg/100g) and LEU (16.28 mg/100g) were the three highest FAAs. And the GLY was 10.84 mg/100g, GLU was 6.05mg/100g, which were lower than the values found by Madruga et al. (2010). The ALA, GLY, GLU and GLN are non-essential FAAs which can be synthesized by body. The essential FAAs, such as LEU play an important role in directly stimulating muscle protein synthesis (Doultani, Turhan, & Etzel, 2004). Apart from those FAAs that contribute to the meat flavor, LEU, VAL, THR and PHE, not much of other FAAs were expected to be seen in the undigested phase (S1). Madruga et al. (2010) used the male goats from British Saanen, however lambs used in this research were from AgResearch Ltd (Hamilton, New Zealand). The difference seen in FAAs may be caused by the use of different animal species (pork and lamb) and different meat cuts (rump and knuckle in lamb, pork loin) (Pereira and Vicente, 2013; Madruga et al., 2010), breeds, sex (Arsenos et al., 2002) and feeding regimes (Williams, 2007).

Penet et al. (1983) used a mixture of methanol, water and chloroform to extract FAAs from beef; they found cooked beef had 120.36 mg/100g of FAAs, which was lower than those in the cooked rump (211.45 mg/100g) and knuckle (235.14 mg/100g). The possible reason for the difference may be caused by short centrifuge time (15 minutes) for cooked beef compared to 30 minutes of vortex with glass beads for cooked rump and knuckle. The longer time of shaking with glass beads allowed an intensive mixing of the meat with methanol, enhancing the extraction of FAAs overall.

Pérez-Palacios et al. (2015) extracted FAAs in fresh pork loin and dry-cured ham by using mixer mill (MM400, Retsch Technology, Haan, Germany). The EAAs such as MET, PHE and LYS were found to be 32.26mg/100g, 27.48 mg/100g and 48.94 mg/100g, respectively. However, the same EAAs of cooked rump and knuckle (control) in my research were all lower than the values reported by Pérez-Palacios et al. (2015). The primary reason for the difference in the FAAs amount is likely to be the extraction method. Pérez-Palacios et al. (2015) used HCl, mixer mill (MM400, Retsch technology, Haan, Germany) with three stainless steel balls to extract the FAAs in the meat samples, however, for the current study, methanol with 5.4 g glass beads were used and vortex for 30 minutes. The mixer mill machine used in the study of Pérez-Palacios et al. (2015) was an effective tool in the extraction procedure compared to the stomacher methods, which had been summarized in Table 3. Another reason may be due to the heating. Heating leads denaturation of protein and reduce the quantities of LEU, SER, GLU, THR, VAL and PHE by 30-50% (Madruga et al., 2010). For example, VAL reduced from 7.6 mg/100g (raw rump of lamb) to 5.1 mg/100g (cooked rump of lamb). GLU decreased significantly from 35.5 mg/100g in raw rump to cooked rump (23.9 mg/100g) (Madruga et al., 2010). The sous vide cooking method (60 °C, 2 h) in my research resulted in protein denaturation that lower the FAAs in cooked lamb compared to the raw lamb samples, which is supported by the study of Madruga et al.(2010).

4.1.1.2. FAAs found in digested HPP rump and knuckle (S2 and S3)

The total FAAs showed an increasing tendency from undigested phase (S1) to intestinal phase (S3), in Figure 12. There was a slight increase from S1 to S2 (gastric phase), however, a massive increase in the total FAAs in the intestinal phase was observed in

both cuts. The control rump, for example, had 211.45 mg/100g of total FAAs in S1, and 452.12 mg/100g in S2, which was 2.13 times higher than that of the S1. The total FAAs in S3 was 1925.31 mg/100g, which was 9.11 times and 4.26 times greater than that of the S1 and S2, respectively. For the control knuckle, the ratio for S2: S1 was 2.15, S3: S1 was 8.74 and S3: S2 was 4.07, which was similar to that of the control rump. It was evident that the S1, S2 and S3 had a significant increase in total FAAs over the course of digestion, indicating for higher digestibility. This was in agreement with the findings from Hur et al. (2015) who found a dramatic increase in digestibility of protein in cooked beef ($p < 0.05$) in the intestinal phase.

Amylase used in the oral phase is an enzyme found in the mouth and it is mainly responsible for converting starches to oligosaccharides and monosaccharaides (glucose) (Hur et al., 2011). The major objective of the study was to find the digestibility of proteins in lamb. Insignificant amount of carbohydrate is present in meat (Williams, 2007). Hence the amounts of total FAAs were not examined in the oral phase (Williams, 2007), assuming that no changes would have been made in the oral phase.

Pepsin is an enzyme secreted in the human stomach that breaks down dietary proteins into smaller peptides and FAAs (Minekus et al., 2014). Pepsin plays an important role in cleaving peptide bonds between hydrophobic and preferably aromatic amino acids such as PHE, TRP and TYR (Dunn, 2001). The same conclusion was found in this study. For control rump, PHE, TYR and TRP were 8.71 mg/100g, 6.47 mg/100g and 1.80 mg/100g, respectively, prior to the digestion. And the FAAs content increased to 52.83 mg/100g (PHE), 22.66 mg/100g (TYR) and 7.66 mg/100g (TRP) due to the effect of pepsin in the gastric phase. The same tendency was found in the knuckle and for each of the pressure conditions.

In the intestinal phase (S3), pancreatin, bile, and lipase have enzymatically digested the lamb samples for 3 hours. Pancreatin contains amylase, proteases and lipase which breakdown the proteins and protein fractions into smaller peptides and FAAs. Trypsin is part of pancreatin which is responsible for the breakdown of the peptide bond linked with carboxyl side of core amino acids, such as LYS and ARG (Krehbiel & Matthews, 2003). The linkage between aromatic amino acids, such as PHE, TYR and HIS, is cleaved with the catalysis of chymotrypsin that is produced and released by the pancreas (Sitrin, 2014). Bile and lipases contribute towards lipolysis (Minekus et al., 2014) in the

intestinal phase for lipid digestion. Lipid content was not measured in the current study. Figure 13 shows the amounts of PHE, TYR and HIS found in S2 and S3, digested by pepsin in the gastric phase and pancreatin in the intestinal phase, respectively, for the control rump and control knuckle. For the control rump, PHE in the intestinal phase was 311.55 mg/100g, TYR was 273.57 mg/100g and HIS was 123.7 mg/100g, which were all much higher than the values in the gastric phase (52.83 mg/100g for PHE, 22.66 mg/100g for TYR and 15.54 mg/100g for HIS). For the control knuckle, the same pattern was observed. For example, in S3, PHE was 328.29 mg/100g, TYR was 301.36 mg/100g and HIS was 125.05 mg/100g. In S2, the PHE was 49.63 mg/100g, TYR was 15.72 mg/100g and HIS was 25.51 mg/100g, which were much lower than the values in the S3. This data can be fully explained by the study of Sitrin (2014) who found chymotrypsin released from pancreatin which broke down the linkage between PHE, TYR and HIS to liberate them in form of FAAs.

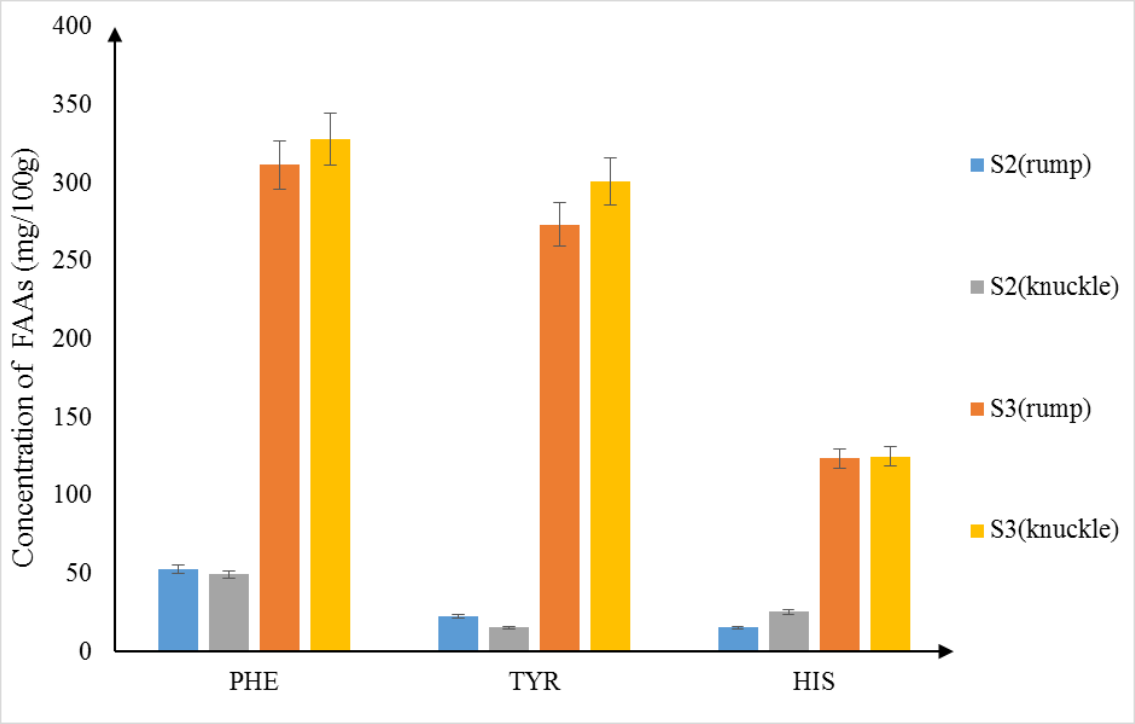

Figure 13. The concentrations of PHE, TYR and HIS in S2 and S3, in rump and knuckle (control).

For the control rump and knuckle, the pepsin broke down 12.14 % of proteins for rump and 13.00% protein for knuckle in gastric phase (S2). For the HPP treated rump and

knuckle, pepsin hydrolyzed about 10-15 % of lamb proteins as well, which was in agreement with the studies by Krehbiel & Matthews (2003) and Restani et al. (1992), who found that pepsin broke down approximately 10 to 20 % of the ingested proteins in the human stomach.

For the control rump and knuckle in the intestinal phase, the mixture of pancreatin, bile and lipase hydrolyzed 69.31% and 67.42 % for the rump and knuckle, respectively, which were higher than the values observed by Krehbiel & Matthews (2003) and Restani et al. (1992). They reported that pancreatic enzymes, such as pancreatin and trypsin, can hydrolyze about 40% protein. This is stronger than hydrolysis by pepsin, which is about10-20%, in gastric phase. Krehbiel & Matthews (2003) and Restani et al. (1992) also reported that the ability of pepsin in degradating proteins is less active compared to that of the pancreatin. For the high-pressure treated lamb, the intestinal enzymes (pancreatin, bile and lipase) hydrolyzed 65-70 % protein. These results confirmed that pepsin and intestinal enzymes were used appropriately in the study, in terms of enzyme activity (concentration and volume) and enzyme source.

Apart from the enzymes, other parameters, such as digestion time (transit time), and volume of digestion fluids also affect the overall digestibility of a sample being tested using an *in vitro* digestion model, as suggested by Hur et al. (2011) and Yoo (2009).

Considering the study of Hur et al. (2011) who stated the digestion time, also known as transit time, is important during the *in vitro* digestion, the reaction time in the current study took 2 hours in the gastric phase and 3 hours in the intestinal phase. The actual digestion time in human is about 3-4 hours for the gastric phase and 7-8 hours for the intestinal phase to pass through the colon (Minekus et al., 2014; Guerra et al., 2012). Due to the experiment time limitation, the reaction time for *in vitro* digestion was shortened. The digestion time depends on the individual characteristics (age, sex, health states, time of day) and food properties such as total amount, composition and particle size (Hur et al., 2011). In view of total amount of lamb sample (5 g) and lamb composition (mainly in protein), the 2 and 3 hours for gastric and intestinal phase seemed proper for simulation of digestion. The similar transit times have also been used by the studies of Hur et al. (2015), Diaz et al. (2002) and Minekus et al. (2014). The significant difference of FAAs amount between gastric phase and intestinal phase also proved that the digestion simulation took place appropriately.

In consideration of the digestive fluids in the study, for the real-life scenario in the human digestion system, the saliva in the mouth is produced approximately 1-1.5 L daily (Humphrey and Williamson, 2001) which mainly composed of 99.5 wt % water. The gastric juice is produced 2-3 L daily which contains 95 wt % water and about 5 wt% glycoprotein (Yoo, 2009). In the small intestine, 3L of intestinal juice is produced daily by cells in the walls of the duodenum (Sircus, 1953). Compared with the digestive fluids of human with the volumes used in this study (5 ml in S1, 10 ml in S2 and 20 ml in S3), the volumes were significantly lower. The simulation of digestion was a scaled down model of the human digestion, where the sample size of 5 g was used. Volumes of digestive secretions were also reduced accordingly. However, the results of FAAs from my study confirmed that the volumes of digestive fluids used was appropriate for lamb samples. The method in this research closely followed the protocol published by Minekus et al. (2014).

Many studies have focused on analyzing fatty acids and lipid digestion in meat (Hur et al., 2015; Oueslati et al., 2016). The studies examining FAAs in meat after *in vitro* digestion is absent to date. Some studies have focused on examining the protein content by using SDS-PAGE after *in vitro* digestion. Farouk et al. (2014) found pancreatin in the small intestinal phase produced more peptides than the pepsin in the gastric phase of fresh raw beef samples. This was in agreement with the study of Kaur et al. (2016) who found that the soluble protein (%) of raw beef samples increased after 60 minutes of digestion by pancreatin in the intestinal phase than by pepsin in the gastric phase. The conclusion in the studies above can fully explain the reason why higher amounts of FAAs were detected in the intestinal phase compared to the gastric phase in the current study.

There was an increase in the amounts of total FAAs between S1, S2 and S3 demonstrating that the digestive enzymes used in the study have worked to breakdown the protein in lamb into peptides and amino acids. The effect of different lamb cuts and high-pressure treatments on digestibility of proteins in lamb is discussed in the next two sections.

4.1.2. Digestibility of different muscles (rump and knuckle) of control and HPP treated lamb

The amounts of total FAAs (mg/100g) of the knuckle in all of the three phases (S1, S2 and S3) were all significantly higher than those found in the rump, except for 400 MPa in S3, as shown in Tables 11, 12 and 13 and Figure 12. The digestibility was influenced by different lamb cuts significantly, at different phases of digestion; ($P<0.001$), ($P<0.01$) and ($P<0.001$) for S1, S2 and S3, respectively.

4.1.2.1. Effect of different cuts in undigested phase (S1)

Different cuts (rump and knuckle) contained different amounts of total FAAs prior to digestion as shown in Table 11 and Figure 12. The amount of total FAAs in knuckle was significantly higher ($P <0.0001$) than that of the rump across the control and different pressures. This is in agreement with Franco et al. (2010) who found a significant difference ($P < 0.05$) in FAAs between difference muscles of raw beef (Refer to the section 2.3.). Cornet and Bousset (1999) found that the type of FAAs and dipeptides were the same in three different muscles of pig (*masseter*, *trapezius* and full back) but in different amounts. The FAAs of the three muscles in the study of Cornet and Bousset (1999) differed significantly ($p < 0.001$), and these were ALA, ASP, GLU, GLY, ORN and VAL. Similar results were also seen in the current study. For the samples of rump and knuckle in S1 (control and HPP treated), the essential FAAs such as VAL, LEU, ILE and LYS differed significantly ($p<0.05$) between the two cuts. The rump is located in the haunch of lamb; however, the knuckle is mainly located in the leg of lamb, which exercised everyday through the walking and running (Refer to Figure 4). The muscle in rump and knuckle are different in function, which may have contributed to a difference in FAAs between these two cuts.

The FAAs between rump and knuckle in HPP treated and non-HPP treated had a significant difference ($p<0.05$). When the rump and the knuckle of the control sample were compared, the cuts influenced each one of the FAAs significantly ($P < 0.05$),

especially for ILE and GLN ($P < 0.0001$). No effect of cuts on digestibility was seen in MET, PHE, TRP for EAAs and ASN, SER for non-EAAs. ILE of rump was higher than that of the knuckle, 16.24 and 11.48 mg/100g, respectively. These amounts were higher than the values obtained from Franco et al. (2010), who reported on ILE for the heel (10.43 mg/100g), inside cut (5.55 mg/100g), full back (2.16 mg/100g) and cardiac muscle (3.32 mg/100g) in raw beef. The difference may be attributed to the different type of animals (Franco et al., 2010), the age of slaughter (Watanabe, Ueda, & Higuchi, 2004) and the type of feed method (Koga et al., 1985). The GLN in knuckle (72.86 mg/100g) was almost twice as high when compared to the rump (37 mg/100g).

Different cuts of lamb are composed of different types of muscular fibres. According to Talmant et al. (1986), various cuts of muscles profoundly affect the oxidative enzymatic activities and glycolytic enzyme activities. Franco et al. (2010) who analysed the FAAs in veal reported the oxidative ability of veal cuts in decreasing order as masseter > cardiac muscle > knuckle > heel > full back > inside cut, where the masseter was the most oxidative and the inside cut was the most glycolytic. The oxidative muscles have more FAAs such as ASP, GLN and TAU. On the contrary, the contents of β-ALA and carnosine are high in glycolytic muscles (Cornet and Bousset, 1999). From my results, GLN (72.86 mg/100g) and ASP (2.21 mg/100g) in knuckle were all higher than the rump (GLN: 37 mg/100g, ASP: 0.88 mg/100g). The results reinforce that the knuckle was more oxidative than the rump, and amino acid composition of the different cuts of the same species significantly differ. Furthermore, the TAU content has been shown to be highly related to the different oxidative ability of beef muscles (Franco et al., 2010). The muscles with high oxidative ability contained more TAU in raw beef, which was in agreement with Cornet and Bousset (1999). TAU can prevent oxidative stress induced by exercise (Zhang et al., 2004). The different cuts (rump and knuckle) contained different FAAs contents such as VAL, PHE, LEU and THR which led to the different flavour of meat (Madruga et al., 2010). For example, the contents of VAL was 10.35 mg/100g (rump) and 13.81 mg/100g (knuckle) of 300 MPa in S1, which is significantly different ($P < 0.05$).

4.1.2.2. Effect of different cuts in digested phases (S2 and S3)

As shown in Figure 12 and Table 12, the total FAAs of knuckle was higher than that of the rump, except for 600 MPa treated samples in the gastric phase. In 600 MPa, the rump contained 426.23 mg/100g of FAAs, and the knuckle contained 422.75 mg/100g. The possible reason for the no significant difference between the rump and the knuckle was may be due to high-pressure treatment (600 MPa) resulting in the protein deterioration and lipid oxidation in the meat (Simonin et al., 2012). The cuts influenced the amount of total FAAs significantly ($P < 0.001$) and the individual FAAs ($P < 0.05$) in the gastric phase.

In the gastric phase for both control rump and knuckle, HIS of rump was 15.54 mg/100g however the value in knuckle was higher than the rump which was 25.51 mg/100g. The TYR in rump (control) was 22.66 mg/100g which was higher than the knuckle (15.72 mg/100g). The HIS and TYR play an important role in the brain functions. Deficiency in HIS and TYR can lead to anemia, mood swing and stunted growth (Wu, 2013; Fernstrom, 2013; Friedman and Levin, 2012).

The cuts influenced the total FAAs significantly in S3 ($P < 0.0001$). This was in agreement with the conclusion obtained from Bax et al. (2012) who found the different muscle composition affected the *in vitro* digestion of cooked carcass of beef. The reason may due to various muscles in the same species containing different amounts of several contractile and structural proteins such as actin, myosin light chain and tubulin-α. The contents of detoxification enzymes and regulatory muscle contraction proteins were different as well, such as slow TnT and calsarcin 2, which led to the the difference in protein solubility during digestion (Bax et al., 2012). Morzel et al. (2006) also drew the same conclusion. They reported that the intermolecular cross-links and the formation of aggregation may disturb the recognition of proteins by the protease, causing changes in protein hydrophobicity and solubility, and in turn, decreased the protein digestibility.

As shown in Figure 12 and Table 13, the total FAAs of the knuckle was higher than the rump, except for 400 MPa, in the intestinal phase. In 400 MPa, the total FAAs in the

knuckle was smaller than that of the rump, 2053.18 and 2148.97 mg/100g, respectively. The knuckle may have had high amount lipid oxidation and protein denaturation compared to the rump during 400 MPa. This was briefly explained by the studies of Rastogi et al. (2007) and Simonin et al. (2012) who claimed that the high pressure denatures meat protein and lipid oxidation occurred when above 300 MPa.

The composition of FAAs differed a lot ($P < 0.05$) between rump and knuckle in the intestinal phase. ILE (103.29 mg/100g) and TRP (101.81 mg/100g) of knuckle (control) were much higher than the values (ILE: 60.58 mg/100g; TRP: 74.83 mg/100g) in the rump (control) in the intestinal phase (Table 13). This means that more ILE and TRP are available in knuckle than rump to be absorbed and utilised in the body. ILE are branched chain amino acid that supports muscle synthesis (Kohlmeier, 2015; Yang et al., 2015). TRP acids the memory and brain functions to help with mood swing and mood change (Fernstrom, 2013; Friedman and Levin, 2012).

In conclusion, the total FAAs, indicating digestibility of proteins, was influenced by different cuts and this may be due to the difference in composition and structure of muscle proteins for different cuts.

4.1.3. Effect of high-pressure treatments on digestibility of proteins in lamb

The amount of FAAs found in rump and knuckle cuts treated with high-pressure are shown in Tables 11, 12 and 13 for S1, S2 and S3, respectively. For both rump and knuckle, HPP had a significant ($P < 0.0001$) impact on the total FAAs compared to that of the control in all three phases (S1, S2 and S3).

With pressure treatments, in each digestion phase, the total FAAs of rump and knuckle increased from the control to 300 MPa, and decreased from 300 MPa to 600 MPa. The highest amount of total FAAs was seen at 300 MPa across all digestion phases, for both rump and knuckle except for S3 of rump. This is in agreement with the study of Toldrá et al. (1997) and Ohmori et al. (1991), who has observed the highest amount of FAAs in beef being presented at 300 MPa, which had been mentioned in Section 2.5.2.2. In the

gastric phase (S2) of rump, the total FAAs increased from the control (452.12mg/100g) to 300 MPa (481.69 mg/100g), and then decreased to 466.92 mg/100g in 400 MPa, and further decreased to 426.23 mg/100g in 600 MPa. However, for the intestinal phase (S3) of knuckle, the total FAAs decreased from 300 MPa (2204.30 mg/100g) to 400 MPa (2053.18 mg/100g) and slightly increased to 2133.48 mg/100g in 600 MPa.

Furthermore, the high-pressure condition influenced most free EAAs and non-essential AAs of rump and knuckle significantly ($P < 0.0001$) as well. The amount of most free EAAs and non-essential AAs increased from 200 to 300 MPa, and decreased from 300 to 600 MPa. For instance, for the rump in S1 (Table 11), the TYR increased from 7.41 (200 MPa) to 7.53 mg/100g (300 MPa), and decreased to 6.79 mg/100g (400 MPa) and then to 5.93 mg/100g (600 MPa). MET and ORN of rump in S3 have shown no significant difference to high pressure treatment. For LYS of knuckle in S1, there was a slight increase for 8.96mg/100g to 9.74 mg/100g for pressure 400 MPa and 600 MPa, respectively.

4.1.3.1. Effect of high-pressure treatments on lamb prior to simulation of digestion (S1)

Studies of HPP treatments in foods have been mainly focused in determining the presence of microorganisms (Kruk et al., 2011), changes in texture (Rodríguez-Calleja et al., 2012), color (Jung & de Lamballerie-Anton, 2003) and lipid oxidation (Souza et al., 2011). The effect of HPP treatment in digestion of meat has not been studied before. As shown in Table 11, the total FAAs for both rump and knuckle showed a significant difference ($P<0.001$) with HPP treatment when compared to the control. Most free EAAs and non-essential AAs increased from 200 to 300 MPa, and then decreased from 300 to 600 MPa that had been mentioned. However, ORN in rump and LYS and GLU in knuckle have shown no significant difference. Suzuki et al. (1994) stated that there were no significant differences observed in all of FAAs and peptide content after 100 to 300 MPa HPP treatment (5 minutes, 2 °C) and after being stored for 7 days in chilled condition. Campus et al. (2008) also reported that high-pressure treatment (300 to 400 MPa for 10 minutes at 20 °C) did not have an effect on total FAAs content in dry-cured pork, because the high pressure reduced the activity of amino peptidases. However, the SER, GLU, GLN, GLY and ALA increased when the pressure increased to 200 MPa

(Suzuki et al., 1994). This was in agreement with the FAAs of lamb (rump and knuckle) in this experiment. GLY, GLU and GLN also showed an increase ($P < 0.05$) from the control to the 200 MPa. For example, for the rump in S1, GLY increased from 10.84 mg/100g (control) to 13.99 mg/100g (200 MPa). GLN increased from 37.00 mg/100g (control) to 41.36mg/100g (200 MPa).

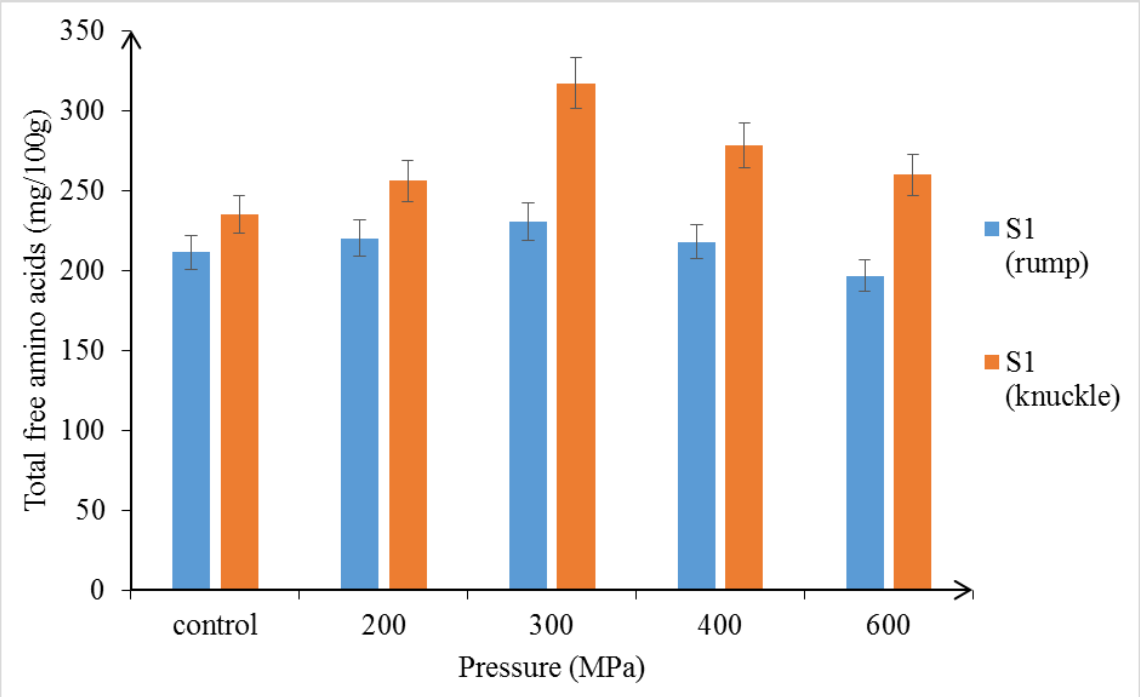

Figure 14. A graph showing the amount of total FAAs of HPP treated rump and knuckle found in undigested phase (S1). Mean values are plotted with error bars with S.D.

As shown in Table 11 and Figure 14, the total FAAs of rump and knuckle increased from the control to the 300 MPa, and decreased from the 300 MPa to the 600 MPa. The change in total FAAs is in agreement with the trend demonstrated by Ohmori et al. (1991), who found an incremental increase in total FAAs between 100 and 300 MPa and a decrease from 400 to 500 MPa, with 10 min of treatment time at 25°C in beef rounds, and measured FAAs after one-week storage. The increment of total FAAs in 100 MPa was larger than other pressure treatments. The reason for higher amount of total FAAs, indicating for higher digestibility, may be due to the denaturation of proteins caused by high-pressure treatments (Kaur et al., 2016). Toldrá et al. (1997) who analysed the enzymatic generation and process influence of dry-cured ham and stated that the muscle protein of raw ham undergoes an excessive proteolysis when processed into the dry-cured ham, resulting to a high amount of small peptides and FAAs. The

processing conditions such as temperature, time, water activity, redox potential and salt content, affect the enzymes activity of pepsin and pancreatin in raw meat (Toldrá et al., 1997). For 200 to 300 MPa treated on the raw beef, the remarkable increase in the total FAAs was found to be due to the growing activity of endogenous proteases (cathepsins B, D, H and L and calpains) and exopeptidases (peptidases and aminopeptidases) (Toldrá et al., 1997) that leaked from the lysosomes. For rump (S1) in my data, VAL increased from 9.36 mg/100g (200 MPa) to 10.35 mg/100g (300 MPa). LEU of knuckle (S1) also showed an increasing tendency from 200 MPa (15.2 mg/100g) to 300 MPa (17.22 mg/100g). These were also observed by the Ohmori et al. (1991) who found the FAAs such as VAL, LEU, ILE, PHE and LYS increased from 200 to 300 MPa in cooked beef. The enzymes in the meat such as proteinases and exopeptidases (peptidases and aminopeptidases) were responsible for this change (Ohmori et al., 1991). Moreover, when the pressure reached up to 400-500 MPa, the aminopeptidase and carboxypeptidase in raw beef were inactive (Ohmori et al., 1991), which explains the reason why the total FAAs decreased from 400 to 600 MPa in the current study.

To sum up, the total and individual FAAs increased with the pressure treatments from 200 to 300 MPa. The lamb flavour increased due to the incremental increases of LEU, VAL, THR and PHE that contributed to the meaty flavour (Madruga et al., 2010). However, the lamb flavour decreased from 300 to 600 MPa because of those flavour carrying FAAs reduced. In order to maximise the benefit of making the lamb more flavourful, 300 MPa HPP treatments should be used. Ohmori et al. (1991) and Toldrá et al. (1997) also stated that shelf life extension was observed at 300 MPa treatment in beef (Table 5). Hence 300 MPa seemed to be the best pressure for increasing the value of lamb.

4.1.3.2. Effect of high-pressure treatments on digestibility of lamb (S2 and S3)

As shown in Tables 12 and 13, the high-pressure treatments influenced the total FAAs sufficiently ($P < 0.0001$) under the digested conditions, S2 and S3. In particular, the HPP treatment has significantly ($P < 0.0001$) contributed in the release of EAAs from the protein complex of the lamb in S2, such as, VAL, LEU, ILE, PHE and HIS. For the

rump in S2, the contents of VAL increased from 12.96 mg/100g (control) to 20.64 mg/100g (300 MPa), and dropped to 14.98 mg/100g in 600 MPa. No significant difference was seen between the control and the HPP treated (200-600 MPa) samples for MET and ORN in S3. MET, as an EAA, plays an important role in building up of hydrogen peroxide in hair follicles (Wood et al., 2009).

To date, studies on the effect of high-pressure treatments on FAAs during *in vitro* digestion are absent. Rastogi et al. (2007) claimed that the high pressure denatures protein depending on the protein type and the applied pressure. The protein may dissolve or precipitate upon the application of high pressure. These changes are known to be reversible in lower pressure range of 100 to 300 MPa but irreversible for the pressure of greater than 300 MPa. The oligomeric proteins are dissociated and vulnerable to proteolysis. The same conclusion was found in the study of Kaur et al. (2016). They reported that the pepsin can breakdown more proteins in HPP (175 MPa) treated beef compared to untreated beef. The HPP (175 MPa) increased protein breakdown and protein solubility (%) during digestion. However, high-pressure (400-600 MPa) led to the protein deterioration and lipid oxidation in meat (Simonin et al., 2012) which was mentioned in section 2.5.2.2. In addition, muscle proteins susceptible to oxidative reactions may have resulted in the loss of EAAs and decreased protein digestibility. This fully explains the reason why the amount of total FAAs increased from the control to 300 MPa, and decreased from 300 to 600 MPa. As mentioned in section 4.2.1.1, the activity of enzymes, such as proteinases and exopeptidases (peptidases and aminopeptidases) in raw beef increased when treated by 200 to 300 MPa (Ohmori et al., 1991), resulting in a high amount of total FAAs. This means that 200-300 MPa treated lamb can be more easily digested to liberate more FAAs compared to the control.

In this study, the 300 MPa condition was the most effective pressure compared to others. With the increasing pressure (400-600 MPa), the total FAAs contents decreased. The same conclusion was found in the study of Ohmori et al. (1991) which is mentioned above. They observed an increment in total FAAs when 100 to 300 MPa was applied compared to the 400 and 500 MPa. Similar results have been observed by Barba, Esteve, & Frigola (2013) where an increase in total phenolic content in juice was seen mainly after 200 MPa. From their study, 400 MPa and 600 MPa treatment for all had the lowest values for total antioxidant capacity. Therefore, it can be acknowledged that 100-300

MPa was more effective than 400-600 MPa for HPP treatment of foods to maintain its functional property.

Not only the total FAAs, but also most of FAAs in rump and knuckle also showed the same response to the application of pressure. For example, PHE of the rump in the S2, the amounts increased from 200 MPa (55.38 mg/100g) to 300 MPa (56.76 mg/100g), and then decreased to 400 MPa (55.4 mg/100g) to 600 MPa (47.14 mg/100g). The same pattern was seen in the S3, increasing from the 324.32 mg/100g (200 MPa) to 346.51 mg/100g (300 MPa), and decreased to 331.57 mg/100g (400 MPa) to 319.82 mg/100g (600 MPa). PHE is one of the EAA which contribute for the brain function and memory (section 2.1). So 300 MPa seemed to be the most useful pressure to apply treatments for lambs to maximise the availability of its functional fragments for the body to utilise.

In conclusion, the results showed that HPP processing increased the contents of total FAAs from 200 to 300 MPa and decreased the amount of total FAAs from 400 to 600 MPa. 300 MPa was the most efficient pressure that led to the highest amount of total FAAs for both rump and knuckle compared to other high-pressure treatments.

Table 11: Concentration of FAAs (mg/100g) in New Zealand lamb (rump and knuckle) subjected to different pressure treatments (Control, 200 MPa, 300 MPa, 400 MPa and 600 MPa) prior to digestion (S1). Values are presented as Mean ± S.D.

	Rump						**Knuckle**					
	Control (0 MPa)	200(MPa)	300(MPa)	400(MPa)	600(MPa)	P-values	Control(0 MPa)	200(MPa)	300(MPa)	400(MPa)	600(MPa)	P-values
EAAs												
VAL	8.35±0.14^{c}	9.36±0.19^{b}	10.35±0.36^{a}	9.3±0.07^{b}	7.12±0.12^{d}	****	10.08±0.16^{b}	10.27±0.15^{b}	13.81±0.46^{a}	7.80±0.31^{c}	7.30±0.17^{c}	****
LEU	16.28±0.15^{b}	16.64±0.38^{b}	18.69±0.63^{a}	17.96±0.28^{a}	17.85±0.63^{a}	**	13.52±0.29^{c}	15.2±0.01^{b}	17.22±0.17^{a}	17.12±0.31^{a}	13.33±0.16^{c}	****
ILE	16.24±0.16^{a}	12.69±0.08^{c}	12.08±0.07^{d}	13.36±0.07^{b}	11.52±0.05^{e}	****	11.48±0.06^{b}	11.61±0.11^{b}	13.41±0.22^{a}	13.75±0.11^{a}	13.41±0.22^{a}	****
MET	3.89±0.31^{d}	4.29±0.12^{cd}	6.16±0.70^{a}	5.48±0.23^{ab}	4.88±0.34^{bc}	**	3.67±0.25^{d}	3.52±0.20^{d}	5.96±0.02^{a}	5.45±0.09^{b}	4.34±0.28^{c}	****
PHE	8.71±0.44^{b}	10.76±0.44^{a}	10.41±0.09^{a}	10.56±0.65^{a}	9.02±0.58^{b}	**	9.02±0.30^{bc}	9.66±0.47^{b}	14.01±0.22^{a}	8.62±0.06^{c}	8.98±0.42^{bc}	****
LYS	13.71±0.34^{a}	12.57±0.37^{b}	9.72±0.44^{c}	9.65±0.75^{c}	9.57±0.37^{a}	****	9.63±0.36^{a}	9.29±0.16^{ab}	8.97±0.35^{b}	8.96±0.10^{b}	9.74±0.16^{a}	**ns**
HIS	7.71±0.24^{b}	7.59±0.07^{b}	7.25±0.07^{b}	9.14±0.43^{a}	5.93±0.31^{c}	****	5.99±0.35^{c}	7.83±0.42^{b}	10.00±0.35^{a}	7.96±0.34^{b}	5.67±0.38^{c}	****
THR	8.44±0.39^{b}	10.56±0.22^{a}	10.41±0.36^{a}	9.14±0.19^{b}	8.65±0.78^{b}	**	7.63±0.37^{c}	12.19±0.69^{b}	12.07±0.09^{b}	13.34±0.52^{a}	7.96±0.34^{c}	****
TRP	1.80±0.06^{a}	1.71±0.23^{a}	1.23±0.05^{b}	1.43±0.05^{bc}	1.14±0.05^{c}	**	1.83±0.12^{a}	1.28±0.04^{b}	1.21±0.04^{b}	1.26±0.04^{b}	1.28±0.09^{b}	****
NEAAs												
AlA	36.87±0.25^{b}	31.35±0.22^{c}	36.81±0.27^{b}	29.47±1.08^{d}	29.74±0.82^{a}	****	33.03±0.37^{e}	39.37±0.07^{c}	45.52±0.33^{a}	41.35±0.05^{b}	35.67±0.23^{d}	****
GLY	10.84±0.16^{b}	13.99±0.30^{a}	14.18±0.11^{a}	14.15±0.23^{a}	11.32±0.55^{b}	****	12.80±0.07^{d}	14.17±0.36^{c}	20.85±0.59^{a}	16.28±0.18^{b}	14.71±0.09^{c}	****
ASN	5.95±0.27^{d}	8.93±0.01^{a}	7.72±0.05^{b}	8.77±0.32^{a}	6.87±0.39^{c}	****	5.51±0.37^{c}	5.2±0.15^{c}	6.79±0.50^{a}	6.60±0.50^{ab}	5.87±0.03^{bc}	**
ASP	0.88±0.13^{e}	1.18±0.03^{d}	3.08±0.16^{a}	1.82±0.06^{c}	2.75±0.03^{b}	****	2.21±0.07^{c}	2.22±0.16^{c}	5.50±0.21^{a}	2.22±0.04^{c}	3.21±0.16^{b}	****
GLU	6.05±0.25^{c}	6.76±0.20^{ab}	6.95±0.23^{ab}	6.45±0.33^{bc}	7.38±0.47^{a}	*	6.94±0.21^{a}	6.96±0.25^{a}	6.93±0.41^{a}	7.55±0.47^{a}	7.40±0.29^{a}	**ns**
TYR	6.47±0.38^{bc}	7.41±0.05^{a}	7.53±0.37^{a}	6.79±0.08^{b}	5.93±0.21^{c}	**	4.95±0.29^{b}	6.42±0.38^{a}	7.13±0.31^{a}	6.97±0.43^{a}	4.99±0.26^{b}	***
GLN	37.00±0.39^{c}	41.36±0.21^{a}	41.85±0.27^{a}	36.54±0.14^{c}	39.54±0.56^{b}	****	72.86±0.04^{d}	78.92±0.17^{c}	88.00±0.28^{a}	87.18±0.16^{b}	88.29±0.33^{a}	****
ORN	1.78±0.05^{a}	1.85±0.44^{a}	1.80±0.11^{a}	1.93±0.06^{a}	1.65±0.14^{a}	**ns**	2.94±0.14^{c}	4.16±0.13^{b}	5.20±0.15^{a}	4.35±0.22^{b}	4.29±0.24^{b}	****
SER	15.11±0.06^{d}	15.91±0.01^{c}	19.06±0.12^{a}	17.65±0.44^{b}	11.51±0.5^{e}	****	14.57±0.41^{d}	12.60±0.57^{e}	23.30±0.06^{a}	15.85±0.21^{c}	17.50±0.20^{b}	****
PRO	5.38±0.24^{b}	5.33±0.20^{b}	5.03±0.74^{bc}	8.37±0.48^{a}	4.37±0.16^{c}	****	6.46±0.35^{b}	4.97±0.34^{c}	11.05±0.65^{a}	5.51±0.26^{c}	5.76±0.18^{bc}	****
Total FAAs	211.45±2.46^{d}	220.23±2.29^{b}	230.32±2.07^{a}	217.95±1.10^{c}	196.75±0.72^{cd}	****	235.14±1.59^{e}	255.84±0.70^{d}	316.93±1.31^{a}	278.12±0.45^{b}	259.69±0.83^{c}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for pressure within the same cut. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 12. Concentration of FAAs (mg/100g) in New Zealand lamb (rump and knuckle) subjected to different pressure treatments (Control, 200 MPa, 300 MPa, 400 MPa and 600 MPa) post to the gastric phase of *in vitro* digestion (S2). Values are presented as Mean ± S.D

	Rump						**Knuckle**					
	Control(0 MPa)	200(MPa)	300(MPa)	400(MPa)	600(MPa)	P-values	Control(0 MPa)	200(MPa)	300(MPa)	400(MPa)	600(MPa)	P-values
EAAs												
VAL	12.96±0.53^{c}	15.56±0.27^{b}	20.64±1.28^{a}	15.58±0.87^{b}	14.98±0.39^{b}	****	12.45±0.47^{c}	15.4±0.49^{b}	24.91±0.15^{a}	11.78±0.33^{c}	8.55±0.48^{d}	****
LEU	52.27±0.42^{b}	55.30±1.35^{a}	48.35±0.42^{c}	53.24±0.45^{b}	49.91±1.06^{c}	****	54.95±0.48^{b}	59.89±0.25^{a}	53.11±0.73^{c}	55.61±1.3^{b}	42.55±0.1^{d}	****
ILE	22.32±0.78^{a}	20.42±1.11^{b}	17.15±0.51^{c}	17.32±0.89^{c}	14.87±0.18^{d}	****	20.59±0.41^{b}	18.89±0.36^{c}	25.4±1.23^{a}	15.91±0.7^{d}	12.28±0.32^{e}	****
MET	21.17±0.72^{c}	23.21±0.48^{ab}	21.8±0.83^{bc}	23.68±0.80^{a}	22.52±0.81^{abc}	*	21.19±1.29^{b}	20.78±0.15^{bc}	22.35±0.23^{b}	25.88±0.58^{a}	18.87±0.61^{c}	***
PHE	52.83±0.18^{b}	55.38±1.49^{a}	56.76±0.66^{a}	55.4±0.09^{a}	47.14±0.62^{c}	****	49.63±2.76^{c}	61.46±0.35^{a}	53.08±0.43^{b}	54.78±0.75^{b}	48.82±0.45^{c}	****
LYS	24.19±0.87^{b}	21.56±0.80^{c}	25.77±0.59^{a}	26.4±0.46^{a}	23.53±0.55^{b}	***	24.40±0.72^{b}	27.40±0.95^{a}	24.26±1.22^{b}	22.12±0.32^{c}	17.90±0.23^{d}	****
HIS	15.54±0.63^{c}	23.13±0.06^{a}	21.33±0.65^{b}	20.91±0.85^{b}	20.35±0.63^{b}	****	25.51±1.04^{a}	23.01±0.21^{b}	19.94±0.51^{c}	18.26±1.08^{d}	14.54±0.28^{e}	****
THR	14.22±0.50^{d}	16.08±0.22^{c}	21.95±0.69^{a}	18.16±0.24^{b}	17.80±0.57^{b}	****	18.15±0.46^{b}	14.74±0.59^{c}	21.81±0.13^{a}	15.54±0.17^{c}	10.68±0.52^{d}	****
TRP	7.66±0.21^{c}	10.99±0.27^{a}	8.85±0.41^{b}	8.44±0.13^{bc}	8.61±0.72^{b}	***	6.39±0.04^{b}	8.41±0.14^{a}	8.05±0.68^{a}	6.76±0.10^{b}	6.57±0.13^{b}	***
NEAAs												
AlA	40.19±0.80^{c}	49.13±0.44^{a}	49.86±0.62^{a}	43.31±0.91^{b}	39.22±1.23^{c}	****	50.97±0.62^{c}	57.70±0.80^{a}	54.8±0.45^{b}	52.3±0.77^{c}	41.70±0.4^{d}	****
GLY	31.02±0.50^{b}	31.11±1.21^{b}	35.39±0.67^{a}	34.96±0.39^{a}	23.97±1.11^{c}	****	29.40±0.16^{c}	34.94±0.45^{a}	32.32±0.32^{b}	32.3±0.92^{b}	23.56±0.33^{d}	****
ASN	20.92±0.46^{b}	13.99±0.70^{e}	22.8±0.40^{a}	16.24±0.9^{d}	18.40±0.47^{c}	****	13.62±0.47^{b}	17.44±0.37^{a}	18.16±0.80^{a}	14.1±0.13^{b}	9.54±0.24^{c}	****
ASP	16.33±0.81^{ab}	14.81±0.95^{b}	8.95±0.41^{c}	16.88±0.83^{a}	9.91±0.12^{c}	****	13.86±0.37^{a}	8.58±0.54^{d}	14.21±0.78^{a}	12.43±0.4^{b}	10.15±0.62^{c}	****
GLU	12.79±0.44^{b}	12.57±0.73^{b}	14.40±0.46^{a}	8.18±0.15^{c}	8.02±0.36^{c}	****	9.45±0.24^{b}	8.25±0.54^{c}	9.29±0.08^{b}	9.60±0.15^{b}	10.50±0.4^{a}	**
GLN	41.19±0.80^{c}	47.16±1.28^{a}	45.65±0.63^{ab}	44.4±1.07^{b}	45.56±0.34^{ab}	**	89.20±0.54^{c}	88.62±1.10^{c}	104.78±0.29^{a}	106.58±1.06^{a}	96.68±0.53^{b}	****
ORN	7.45±0.17^{b}	6.92±0.11^{c}	7.23±0.12^{bc}	8.17±0.23^{a}	7.04±0.18^{c}	***	10.16±0.37^{c}	12.33±0.24^{b}	14.39±0.10^{a}	10.53±0.63^{c}	8.50±0.21^{d}	****
TYR	22.66±0.69^{b}	24.57±0.55^{a}	19.3±1.09^{c}	19.31±0.61^{c}	21.41±0.70^{b}	***	15.72±0.14^{c}	21.65±0.49^{a}	20.64±1.20^{ab}	19.92±0.53^{b}	16.40±0.24^{c}	****
SER	27.91±1.47^{a}	26.78±0.23^{a}	22.70±0.56^{c}	23.46±0.2^{bc}	24.75±0.22^{b}	***	28.55±0.28^{c}	27.49±0.59^{c}	30.11±0.51^{b}	31.71±0.59^{a}	18.09±0.79^{d}	****
PRO	10.5±0.61^{b}	10.58±0.26^{b}	12.80±0.59^{a}	12.87±0.45^{a}	8.28±0.38^{c}	****	11.23±0.49^{c}	12.67±0.71^{b}	15.85±0.41^{a}	9.94±0.53^{d}	6.87±0.23^{e}	****
Total FAAs	454.12±+3.62^{a}	479.29±3.12^{a}	481.69±3.74^{a}	466.92±1.53^{a}	426.23±3.74^{b}	****	505.42±3.37^{d}	539.64±2.51^{b}	567.46±2.25^{a}	526.03±3.82^{c}	422.75±2.66^{e}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for pressures within the same cut. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 13. Concentration of FAAs (mg/100g) in New Zealand lamb (rump and knuckle) subjected to different pressure treatments (Control, 200 MPa, 300 MPa, 400 MPa and 600 MPa) post to the intestinal phase of *in vitro* digestion (S3). Values are presented as Mean ± S.D.

	Rump						**Knuckle**					
	Control(0 MPa)	200(MPa)	300(MPa)	400(MPa)	600(MPa)	P-values	Control(0 MPa)	200(MPa)	300(MPa)	400(MPa)	600(MPa)	P-values
EAAs												
VAL	34.46±0.49^{d}	37.06±0.40^{c}	38.62±1.21^{c}	43.83±0.52^{a}	40.75±0.92^{b}	****	41.45±0.53^{a}	37.56±0.11^{c}	36.56±1.26^{cd}	39.72±0.15^{b}	35.61±0.18^{d}	****
LEU	372.20±0.82^{d}	384.7±0.82^{c}	394.51±0.82^{a}	388.01±0.82^{b}	386.89±0.93^{b}	****	384.24±0.41^{c}	378.48±0.97^{d}	385.77±0.39^{b}	356.52±0.67^{e}	408.16±0.53^{a}	****
ILE	60.58±0.53^{d}	68.03±1.06^{b}	65.42±1.05^{c}	109.64±0.79^{a}	110.05±0.97^{a}	****	103.29±0.50^{b}	101.48±0.39^{c}	109.35±0.61^{a}	97.37±1.17^{d}	82.26±0.18^{e}	****
MET	55.93±1.14^{a}	56.89±0.97^{b}	58.17±1.55^{ab}	54.05±0.18^{a}	54.81±0.87^{a}	ns	60.83±0.71^{b}	62.38±0.11^{a}	65.42±0.55^{c}	61.58±1.10^{ab}	58.39±0.78^{d}	****
PHE	311.55±0.54^{e}	324.32±1.52^{c}	346.51±0.22^{a}	331.57±0.51^{b}	319.82±1.03^{d}	****	328.29±0.57^{d}	348.44±1.19^{c}	364.94±0.51^{a}	315.91±1.12^{e}	356.81±1.23^{b}	****
LYS	244.19±1.35^{c}	245.54±0.59^{bc}	249.73±0.85^{a}	245.58±0.43^{bc}	247.86±0.40^{ab}	*	238.25±0.51^{e}	261.08±0.65^{d}	273.58±0.72^{b}	268.82±0.67^{c}	291.11±0.41^{a}	****
HIS	123.70±0.82^{d}	143.88±1.57^{b}	150.81±0.54^{a}	149.14±0.67^{a}	137.03±0.97^{c}	****	125.05±0.4^{c}	134.32±0.4^{b}	136.30±0.76^{a}	114.32±0.79^{d}	123.89±0.52^{c}	****
THR	26.28±0.71^{c}	28.96±1.20^{b}	28.75±0.82^{b}	32.74±0.21^{a}	29.68±1.07^{b}	***	23.82±0.52^{b}	26.88±0.62^{a}	25.92±0.60^{a}	27.07±0.54^{a}	23.81±0.53^{b}	***
TRP	74.83±0.68^{d}	89.84±1.08^{b}	95.79±0.90^{a}	87.92±1.48^{bc}	87.18±0.62^{c}	****	101.81±0.54^{b}	98.67±0.11^{c}	112.37±0.4^{a}	95.66±0.45^{d}	95.83±0.76^{d}	****
NEAAs												
AlA	61.26±1.07^{d}	66.37±0.92^{c}	68.42±1.12^{b}	73.43±0.39^{a}	70.24±0.37^{b}	****	61.05±0.53^{d}	66.13±0.65^{b}	68.73±0.44^{a}	62.78±0.26^{c}	58.84±0.59^{e}	****
GLY	55.17±0.96^{c}	59.78±0.81^{b}	63.81±0.74^{a}	64.77±0.50^{a}	64.54±0.56^{a}	****	45.32±0.85^{d}	57.02±0.56^{a}	52.24±0.25^{c}	45.92±0.46^{d}	54.15±0.94^{b}	****
ASN	24.06±0.57^{b}	21.48±0.10^{d}	22.49±0.8^{cd}	26.94±0.11^{a}	22.85±0.66^{c}	****	19.42±0.71^{b}	19.05±0.4^{b}	19.04±0.99^{b}	21.82±0.88^{a}	21.68±1.04^{a}	*
ASP	36.25±0.34^{a}	32.58±0.25^{b}	31.89±0.14^{b}	37.76±0.38^{a}	34.51±0.40^{c}	***	24.00±0.62^{a}	26.40±0.84^{b}	20.47±0.34^{c}	17.84±0.53^{d}	17.88±0.14^{d}	****
GLU	22.88±0.64^{d}	18.73±0.54^{e}	57.72±1.49^{a}	46.65±0.68^{b}	38.07±0.03^{c}	****	34.77±1.39^{a}	28.41±0.43^{b}	25.65±0.72^{c}	29.61±0.62^{b}	25.92±0.29^{c}	****
GLN	100.63±1.06^{d}	109.36±1.28^{c}	113.73±0.64^{b}	115.55±0.26^{ab}	117.50±0.74^{a}	****	110.61±0.69^{d}	126.62±1.21^{c}	136.04±0.7^{a}	137.17±1.29^{a}	133.51±1.28^{b}	****
ORN	8.44±0.38^{a}	8.88±0.49^{a}	9.18±0.38^{a}	9.19±0.31^{a}	9.10±0.89^{a}	ns	13.71±0.53^{ab}	12.43±0.7^{bc}	12.15±0.75^{c}	14.6±0.34^{a}	14.28±0.49^{a}	**
TYR	273.57±0.42^{d}	276.39±1.58^{c}	294.31±0.69^{a}	283.81±0.58^{b}	264.52±1.49^{e}	****	301.36±0.64^{d}	303.08±0.32^{c}	316.83±0.51^{a}	305.09±1.34^{b}	284.79±0.29^{e}	****
SER	28.23±1.16^{b}	26.82±1.16^{b}	27.66±0.67^{b}	32.24±1.08^{a}	28.79±1.5^{b}	**	27.62±1.08^{b}	27.62±0.45^{b}	29.5±0.91^{a}	29.55±0.49^{a}	23.39±0.38^{c}	****
PRO	11.11±0.31^{c}	11.77±0.38^{bc}	12.79±0.32^{b}	16.17±0.16^{a}	12.31±0.97^{b}	****	11.95±0.76^{c}	18.45±0.53^{a}	13.43±0.12^{b}	11.84±0.11^{c}	13.16±0.14^{b}	****
Total FAAs	1925.31±1.67^{e}	2011.40±2.98^{d}	2130.29±3.95^{b}	2148.97±2.40^{a}	2076.50±2.68^{c}	****	2056.80±3.10^{c}	2134.50±3.50^{b}	2204.30±2.50^{a}	2053.18±0.72^{c}	2133.48±3.65^{b}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for pressures within the same cut. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

4.1.4. Physical properties of HPP treated lamb

4.1.4.1. pH of cooked HPP lamb

Table 14. The pH of HPP treated cooked lamb

pH	control	200 MPa	300 MPa	400 MPa	600 MPa	P-values
Rump	6.20 ± 0.08^{a}	6.19 ± 0.03^{a}	6.08 ± 0.01^{b}	6.14 ± 0.03^{b}	5.86 ± 0.02^{c}	***
knuckle	6.40 ± 0.03^{a}	6.46 ± 0.02^{a}	6.06 ± 0.44^{b}	6.04 ± 0.01^{b}	5.76 ± 0.05^{c}	**

Values with different superscripts (a,b,c,d,e) in the same row differ significantly. $P < 0.001$ presented as $***$ for level of significance; $P < 0.01$ presented as $**$ level of significance.

Table 14 shows the pH of HPP treated rump and knuckle. The high-pressure condition affected the pH significantly ($P < 0.05$) for both cooked rump and knuckle lamb cuts. The pH decreased with increase in pressure. For example, in the cooked rump, the pH values from 200 MPa to 300 MPa were around 6.10. The 200 MPa had the highest pH value (6.19) among the HPP treated lambs. The 600 MPa rump had the lowest pH (5.86) which was significantly different from the other pressure conditions. Compared with rump, the knuckle treated with 600 MPa also showed the lowest pH value, which was 5.76. The pH of knuckle in 200 MPa was 6.46, which was the highest among all high pressure.

In general, the pH of cooked lamb, in my data, was between 5.76 and 6.4, which means the meat was in the normal pH range. This is in agreement with the conclusion by Zhang et al. (2005) who reported that the normal pH of raw beef was between 5.5 and 7.5. The pH of cooked beef (*pectoralis profundus*) was 5.71 in 400 MPa and 5.76 in 600 MPa (McArdle et al., 2013) which were similar to the values of the lamb samples in 600 MPa of the current study. However no significant difference was observed between 400 MPa and 600 MPa (McArdle et al., 2013), which was different from my data. The possible reason may due to the different types of meat (beef vs. lamb) and cuts.

4.1.4.2. Moisture

Table 15. Moisture content of HPP treated cooked lamb.

Moisture %	control	200 MPa	300 MPa	400 MPa	600 MPa	P-values
rump	51 ± 0.01^{cA}	65 ± 0.02^{aA}	59 ± 0.14^{eA}	53 ± 0.08^{bA}	47 ± 0.1^{dA}	****
knuckle	62 ± 0.04^{bB}	62 ± 0.01^{bB}	67 ± 0.01^{aB}	56 ± 0.03^{cB}	46 ± 0.03^{dB}	****
P-values	****	***	****	**	ns	

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for pressures. Values with different superscripts (^{A,B}) in the same column differ significantly for the 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; and ns meaning not statistically significant.

A significant difference was observed in the moisture content of HPP treated lamb meat ($P < 0.05$) (Table 15). For rump, the moisture content increased from the control (51 %) to 65 % (200 MPa), and then dropped to 59 % (300 MPa) and then to 47 % (600 MPa). Similar pattern was found in the knuckle, where the moisture increased from the control (62%) and reached to 67 % in 300 MPa, and then decreased to 46% in 600 MPa. For both rump and knuckle, the 600 MPa had the lowest moisture content, which was 47 % for rump and 46 % for knuckle. The moisture contents also showed the significant difference ($P < 0.05$) between the two cuts except in 600 MPa. This may be related to a different amount of water being bound to the muscle fiber bundles from the denaturation of proteins caused by high pressure. Yet, moisture content has not been studied on HPP treated lamb.

4.1.4.3. Color

Table 16. The color of HPP treated cooked lamb.

		L* (lightness)	a* (redness)	b* (yellowness)
Rump	control	37.92 ± 0.06^{d}	4.94 ± 0.1^{a}	11.65 ± 0.15^{d}
	200 MPa	38.44 ± 0.07^{e}	4.89 ± 0.16^{a}	11.75 ± 0.16^{d}
	300 MPa	40.26 ± 0.04^{c}	4.24 ± 0.03^{b}	12.73 ± 0.21^{c}
	400 MPa	42.23 ± 0.02^{b}	3.56 ± 0.10^{c}	13.31 ± 0.33^{b}
	600 MPa	42.62 ± 0.03^{a}	3.45 ± 0.21^{c}	15.04 ± 0.36^{a}
	P-values	****	****	****
Knuckle	Control	37.75 ± 0.53^{d}	6.65 ± 0.19^{a}	9.77 ± 0.28^{e}
	200 MPa	37.96 ± 0.09^{d}	5.89 ± 0.09^{b}	11.56 ± 0.1^{d}
	300 MPa	39.50 ± 0.01^{c}	4.47 ± 0.07^{c}	16.42 ± 0.18^{b}
	400 MPa	41.51 ± 0.02^{b}	4.18 ± 0.10^{d}	15.55 ± 0.38^{c}
	600 MPa	44.40 ± 0.05^{a}	3.49 ± 0.12^{e}	18.02 ± 0.15^{a}
	P-values	****	****	****

Values with different superscripts (^{a,b,c,d,e}) in the same column differ significantly within the same cuts. $P < 0.0001$ presented as ****for level of significance.

Significant difference in L*, a * and b * values were observed between control and pressure treated samples (Table 16). Lamb samples processed at higher pressure levels (600 MPa) had higher L* and b * value, manifesting as a whitening effect. McArdle et al. (2013) reported an increase in L* and b* values from 200 to 600 MPa treated lamb. The same conclusion was found by McArdle et al. (2011) who reported an increase value in L* from cooked beef sample after HPP (400-600 MPa at 55 ^{0}C). The decrease in the redness for both rump and knuckle samples was in agreement with the conclusion found by Jung et al. (2003). They reported that the redness of the raw beef was maintained with HPP treatments at 130 MPa, however, it decreased when the pressure was raised to 520 MPa. The whitening effect was due to the denaturation of myoglobin, and a haem displacemnet or release (Campus et al., 2008; Carlez et al., 1995), as mentioned in section 2.5.1.2. Secondly, oxidation of ferrous myoglobin to ferric state, under high pressure of above 400 MPa may also have contributed towards in redness.

Myoglobin upon the exposure to oxygen forms metmyoglobin in ferric state, with colour turning to brownish red (Young and West, 2001).

The cuts affected the color significantly as well ($P < 0.05$). The knuckle (600 MPa) had the higher L* (lightness) value than rump (600 MPa). The b* (yellowness) value of knuckle (300 to 600 MPa) were all higher than the rump (300 to 600 MPa). The control knuckle was higher than rump (control) in redness (a*). This is explained by the study of Talmant et al. (1986) who stated that different cuts of lamb are composed of different types of muscular fibres and various cuts of muscles have different oxidative ability which affect the denaturation of myoglobin.

4.2. Pulsed electric field (PEF)

Apart from the HPP treatment, another non-thermal processing treatment, pulsed electric field (PEF) was applied on seven lamb cuts. The amounts of FAAs present in different cuts with non-PEF and PEF treated lamb, stored for 0 day and 7 days, are presented as mean ± S.D. in mg/100g in Tables 17 to 30. In undigested phase (S1) and digested phases (S2 and S3), nine EAAs and ten NEAAs have been identified and quantified with the use of the EZ:Faast amino acid analysis kit (Phenomenex®, USA) and GC. The nine EAAs were VAL, LEU, ILE, MET, PHE, LYS, HIS, THR and TRP. The ten non-essentials amino acids were ALA, GLY, TYR, SER, PRO, ASP, GLU, ORN, ASN and GLN, which were same FAAs found in HPP treated lamb. The results and discussion of PEF data are separated into three sections:

1. Extent of digestibility of proteins in lambs in different phases of simulated digestion
2. Digestibility of different muscles (meat cuts) of non-PEF and PEF treated lamb
3. Effect of the storage time (0 day and 7 days) in FAAs content of non-PEF and PEF treated lamb

4.2.1. Extent of digestibility of proteins in lamb

The extraction and the digestion method for both HPP and PEF lamb were the same. As mentioned in section 4.1.1.1, the total FAAs in S1 was extracted by methanol. For the

seven non-PEF treated lamb cuts (0 day and 7 days), in the undigested phase, VAL, LEU, ILE, PHE, LYS and HIS were the most abundant EAAs. And a high amount of NEAAs including ALA, GLY, GLN and PRO were found. For example, in the non-PEF treated shanks (0 day) in S1, the VAl was 9.97 mg/100g which was the highest amount of EAAs, followed by the LEU and HIS which was 8.07 mg/100g and 8.90 mg/100g respectively. In the non-PEF treated shanks (7 days) in S1, the LEU, HIS, VAL and LYS were the four highest EAAs, which was 13.96 mg/100g, 14.02 mg/100g, 11.91 mg/100g and 11.35 mg/100g respectively. LEU, VAL, THR and PHE play an important role in the meat flavor (Madruga et al., 2010). The high amount of LEU, VAL, THR and PHE contribute the strong smell of lamb that people can smell.

For the seven PEF treated lamb cuts (0 day and 7 days), VAL, LEU, ILE, PHE, LYS and HIS were the most abundant EAAs in the undigested phase. And high amount of NEAAs including LEU, HIS, VAL and LYS were found. Comparing the FAAs between non-PEF and PEF treated shanks (7 day) in S1, the VAL. LEU. LYS and TYR of PEF treated shanks were all higher than the values in non-PEF treated shanks, which means that the PEF treated shanks have more lamb flavor than non-PEF treated shanks. The most FAAs in other PEF treated cuts also showed higher values than in the non-PEF treated lamb.

Figure 15 illustrates the increasing tendency from S1 to S3 for both non-PEF and PEF treated lamb at 0 day and 7 days. For non-PEF treated shanks in 0 day, the total FAAs increased from 205.66 mg/100g (S1) to 348.38 mg/100g (S2). There was a significant increment from S2 to S3, which was 1497.55 mg/100g. For 7 days of storage under chilled condition, the total FAAs increased slightly from 304.63 mg/100g (S1) to 466.07 mg/100g (S2). But the total FAAs in S3 (2247.09 mg/100g) was 4.8 times higher than that of the S2. The PEF treated shanks showed the same trend to the non-PEF lamb, which total FAAs from S1 to S2 was a slightly increase, but a significant increment from S2 to S3. Not only the shanks, but also other six lamb cuts showed the increasing trend from S1 to S3. And a significant increment was seen from S2 to S3 for all of the cuts.

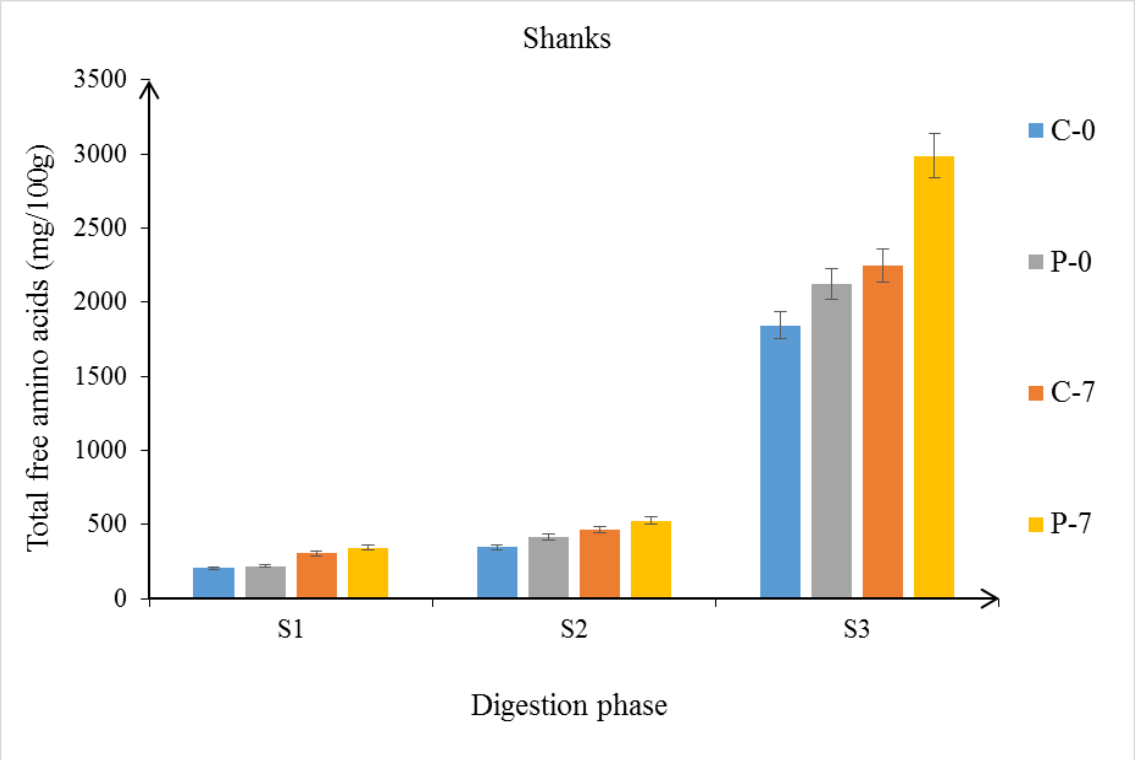

Figure 15. The total FAAs of shanks (non-PEF and PEF) at 0 day and 7 days of storage at three stages (S1, S2 and S3).

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)
S1: Undigested phase; S2: Gastric phase; S3: Intestinal phase

Not only the total FAAs, but many individual FAAs showed a significant difference ($P < 0.05$) between different phases. For example, the shanks (non-PEF treated) in 0 day, LEU increased from 8.07 mg/100g (S1) to 33.45 mg/100g (S2), and increased sharply to 275.90 mg/100g in S3. For 7 days of storage under chilled condition, LEU showed the same increasing tendency with the non-PEF treated lamb, which was from 13.96 mg/100g (S1) to 39.91 mg/100g (S2) and reached 399.54 mg/100g (S3). PHE and LYS showed a significant increase from S2 to S3. The increment of LEU, PHE and LYS in the gastric and intestinal phase does benefit to the human. LEU supports muscle synthesis (Kohlmeier, 2015; Yang et al., 2015), and PHE and LYS play an important role in the memory and brain function (Wu, 2013; Fernstrom, 2013), as mentioned in the section 2.1.

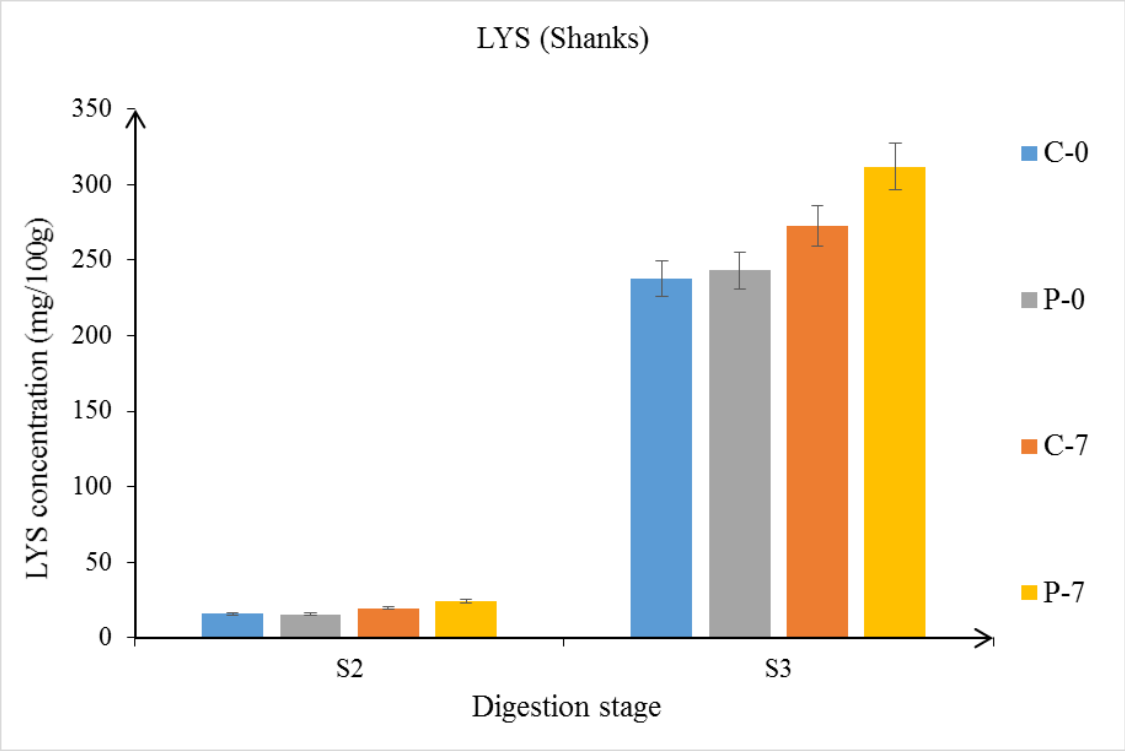

Figure 16. The LYS concentration of non-PEF and PEF treated shanks at 0 day and 7 days storage in gastric phase (S2) an intestinal phase (S3).

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)

As seen in Figure 16, the content of LYS in S3 were much higher than in S2, which was also observed by Krehbiel & Matthews (2003), who found that the pancreatin in the intestinal phase was resposibile for the breakdown of carboxyl side of core amino acids, such as LYS and ARG. From the results, LYS in S2 was 15.78 mg/100g for 0 day and 19.63 for 7 days. In S3, LYS increased sharply to 237.70 mg/100g in 0 day and 272.66 mg/100g in 7 days, which demonstrated a strong ability of pancreatin to hydrolyze and liberate FAAs for the proteins compared to pepsin. For the non-PEF treated shanks in 0 day of storage, pepsin broke down 29.40% of protein in the gastric phase and pancreatin hydrolyzed 89.35 % lamb protein in the intestinal phase. For 7 days of storage, the digestibility of protein was 15.64 % in S2 and 85.60 % in S3. This was higher than the values found in the studies of Krehbiel & Matthews (2003) and Sitrin (2014). They reported that pepsin can only breakdown 10-20% of protein, however, pancreatic can hydrolyse about 40% protein into FAAs. The significant increment of LYS from S2 to S3 was observed in other cuts as well. LYS plays an important role in producing the blood and growth (Wu, 2013). Compared with the digestibility of HPP mentioned in section 4.1.1.2, PEF treated lamb (Shanks) had higher digestibility in the intestinal

phase than HPP treated lambs. The possible reason may be due to the different treatments methods. In general, the PEF treatments had higher impact on the muscle protein than the HPP treatments, resulting in a higher digestibility, shown by higher amount of FAAs hydrolyzed from protein.

The enzymes (amylase, pepsin, pancreatin and bile) used in the study, digestion time (transit time), sample amount and volume of digestion fluids were appropriate, which had been mentioned in section 4.1.1.2. With comparison to HPP data, the results of FAAs in PEF confirmed again the whole method for digestion was appropriate for lamb samples. The method in this research closely followed the protocol published by Minekus et al. (2014).

4.2.2. Digestibility of different muscles (meat cuts) of non-PEF and PEF treated lamb

Different muscles cuts used in the study had an impact on the digestibility of PEF and non- PEF treated lambs. This was observed as a significant difference ($P<0.0001$) in the amount of total FAAs across the three stages of digestion.

4.2.2.1. Effect of different cuts on FAAs content in the undigested phase (S1)

From Figure 17 and Figure 18, it is clear that the different cuts had significant influence on the total FAAs in both non-PEF and PEF treated lamb. From Figure 17, it can be seen that total FAAs in rump (409.01 mg/100g) was the highest in 7 days and followed by flat (383.67mg/100g). The total FAAs in the knuckle (0 day) was the lowest (161.41 mg/100g).

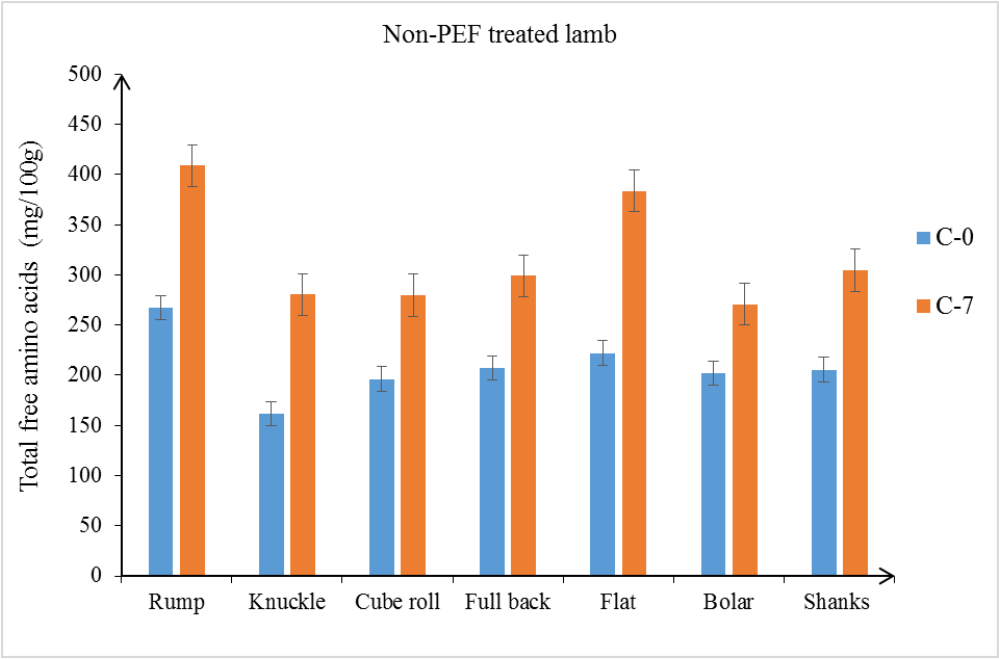

Figure 17. The total FAAs (mg/100g) in non-PEF treated lamb at 0 day and 7 days of storage time, prior to digestion (S1).

C-0: non-PEF (0 day) C-7: non-PEF (7 days).

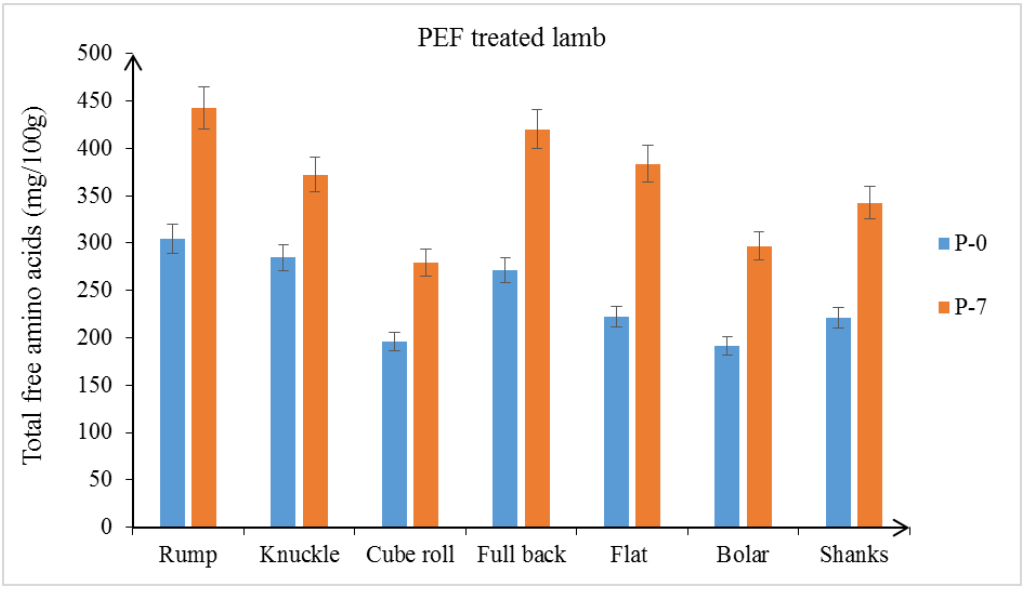

Figure 18. The PEF treated lamb at 0 day and 7 days storage time in undigested phase.

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)

Figure 18 shows the total FAAs in PEF treated lamb in S1. The total FAAs in rump was the highest in both 0 day (304.89 mg/100g) and 7 days (442.8 mg/100g) of storage. The total FAAs of full back was 420.41 mg/100g in 7 days, which was lower than that of the rump. The total FAAs of bolar in both 0 day (191.14 mg/100g) and 7 days (296.57 mg/100g) were lowest among seven cuts. As mentioned in section 4.1.3.1, the muscular

fibers are different in various cuts of the same species can affect the oxidative enzymatic activities and glycolytic enzyme activities (Talmant et al., 1986). Franco et al. (2010) reported the oxidative ability in decreasing order are masseter>cardiac muscle> knuckle> heel >full back>inside cut, where the masseter is the most oxidative and the inside cut is the most glycolic. The oxidative muscles have more FAAs such as ASP, GLN and TAU. The oxidative ability of knuckle was stronger than full back was also seen in my results. Refering Figure 4, the location of seven cuts are different. For example, the rump is located in the haunch of lamb; however, the knuckle is mainly located in the leg of lamb, which is exercised everyday through the walking and running. Seven cuts are different in function, which may have contributed to a difference in FAAs.

TAU content has been reported to be highly related to the different oxidative ability of porcine muscles (Cornet and Bousset, 1999). The high oxidative ability muscles have been found to have more in fresh beef which was in agreement with Cornet and Bousset (1999). In addition, oxidative muscles have more ASP, GLN and taurine. For the non-PEF and PEF treated rump, GLN was 85.28 mg/100g and 104.03 mg/100g respectively, which was higher than the values found in the full back (0 day: 52.67 mg/100g and 7 days: 68.04 mg/100g).

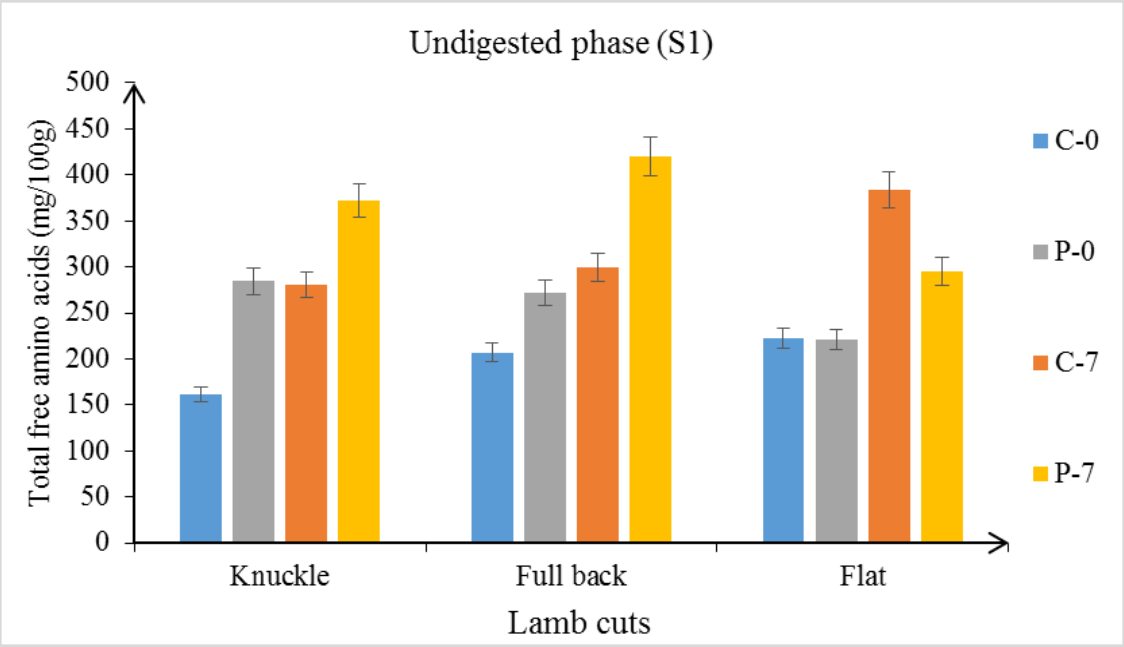

Figure 19. Comparison of total FAAs in 0 day and 7 days of non-PEF and PEF treated cuts, in undigested phase (S1).

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)

Comparing the non-PEF and PEF treated lamb cuts (Figure 17 and Figure 18), the total FAAs in PEF treated lamb were higher than the values in non-PEF treated lamb. Figure 19 shows that the knuckle and full back had significant increase in total FAAs between non-PEF and PEF treatment. For the knuckle, the total FAAs was 284.58 mg/100g for P-0 and 161.41 mg/100g for C-0. That is, P-0 contained 123.17 mg/100g more of total FAAs than C-0. Furthermore, after 7 days of storage, the total FAAs in the full back increased a lot from C-7 (299.18 mg/100g) to P-7 (420.41 mg/100g). As PEF treatment may cause deterioration of the myofibrils and increase the activities of proteolytic enzymes in meat (Faridnia et al., 2015), the total FAAs in PEF were higher than that of the non-PEF treated lamb. And from Figure 18 it can also be concluded that PEF had different level of effects on the various cuts. There is no reference related to the FAAs of PEF meat, and this was the first study to investigate them.

4.2.2.2. Effect of different cuts in FAAs content during *in vitro* digestion (S2 and S3)

From Figure 20, it can be seen that the cuts have an effect on the total FAAs. In the 0 day, the bolar contained the highest amount of total FAAs, which was 391.43 mg/100g, followed by the rump (379.32 mg/100g) and cube roll (366.06 mg/100g). And full back contained the lowest amount of total FAAs, which was 206.88 mg/100g. After 7 days of storage, the rump and bolar increased a lot in total FAAs, which were 516.13 mg/100g and 491.78 mg/100g separately. The full back and flat increased very little which were 312.18mg/100g and 363.29mg/ 100g respectively. It can be concluded that the digestibility differed depending on the cuts as shown by significantly different amounts of the total FAAs being released in the gastric phase alone. And the different cuts also had different increments in total FAAs during 7 days of storage.

The cuts and storage time affected the individual FAAs significantly ($P < 0.05$) as well. For instance, in the gastric phase of non-PEF treated lamb, rump had the highest content of LEU in 0 day (44.40 mg/100g) and 7 days of storage (55.20 mg/100g) (Table 17), followed by bolar whose LEU was 40.91 mg/100g in 0 day and 52.22 mg/100g in 7 days of storage (Table 25). Cube roll contained the lowest amount of LEU which was 32.86 mg/100g and 34.57 mg/100g in 0 day and 7 days, respectively (Table 29). Furthermore, LEU is a branched chain amino acid that supports muscle synthesis of human (Yang et al., 2015). So in the gastric phase, people can absorb more LEU from rump than any other lamb cuts.

Figure 21 shows the total FAAs (mg/100g) in the PEF-treated lamb at 0 day and 7 days storage times, collected in the gastric phase. The total FAAs of knuckle was the highest in both 0 day (523.10 mg/100g) and 7 days (594.23 mg/100g), followed by the rump which was 458.66 mg/100g (0 day) and 560.97 mg/100g (7 days). Total FAAs of bolar in 0 day was the lowest which was 334.19 mg/100g. The concentration of PHE in the seven cuts differed significantly ($P < 0.05$) as well. For the PEF treated lamb in the gastric phase, the concentration of PHE in knuckle was the highest which was 68.77 mg/100g in 0 day and increased to 73.91 mg/100g in 7 days (Table 20). However, the PHE in the bolar was much lower than the values in the knuckle, which was 23.70 mg/100g (0 day) and 38.60 mg/100g (7 days) (Table 26). The PHE is an essential FAAs

for human which can be not be produced by human. Deficiency in PHE may result in the brain problems such as mood swing and mood change (Fernstrom, 2013; Friedman and Levin, 2012).

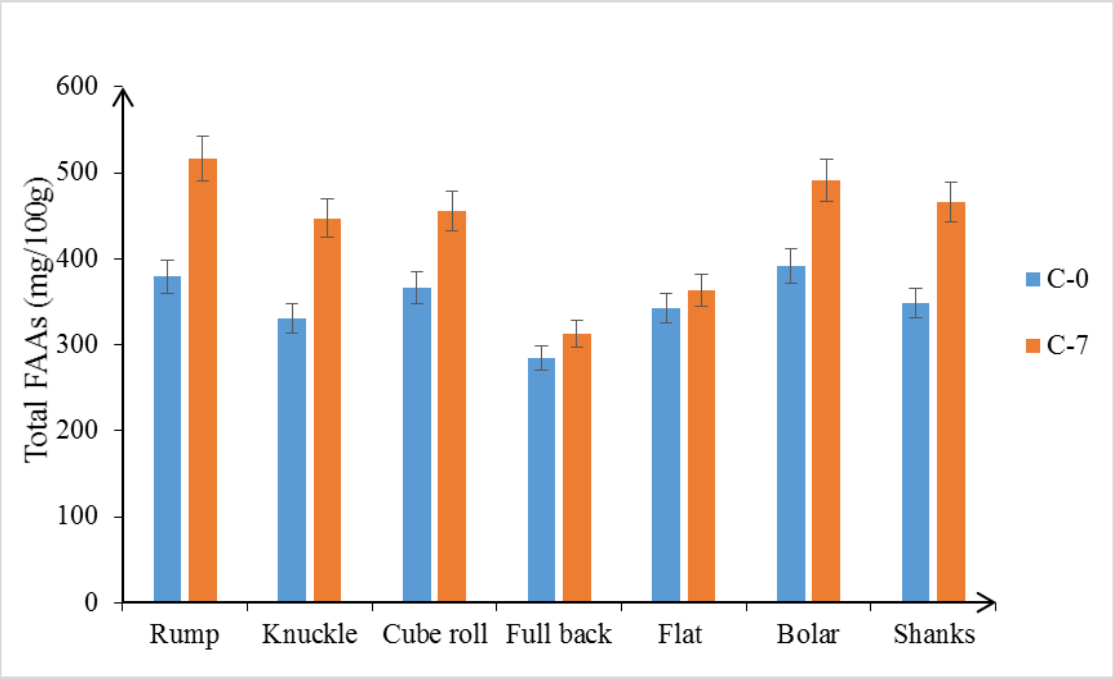

Figure 20. A graph showing total FAAs found in the gastric phase of non-PEF treated lamb at 0 day and 7 days of storage time

C-0: non-PEF (0 day); C-7: non-PEF (7 days).

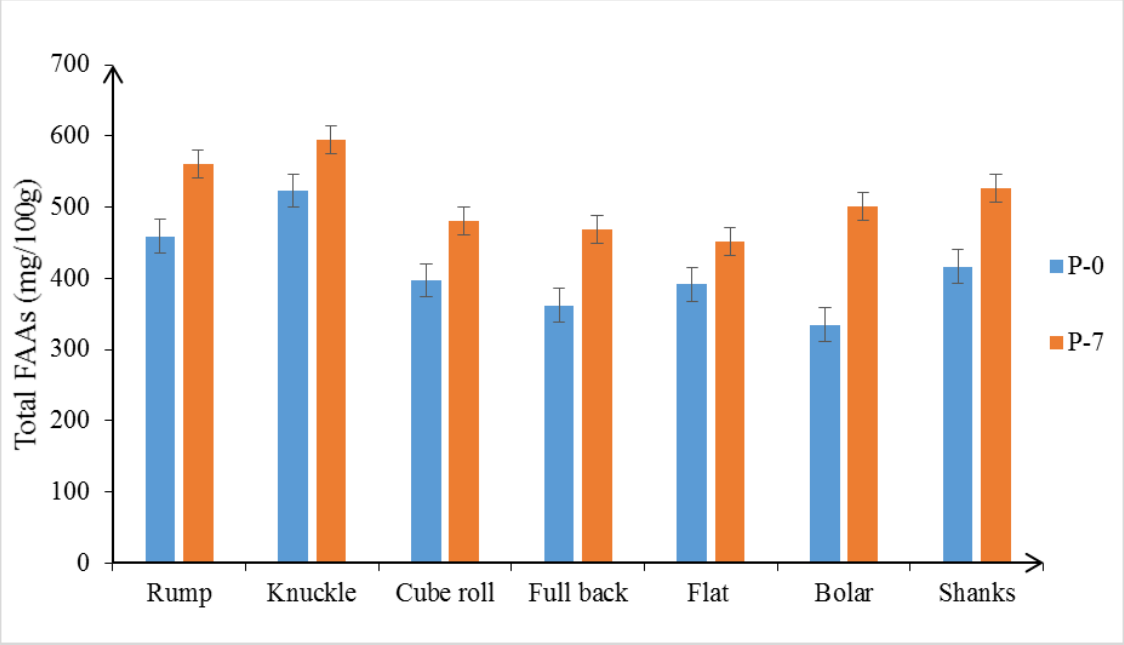

Figure 21. A graph showing total FAAs found in the gastric phase of PEF treated lamb at 0 day and 7 days of storage time

P-0: PEF (0 day); P-7: PEF (7 days)

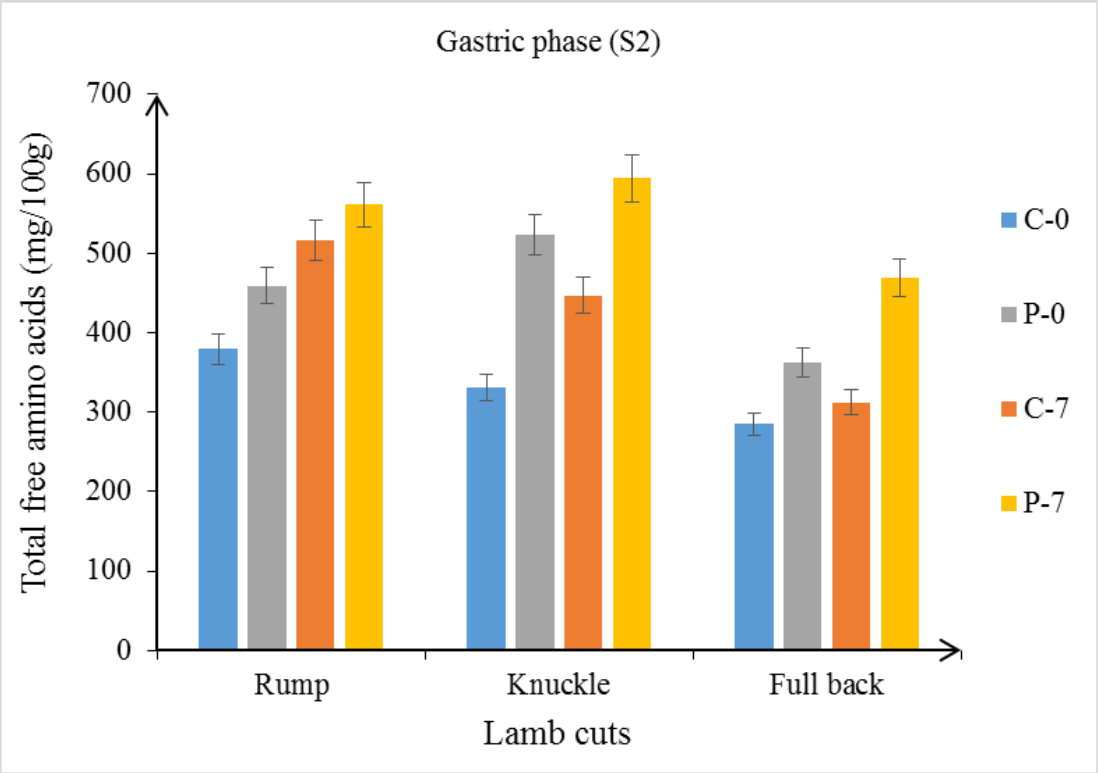

Figure 22. The comparison of non-PEF and PEF treatment in 0 day and 7 days storage in gastric phase

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)

When PEF and non-PEF treated lamb in the gastric phase were compared (Figure 22), it was clear that the lamb processed by the PEF has shown higher digestibility, releasing more FAAs during the gastric phase. For example, the non-PEF treated full back in 0 day and 7 days were 284.71 mg/100 g and 312.18 mg/100g. And the total FAAs increased to 362.12 mg/100g and 468.45 mg/100g in the PEF treated full back. Furthermore, the PEF treated cuts were affected by digestion as well. The FAAs in 0 day and 7 days of PEF- treated knuckle were highest, which was 523.10 mg/100g and 594.23 mg/100g, respectively.

The lamb cuts contained different amounts of total FAAs for both non-PEF and PEF treated lamb in the intestinal phase as well ($P < 0.05$). For the PEF treated lamb in the intestinal phase, LYS of knuckle in 0 day was 347.71 mg/100g (Table 20) which was the highest among seven lamb cuts studied, then increased to 421.45 mg/100g during 7 days storage. However, LYS in flat (PEF) was the lowest, which was 232.66 mg/100g (0 day) and 229.60 mg/100g (7 days).

So it can be concluded that the digestibility of LYS in different cuts was different. Moreover, the shortage of LYS can lead to anemia, mood swing and stunted growth in human (Wu, 2013). Post to the PEF treatment, available of LYS for absorption in knuckle is greatly enhanced as shown by the results.

Figure 23 shows four significantly different ($P < 0.05$) cuts during the *in vitro* digestion in the intestinal phase, the four cuts different significant in total free amino acids ($P<0.05$). The total FAAs of non-PEF and PEF-treated flat were lower than the other cuts, which was 1305.55 mg/100g (0 day) and 1716.57 mg/100g (7 days) for the non-PEF treated lamb. And in the PEF treated flat, the 0 day was 1822.42 mg/100g and 7 days was 1938.12 mg/100g in the intestinal phase. The total FAAs of PEF treated shanks in 7 days storage was 2986.13 mg/100g in intestinal phase which was higher than the values of other cuts in the same condition. As mentioned in the HPP section (4.1.3.2), the different muscles composition affected the digestibility of cooked carcass of beef (Bax et al., 2012). Various muscles in the same species contained different amounts of several contractile and structural proteins such as actin, myosin light chain and tubulin-α. The contents of detoxification enzymes and regulatory muscle contraction proteins were different as well, such as slow TnT and calsarcin 2, which lead to the the difference in protein solubility during digestion (Bax et al., 2012). The same conclusion was also observed by Morzel et al. (2006), they reported that the intermolecular cross-links and the formation of aggregation might disturb the recognition of proteins by the protease, causing changes in protein hydrophobicity and solubility, and in turn, decreased the protein digestibility.

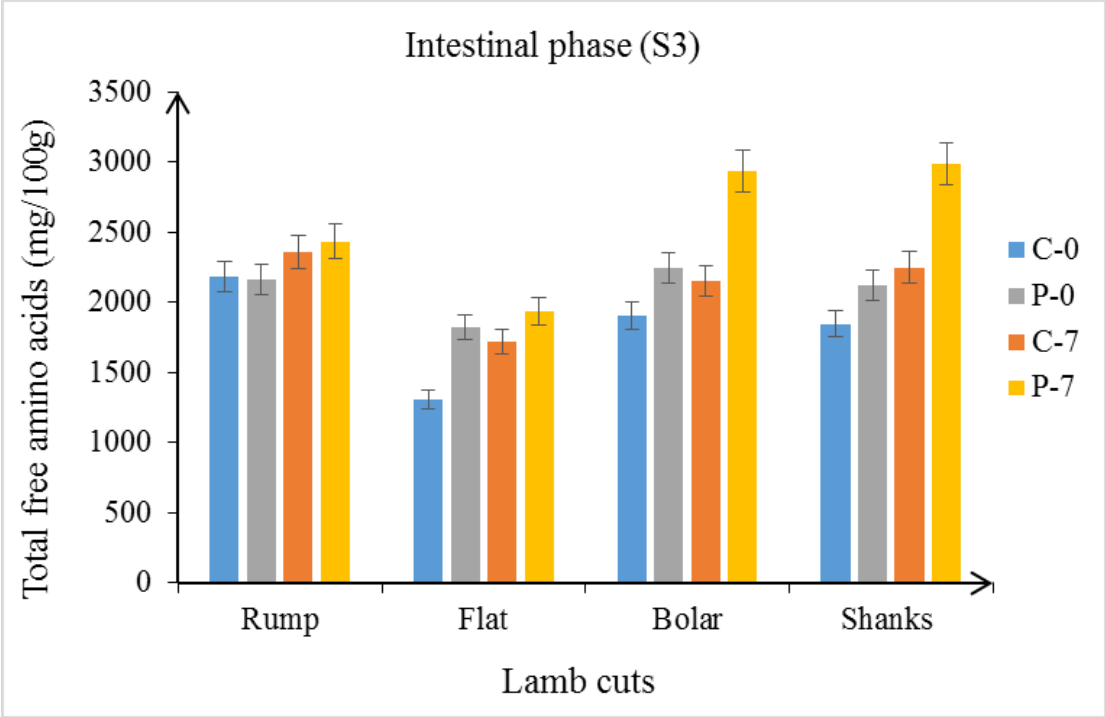

Figure 23. The comparison of non-PEF and PEF treatment in 0 day and 7 days storage in intestinal phase

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)

4.2.3. Effect of the storage time (0 day and 7 days) in FAAs content of PEF treated lamb.

The storage time (0 day and 7 days) had a significant effect ($P < 0.0001$) on the total FAAs at three different stages (S1, S2 and S3) for both non-PEF and PEF treated lamb. The effect of storage time at different stages is discussed separately as follows.

4.2.3.1. Effect of the storage time (0 day and 7 days) in FAAs content in undigested phase (S1)

A significant difference ($P<0.0001$) between storage times for both non-PEF and PEF lamb in S1 for full back was observed (Figure 24 and Tables 27 and 28). For the non-PEF treated full back in S1, the total FAAs increased from 206.88 mg/100g (0 day) to 299.18 mg/100g (7 days). The storage time affected the amount of most of the EAAs

significantly (P<0.0001), and these include LEU, PHE and LYS. For example, LEU increased from 12.55 mg/100g (0 day) to 21.78 mg/100g (7 days) in S1 of non-PEF treated full back. LEU for the PEF full back showed the same increasing tendency from 14.79 mg/100g (0 day) to 26.42 mg/100g (7 days). The total FAAs of PEF treated full back increased from 271.36 mg/100g (0 day) to 420.41 mg/100g (7 days) in S1, which was higher than the value in the non-PEF full back. The storage time also influenced most of non-essential AAs (P<0.001) except for ASP which had no significant difference.

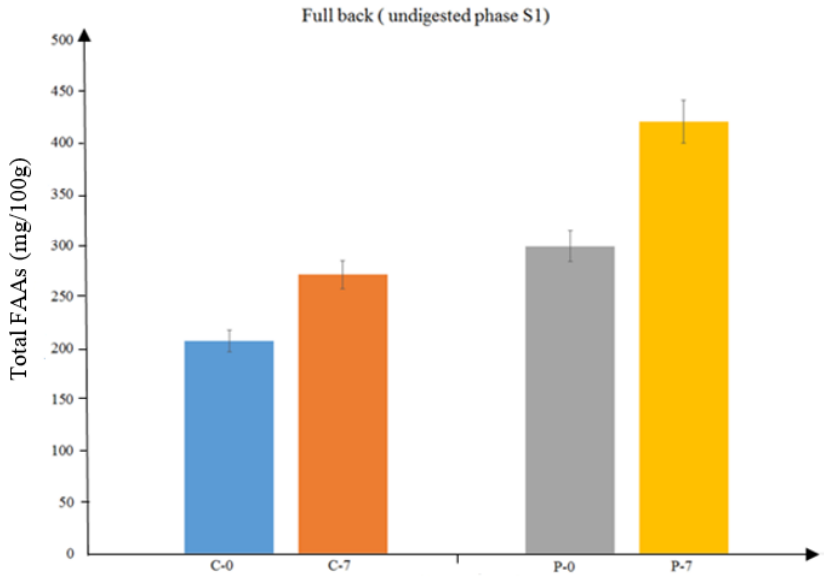

Figure 24. The total FAAs of full back (non-PEF and PEF) in 0 day and 7 days of storage in the undigested phase (S1)

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)

A large increases on ALA, GLU, LEU, SER, HIS and LYS were observed in the non-PEF and PEF treated full back, which was also found in other non-PEF and PEF treated lamb cuts. For example, full back (non-PEF) in the S1, from 0 day to 7 days storage, VAL increased from 28.04 mg/100g to 33.87 mg/100g. GLU increased from 8.19 mg/100g to 17.71 mg/100g. SER increased sharply from 14.06 mg/100g to 34.96 mg/100g. LYS increased from 3.47 mg/100g (0 day) to 10.08 mg/100g (7 days). There was in agreement with Parrish et al. (1969) who found large increments on ALA, THR+LEU and LYS in post-mortern bovine muscle after 312 hours. The same conclusion was found in the study of Nishimura et al. (1988) who reported that the

increments of ALA, LEU, SER, VAL and GLU were especially large after 12 days of storage in raw beef. Increasing FAAs during storage can be explained by endopeptidase, such as calpain and cathepsins, and subsequent reactions by exopeptidase, such as aminopeptidase (Nishimura et al. 1988; Toldrá et al., 1997; Watanabe et al., 2004), where FAAs naturally increase over the course of storage under chilled condition, known as wet-aging. The differences of activity of these proteases may arise from slaughter age, which had been mentioned in HPP section 4.1.2.1.

The PEF treatments influenced the FAAs during the storage time as well. Not only the full back, but also for the other PEF treated cuts, the total FAAs of PEF treated lamb were all higher than the non-PEF treated lamb (0 day and 7 days separately). This can be fully explained by the study of Bekhit et al. (2014) who stated that PEF treatments increased protease enzymes in lamb such as cathepsins B and L that are released from the lysosomes. Those protease enzymes have an important role in the proteolysis and produce high amount of small peptides and FAAs as mentioned in HPP (section 4.1.2.1). Moreover, PEF treatment is also degrade myofibril structure. Faridnia et al. (2015) stated that the ruptures in myofibrils and the breakdown of cells can increase the activities of proteolytic enzymes in meat, which can increase the digestibility of meat as well. This was well observed through the data from the current study.

4.2.3.2. Effect of the storage time (0 day and 7 days) in free amino acids content during *in vitro* digestion (S2 and S3)

Figure 25 shows that the storage time (0 day and 7 day) affected the total FAAs significantly ($P<0.001$) for both non-PEF and PEF treated full back during *in vitro* digestion. The total FAAs in 7 days were higher than the values in 0 days, in general. For instance, the PEF treated full back increased from 362.12 mg/100g (0 day) to 468.45 mg/100g (7 days) in S2, and increased sharply from 1989.61 mg/100g (0 day) to 2480.67 mg/100g (7 days) in S3.

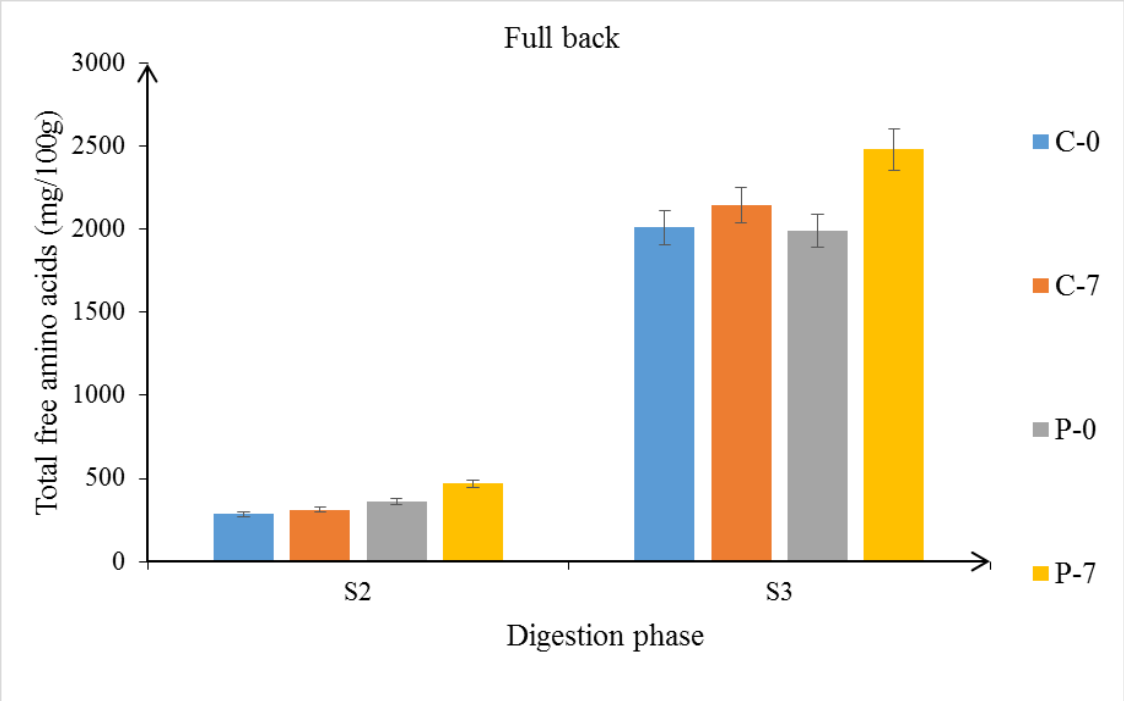

Figure 25. The amount of total FAAs found in the full back of the digested (S2 and S3) non-PEF and PEF treated lambs, stored for 0 day and 7 days.

C-0: non-PEF (0 day); C-7: non-PEF (7 days); P-0: PEF (0 day); P-7: PEF (7 days)

Not only the total FAAs, but many of the individual FAAs also showed significant difference ($P < 0.05$) in terms of storage time (0 day and 7 days). For instance, in the gastric phase of the non-PEF treated full back. PHE was 28.85 mg/100g in 0 day and increased to 38.59 mg/100g after 7 days of storage. LYS increased from 285.53 mg/100g (0 day) to 318.84 mg/100g (7 days) in the intestinal phase of full back (non-PEF). Considering the FAAs of full back (PEF), the increment of LEU and PHE were 10 mg/100g -12 mg/100g in the gastric phase. Especially for the PHE in the intestinal phase, the content increased sharply from 312.13 mg/100g (0 day) to 403.17 mg/100g (7 days). Lacking in PHE may lead to the brain functions such as mood swing and mood change of human (Fernstrom, 2013; Friedman and Levin, 2012). So it can be concluded that the proteins in lamb become more during digestion after 7 days of storage. This in turn would mean that the storage under chilled condition (wet-aging) allows more nutrients (FAAs) to be available for the body to absorb for further utilization.

To the best of my knowledge, the studies on the effect of storage time on FFAs, related to *in vitro* digestion is absent in the literature. The storage time is able to increase the enzyme activity, of calpain and cathepsin, which are endopeptidases that can react with

exopeptidases and produce high amount of small peptides and FAAs (Nishimura et al., 1988; Watanabe et al., 2004). As a result, the activity of protein muscles proteolysis increases (Toldrá et al., 1997; Ohmori et al., 1991). Moreover, when the PEF treatment affect on the lamb, the microstructure changes rupture in myofibrils and the breakdown of cells can also increase the activities of proteolytic enzymes in meat (Dolatowski, 1988), which increased the digestibility of lamb. The same conclusion was found in the study by Faridnia et al. (2015) who reported that PEF treatment in combination with ageing can increase the rate of proteolysis during digestion.

Table 17: Concentration of FAAs (mg/100g) in New Zealand lamb (rump) in different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

	Rump (non-PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	9.10±0.05^{b}	16.27±0.26^{a}	****	9.53±0.23^{b}	14.30±0.82^{a}	**	42.18±1.34^{b}	47.03±0.33^{a}	**
LEU	12.54±0.04^{b}	24.05±0.28^{a}	****	44.40±0.52^{b}	55.20±0.67^{a}	****	387.72±0.58^{b}	398.10±0.12^{a}	****
ILE	8.86±0.29^{b}	18.71±2.20^{a}	**	11.37±0.37^{b}	18.63±0.59^{a}	***	84.37±0.12^{b}	96.75±0.74^{a}	****
MET	5.48±0.33^{b}	9.05±0.29^{a}	***	20.98±0.58^{b}	23.17±0.74^{a}	*	84.27±0.89^{b}	97.93±0.38^{a}	****
PHE	8.82±0.22^{b}	16.86±0.34^{a}	****	46.88±0.58^{b}	54.41±0.41^{a}	***	319.25±0.36^{b}	371.60±0.57^{a}	****
LYS	6.14±0.22^{b}	15.31±0.66^{a}	****	17.92±0.43^{b}	30.18±1.07^{a}	***	319.86±0.36^{b}	314.07±0.59^{a}	***
HIS	14.16±0.26^{b}	18.06±0.52^{a}	**	19.14±0.27^{a}	20.68±0.74^{a}	ns	136.35±0.37^{b}	158.78±0.21^{a}	****
THR	15.92±0.41^{b}	20.98±0.32^{a}	***	12.86±0.50^{b}	18.92±0.55^{a}	***	34.38±0.31^{b}	32.77±0.40^{a}	*
TRP	1.71±0.05^{a}	2.20±0.43^{a}	ns	7.49±0.25^{a}	7.40±0.41^{a}	ns	102.53±0.93^{b}	117.07±0.21^{a}	****
NEAAs									
ALA	30.93±0.45^{b}	57.51±0.08^{a}	****	36.57±0.29^{b}	55.74±0.84^{a}	****	64.56±0.34^{b}	67.97±0.10^{a}	***
GLY	31.05±0.22^{b}	28.85±0.32^{a}	**	32.41±0.81^{b}	34.10±0.01^{a}	*	57.20±0.73^{b}	64.59±0.33^{a}	***
ASN	4.80±0.13^{b}	6.79±0.34^{a}	**	10.94±0.19^{b}	12.19±0.40^{a}	*	16.64±0.20^{a}	17.09±0.49^{a}	ns
ASP	4.22±0.29^{b}	6.51±0.22^{a}	**	9.64±0.36^{b}	12.33±0.47^{a}	**	12.59±0.22^{b}	15.66±1.05^{a}	*
GLU	3.66±0.07^{b}	10.96±0.25^{a}	****	6.51±0.02^{b}	8.44±0.54^{a}	**	14.54±0.01^{b}	17.63±0.10^{a}	****
GLN	64.20±0.21^{b}	85.28±0.18^{a}	****	33.82±0.46^{b}	56.88±0.56^{a}	****	143.51±0.17^{b}	159.68±0.68^{a}	****
ORN	2.51±0.01^{b}	5.17±0.19^{a}	****	4.85±0.22^{b}	11.87±0.36^{a}	****	9.93±0.16^{a}	9.89±0.67^{a}	ns
TYR	5.96±0.22^{b}	8.3±0.22^{a}	***	17.58±0.91^{b}	20.92±0.55^{a}	*	304.92±0.24^{b}	320.82±0.43^{a}	****
SER	22.75±0.24^{b}	36.47±0.32^{a}	****	25.13±0.45^{b}	41.87±0.94^{a}	****	29.51±0.27^{b}	34.33±0.45^{a}	***
PRO	14.13±0.21^{b}	21.68±0.28^{a}	****	11.32±0.49^{b}	18.91±0.52^{a}	***	16.36±0.31^{b}	18.89±0.67^{a}	**
Total FAAs	266.93±0.94^{b}	409.01±3.23^{a}	****	379.32±3.66^{b}	516.13±2.89^{a}	****	2180.65±1.40^{b}	2360.64±3.19^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 18. Concentration of FAAs (mg/100g) in New Zealand lamb (PEF rump) in different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

	Rump (PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	14.41±0.09^{b}	20.66±0.39^{a}	****	14.57±0.23^{b}	16.69±0.77^{a}	*	39.40±0.30^{b}	46.24±0.94^{a}	**
LEU	20.96±0.56^{b}	23.99±0.31^{a}	**	48.71±0.10^{b}	58.96±0.20^{a}	****	372.93±0.34^{b}	375.17±0.57^{a}	**
ILE	18.44±0.51^{b}	21.77±0.17^{a}	**	16.66±0.29^{b}	23.09±0.80^{a}	***	79.37±0.41^{b}	113.84±0.22^{a}	****
MET	7.91±0.20^{b}	8.48±0.16^{a}	*	17.88±0.17^{b}	24.95±0.45^{a}	****	87.03±0.85^{b}	94.63±0.65^{a}	**
PHE	13.69±0.31^{b}	16.69±0.29^{a}	**	42.35±1.36^{b}	57.47±0.65^{a}	***	297.97±0.22^{b}	357.63±0.48^{a}	****
LYS	8.73±0.11^{b}	15.60±0.25^{a}	****	23.31±1.35^{b}	29.69±0.35^{a}	**	305.03±0.42^{b}	337.14±0.40^{a}	****
HIS	19.22±0.40^{a}	19.10±0.13^{a}	ns	22.63±1.52^{b}	30.62±0.45^{a}	**	145.63±0.32^{b}	161.73±0.50^{a}	****
THR	16.70±0.30^{b}	21.18±0.39^{a}	***	19.10±0.71^{a}	18.74±0.34^{a}	ns	28.47±0.57^{b}	39.35±0.11^{a}	****
TRP	1.57±0.03^{b}	3.21±0.17^{a}	***	5.53±0.38^{b}	8.78±0.74^{a}	**	93.35±0.72^{b}	120.69±0.72^{a}	****
NEAAs									
ALA	42.98±0.36^{b}	58.34±0.20^{a}	****	50.52±0.49^{b}	55.70±0.49^{a}	***	67.96±1.34^{b}	73.15±0.59^{a}	**
GLY	25.39±0.08^{b}	28.54±0.18^{a}	****	42.44±0.38^{a}	42.18±0.36^{a}	ns	68.04±0.58^{b}	86.15±0.56^{a}	****
ASN	4.21±0.01^{b}	6.87±0.31^{a}	***	11.45±0.56^{b}	16.78±0.09^{a}	***	15.69±0.97^{b}	20.46±0.28^{a}	**
ASP	6.30±0.10^{a}	6.30±0.03^{a}	ns	9.57±0.83^{b}	14.51±0.28^{a}	**	11.87±0.17^{b}	18.23±0.50^{a}	****
GLU	5.20±0.14^{b}	11.07±0.31^{a}	****	7.60±0.01^{b}	12.06±0.29^{a}	****	28.67±0.76^{b}	31.50±0.18^{a}	**
GLN	45.44±0.24^{b}	104.03±0.20^{a}	****	47.76±1.12^{b}	61.92±0.67^{a}	***	156.76±0.71^{b}	183.81±0.64^{a}	****
ORN	4.06±0.06^{b}	5.17±0.27^{a}	**	3.60±0.07^{b}	5.05±0.26^{a}	**	7.62±0.44^{a}	7.63±0.38^{a}	ns
TYR	5.96±0.24^{b}	10.60±0.40^{a}	***	20.79±1.37^{b}	25.57±0.44^{a}	**	304.02±0.71^{b}	307.67±0.56^{a}	**
SER	25.27±0.17^{b}	39.73±0.19^{a}	****	35.85±0.47^{b}	33.79±0.62^{a}	*	30.73±0.73^{a}	30.96±0.59^{a}	ns
PRO	18.46±0.56^{b}	21.48±0.14^{a}	**	18.35±0.74^{b}	24.43±0.48^{a}	**	18.00±1.16^{b}	27.63±0.17^{a}	***
Total FAAs	304.89±1.56^{b}	442.80±2.11^{a}	****	458.66±2.97^{b}	560.97±1.09^{a}	****	2158.56±2.37^{b}	2433.61±0.62^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 19. Concentration of FAAs (mg/100g) in New Zealand lamb (knuckle) in different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

	Knuckle (non-PEF)								
Phase	S1			S2			S3		
Storage (days)	C0	C7	P-values	C0	C7	P-values	C0	C7	P-values
EAAs									
VAL	6.30±0.08^{b}	11.57±0.35^{a}	****	6.72±0.19^{b}	11.22±0.64^{a}	**	32.46±0.03^{b}	44.85±0.74^{a}	****
LEU	10.20±0.13^{b}	20.38±0.20^{a}	****	39.08±0.38^{b}	52.26±0.68^{a}	****	331.91±1.13^{b}	475.43±1.01^{a}	****
ILE	10.00±0.29^{b}	17.59±0.37^{a}	****	11.75±0.64^{b}	14.31±0.54^{a}	*	86.04±0.48^{b}	99.56±0.98^{a}	****
MET	2.18±0.19^{b}	6.19±0.24^{a}	****	16.50±0.26^{b}	21.68±0.37^{a}	****	82.69±0.85^{b}	119.34±0.54^{a}	****
PHE	6.86±0.22^{b}	11.64±0.08^{a}	****	46.52±0.43^{b}	58.13±0.16^{a}	****	346.24±0.72^{b}	485.84±0.26^{a}	****
LYS	2.36±0.29^{b}	6.34±0.11^{a}	****	16.73±0.53^{b}	19.77±0.14^{a}	**	249.95±0.11^{b}	377.84±0.60^{a}	****
HIS	5.92±0.35^{b}	11.24±0.04^{a}	****	12.50±0.22^{b}	18.47±0.26^{a}	****	118.87±0.32^{b}	173.46±0.84^{a}	****
THR	4.70±0.16^{b}	13.09±0.25^{a}	****	10.74±0.67^{b}	14.90±0.32^{a}	**	23.52±0.04^{b}	37.11±0.14^{a}	****
TRP	2.29±0.09^{a}	2.40±0.14^{a}	ns	6.71±0.21^{b}	9.62±0.59^{a}	**	114.39±0.55^{b}	151.27±0.67^{a}	****
NEAAs									
ALA	24.83±0.06^{b}	38.90±0.18^{a}	****	33.27±0.10^{b}	45.65±0.47^{a}	****	52.93±0.35^{b}	73.91±0.63^{a}	****
GLY	10.77±0.15^{b}	18.27±0.38^{a}	****	20.36±0.41^{b}	31.43±0.11^{a}	****	58.27±0.36^{b}	75.32±0.16^{a}	****
ASN	3.16±0.13^{b}	7.42±0.15^{a}	****	9.02±0.61^{b}	10.48±0.31^{a}	*	22.30±0.67^{b}	26.60±0.09^{a}	**
ASP	2.11±0.03^{b}	3.52±0.15^{a}	***	5.58±0.13^{b}	6.52±0.38^{a}	*	37.10±0.36^{b}	39.51±0.92^{a}	*
GLU	7.57±0.07^{b}	14.39±0.38^{a}	****	7.66±0.30^{b}	14.57±0.09^{a}	****	21.98±0.03^{b}	53.18±0.58^{a}	****
GLN	43.91±0.31^{b}	57.20±0.16^{a}	****	41.87±0.07^{b}	57.54±0.24^{a}	****	165.10±0.63^{b}	145.12±0.27^{a}	****
ORN	1.55±0.04^{b}	2.33±0.29^{a}	*	5.92±0.10^{b}	5.18±0.23^{a}	*	11.57±0.48^{b}	16.04±0.71^{a}	**
TYR	4.23±0.28^{b}	6.53±0.10^{a}	***	16.33±0.23^{b}	19.78±0.81^{a}	**	298.18±0.39^{b}	435.82±0.07^{a}	****
SER	7.70±0.08^{b}	22.44±0.15^{a}	****	15.85±0.54^{b}	25.66±0.09^{a}	****	41.71±1.02^{a}	41.33±0.52^{a}	ns
PRO	4.76±0.19^{b}	8.88±0.35^{a}	***	7.53±0.36^{b}	9.73±0.39^{a}	**	14.20±0.36^{b}	16.85±0.58^{a}	**
Total FAAs	161.41±0.35^{b}	280.30±1.93^{a}	****	330.64±2.89^{b}	446.90±3.10^{a}	****	2109.39±2.93^{b}	2888.37±0.77^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 20. Concentration of FAAs (mg/100g) in New Zealand lamb (PEF knuckle) in different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

	Knuckle (PEF)								
Phase	S1			S2			S3		
Storage (days)	P0	P7	P-values	P0	P7	P-values	P0	P7	P-values
EAAs									
VAL	12.06±0.09^{b}	12.21±0.32^{a}	ns	12.23±0.28^{b}	18.55±0.27^{a}	****	37.64±0.17^{b}	38.52±0.23^{a}	*
LEU	18.12±0.02^{b}	23.23±0.29^{a}	****	53.74±0.02^{b}	60.82±0.08^{a}	****	430.12±0.08^{b}	449.49±0.25^{a}	****
ILE	14.09±0.04^{b}	18.03±0.5^{a}	***	14.46±0.16^{b}	20.40±0.38^{a}	****	98.79±0.39^{b}	113.65±0.34^{a}	****
MET	5.80±0.17^{b}	6.97±0.07^{a}	**	25.13±0.13^{b}	29.69±0.41^{a}	***	103.63±0.31^{b}	108.19±0.11^{a}	****
PHE	13.07±0.22^{b}	15.73±0.36^{a}	**	68.77±0.32^{b}	73.91±0.18^{a}	****	439.02±0.55^{b}	475.99±0.62^{a}	****
LYS	6.93±0.12^{b}	8.91±0.19^{a}	***	19.41±0.77^{b}	27.39±0.11^{a}	***	347.71±0.64^{b}	421.45±0.39^{a}	****
HIS	12.98±0.04^{b}	11.34±0.15^{a}	***	22.25±0.15^{b}	23.38±0.13^{a}	**	172.71±0.43^{b}	162.47±0.48^{a}	****
THR	12.77±0.02^{b}	14.78±0.17^{a}	****	17.74±0.18^{a}	18.79±0.71^{a}	ns	47.70±0.51^{b}	49.40±0.11^{a}	*
TRP	2.57±0.24^{a}	2.61±0.06^{a}	ns	10.82±0.46^{a}	11.29±0.46^{a}	ns	141.05±0.47^{b}	164.83±0.24^{a}	****
NEAAs									
ALA	43.4±0.26^{b}	46.69±0.36^{a}	***	42.94±0.62^{b}	47.69±0.02^{a}	***	64.64±0.28^{b}	66.44±0.28^{a}	**
GLY	22.73±0.10^{b}	19.71±0.03^{a}	****	35.34±0.28^{b}	33.43±0.38^{a}	**	60.97±0.62^{b}	71.21±0.12^{a}	****
ASN	3.80±0.11^{a}	3.78±0.17^{a}	ns	12.14±0.12^{b}	13.46±0.09^{a}	***	24.58±0.06^{b}	42.83±0.66^{a}	****
ASP	3.65±0.21^{b}	3.02±0.08^{a}	*	9.44±0.08^{b}	12.55±0.14^{a}	****	35.73±0.15^{b}	47.96±0.6^{a}	****
GLU	19.62±0.24^{b}	29.37±0.24^{a}	****	12.48±0.46^{b}	29.37±0.12^{a}	****	33.74±0.25^{b}	50.86±0.64^{a}	****
GLN	55.82±0.27^{b}	110.30±0.23^{a}	****	95.51±0.31^{b}	100.78±0.07^{a}	****	131.23±0.08^{b}	190.91±0.8^{a}	****
ORN	1.50±0.02^{b}	3.37±0.02^{a}	****	8.20±0.26^{a}	8.31±0.18^{a}	ns	12.81±0.16^{b}	15.77±0.37^{a}	***
TYR	8.35±0.04^{b}	7.06±0.20^{a}	**	24.42±0.27^{a}	24.44±0.21^{a}	ns	392.32±0.31^{b}	413.47±0.40^{a}	****
SER	19.03±0.16^{b}	25.01±0.34^{a}	****	25.22±0.13^{b}	27.80±0.11^{a}	****	40.45±0.14^{b}	47.09±0.06^{a}	****
PRO	8.29±0.05^{b}	10.20±0.15^{a}	****	12.85±0.18^{b}	12.17±0.23^{a}	*	17.67±0.12^{a}	17.45±0.09^{a}	ns
Total FAAs	284.58±0.75^{b}	372.32±0.91^{a}	****	523.10±1.18^{b}	594.23±0.95^{a}	****	2632.51±0.97^{b}	2947.98±1.14^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 21. Concentration of FAAs (mg/100g) in New Zealand lamb (flat) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

Flat (non-PEF)									
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	7.79±0.16^{b}	12.11±0.31^{a}	****	12.97±0.61^{a}	12.87±1.36^{a}	ns	30.20±0.87^{b}	42.79±0.41^{a}	****
LEU	17.00±0.21^{b}	25.76±0.08^{a}	****	39.57±0.67^{b}	41.68±0.42^{a}	*	205.07±0.81^{b}	271.02±0.26^{a}	****
ILE	12.17±0.15^{b}	15.20±0.04^{a}	****	19.84±0.69^{a}	19.01±0.42^{a}	ns	75.29±0.45^{b}	79.32±0.54^{a}	**
MET	4.44±0.16^{b}	6.64±0.14^{a}	***	15.27±0.31^{a}	14.70±0.24^{a}	ns	59.16±0.68^{b}	73.78±0.59^{a}	****
PHE	10.47±0.33^{b}	15.68±0.75^{a}	**	31.05±0.23^{a}	30.69±0.42^{a}	ns	180.39±1.08^{b}	253.03±0.17^{a}	****
LYS	9.20±0.54^{b}	12.60±0.11^{a}	**	16.46±0.39^{b}	13.84±0.21^{a}	**	142.56±0.87^{b}	191.32±0.59^{a}	****
HIS	6.62±0.31^{b}	14.39±0.14^{a}	****	13.11±0.12^{b}	16.27±0.23^{a}	****	85.89±0.25^{b}	115.77±0.55^{a}	****
THR	11.66±0.65^{b}	16.04±0.31^{a}	**	12.22±0.82^{a}	14.44±0.80^{a}	ns	25.98±0.46^{b}	33.63±0.08^{a}	****
TRP	0.83±0.10^{b}	1.84±0.24^{a}	**	8.43±0.79^{b}	8.24±0.42^{a}	ns	54.63±0.70^{b}	83.14±0.43^{a}	****
NEAAs									
ALA	30.35±0.71^{b}	33.51±0.37^{a}	**	42.48±0.24^{a}	43.06±0.24^{a}	ns	41.86±0.65^{b}	47.85±0.42^{a}	***
GLY	14.54±0.15^{b}	17.58±0.26^{a}	***	19.89±1.13^{a}	20.68±0.42^{a}	ns	40.59±0.67^{b}	44.83±0.81^{a}	**
ASN	7.76±0.52^{b}	9.98±0.32^{a}	**	8.74±0.50^{b}	17.98±0.25^{a}	****	34.06±0.54^{a}	33.39±0.40^{a}	ns
ASP	1.65±0.31^{a}	1.16±0.03^{a}	ns	7.08±1.59^{a}	7.51±0.99^{a}	ns	11.01±0.73^{a}	10.34±0.20^{a}	ns
GLU	5.37±0.24^{b}	10.27±0.25^{a}	****	13.38±0.43^{b}	10.46±0.29^{a}	**	25.78±0.17^{b}	42.31±0.38^{a}	****
GLN	53.55±0.54^{a}	52.73±0.47^{a}	ns	35.58±0.63^{a}	36.40±1.06^{a}	ns	99.23±0.25^{b}	138.39±0.33^{a}	****
ORN	1.90±0.40^{a}	2.84±0.29^{a}	ns	1.74±0.75^{a}	3.38±0.82^{a}	ns	7.14±0.88^{b}	15.47±0.35^{a}	***
TYR	5.91±0.43^{b}	6.84±0.18^{a}	*	14.54±0.81^{b}	14.23±0.22^{a}	ns	144.67±0.48^{b}	194.91±0.64^{a}	****
SER	15.11±0.54^{b}	32.55±0.3^{a}	****	22.74±0.38^{b}	26.34±0.38^{a}	**	28.51±0.36^{b}	24.31±0.19^{a}	***
PRO	5.12±0.26^{b}	7.60±0.22^{a}	**	7.65±0.27^{b}	11.53±0.79^{a}	**	13.53±0.65^{b}	20.96±0.27^{a}	***
Total FAAs	221.46±1.20^{b}	295.31±2.33^{a}	****	342.73±2.31^{b}	363.29±2.29^{a}	*	1305.55±1.18^{b}	1716.57±1.99^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 22. Concentration of FAAs (mg/100g) in New Zealand lamb (PEF flat) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

Flat (PEF)									
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	9.73±0.16^{b}	17.67±0.22^{a}	****	14.54±0.37^{b}	20.42±0.56^{a}	***	37.89±0.72^{b}	48.08±0.38^{a}	****
LEU	9.87±0.22^{b}	22.96±0.36^{a}	****	39.34±0.47^{b}	36.88±0.22^{a}	**	295.61±0.51^{b}	328.01±0.32^{a}	****
ILE	9.68±0.34^{b}	18.35±0.14^{a}	****	12.24±0.32^{b}	22.75±1.04^{a}	***	82.12±0.42^{b}	83.70±0.53^{a}	*
MET	3.19±0.05^{b}	7.31±0.13^{a}	****	14.14±0.46^{b}	12.54±0.04^{a}	**	72.95±0.16^{a}	72.48±0.35^{a}	ns
PHE	7.86±0.50^{b}	14.95±0.25^{a}	****	36.51±0.37^{a}	37.83±0.56^{a}	ns	268.81±1.45^{a}	270.97±0.27^{a}	ns
LYS	7.70±0.08^{b}	20.79±0.13^{a}	****	22.34±0.22^{b}	25.18±0.67^{a}	**	232.66±0.61^{b}	229.60±0.43^{a}	**
HIS	11.34±0.17^{b}	18.80±0.15^{a}	****	18.81±0.31^{b}	21.85±0.77^{a}	**	117.03±0.49^{b}	122.37±0.66^{a}	**
THR	12.06±0.08^{b}	19.89±0.30^{a}	****	14.40±1.29^{b}	22.01±0.61^{a}	**	26.58±0.31^{b}	41.02±0.46^{a}	****
TRP	1.34±0.09^{a}	1.29±0.03^{a}	ns	5.64±0.54^{a}	7.02±0.45^{a}	ns	97.49±1.49^{b}	86.08±0.31^{a}	***
NEAAs									
ALA	24.43±0.31^{b}	41.29±0.36^{a}	****	38.87±0.63^{b}	55.65±0.36^{a}	****	51.68±0.49^{b}	56.87±0.34^{a}	***
GLY	22.99±0.22^{b}	33.09±0.22^{a}	****	37.82±0.58^{b}	31.67±0.84^{a}	**	46.13±0.22^{b}	49.91±0.55^{a}	**
ASN	6.93±0.26^{b}	12.00±0.18^{a}	****	11.69±0.50^{b}	14.02±0.31^{a}	**	49.54±0.99^{b}	36.37±0.96^{a}	***
ASP	2.44±0.13^{b}	3.90±0.31^{a}	**	10.24±0.22^{b}	12.27±0.82^{a}	*	10.84±0.68^{a}	10.83±0.57^{a}	ns
GLU	2.54±0.02^{b}	6.72±0.05^{a}	****	3.88±0.12^{b}	8.72±0.15^{a}	****	35.89±0.13^{b}	51.86±0.48^{a}	****
GLN	46.42±0.38^{b}	68.78±0.31^{a}	****	43.89±0.37^{b}	40.83±0.58^{a}	**	128.67±2.04^{b}	151.11±0.52^{a}	***
ORN	2.57±0.04^{b}	5.45±0.27^{a}	***	5.56±0.36^{b}	3.14±0.76^{a}	*	7.53±0.84^{a}	7.76±0.38^{a}	ns
TYR	7.19±0.16^{b}	8.59±0.05^{a}	***	15.42±0.19^{a}	14.62±0.55^{a}	ns	231.34±0.38^{b}	213.77±0.43^{a}	****
SER	23.98±0.04^{b}	34.96±0.23^{a}	****	31.76±0.65^{b}	44.01±0.97^{a}	***	15.62±0.16^{b}	57.88±0.42^{a}	****
PRO	9.96±0.19^{b}	26.87±0.14^{a}	****	14.26±0.28^{b}	20.59±0.41^{a}	****	14.03±0.71^{b}	19.45±1.03^{a}	**
Total FAAs	222.23±1.18^{b}	383.67±1.63^{a}	****	391.35±2.80^{b}	451.99±1.26^{a}	****	1822.42±2.61^{b}	1938.12±0.65^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 23. Concentration of FAAs (mg/100g) in New Zealand lamb (shanks) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

Cuts	Shanks (non-PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	9.97±0.11^{b}	11.91±0.51^{a}	**	11.45±0.76^{a}	13.14±1.04^{a}	ns	42.93±0.61^{b}	45.71±0.82^{a}	*
LEU	8.07±0.13^{b}	13.96±0.26^{a}	****	33.45±0.16^{b}	39.91±1.77^{a}	**	275.90±0.47^{b}	399.54±0.8^{a}	****
ILE	7.40±0.08^{b}	9.16±0.36^{a}	**	12.61±0.24^{b}	18.48±0.99^{a}	**	77.90±0.65^{b}	82.05±0.55^{a}	**
MET	2.98±0.65^{b}	5.18±0.10^{a}	**	12.45±1.15^{b}	16.90±0.56^{a}	**	91.20±0.93^{b}	98.82±0.52^{a}	**
PHE	7.58±0.15^{b}	10.8±0.09^{a}	****	28.61±0.10^{b}	33.68±0.49^{a}	***	254.01±0.09^{b}	341.17±0.25^{a}	****
LYS	5.57±0.35^{b}	11.35±0.24^{a}	****	15.78±0.41^{b}	19.63±0.73^{a}	**	237.70±0.63^{b}	272.66±0.42^{a}	****
HIS	8.90±0.22^{b}	14.02±0.20^{a}	****	13.36±0.33^{b}	18.10±0.56^{a}	***	145.88±0.62^{a}	144.40±0.61^{a}	ns
THR	6.31±0.17^{b}	15.40±0.26^{a}	****	13.77±0.24^{b}	19.54±0.23^{a}	****	31.11±0.60^{b}	35.32±0.25^{a}	**
TRP	0.66±0.02^{b}	1.29±0.07^{a}	***	4.84±0.60^{a}	5.93±0.36^{a}	ns	103.05±0.67^{b}	122.09±0.70^{a}	****
NEAAs									
ALA	30.90±0.19^{b}	42.61±0.23^{a}	****	35.59±0.27^{b}	43.69±0.53^{a}	****	59.39±0.16^{b}	61.84±0.46^{a}	**
GLY	31.25±0.06^{b}	27.69±0.44^{a}	***	30.55±0.02^{b}	36.11±1.12^{a}	**	52.42±0.80^{a}	53.74±1.04^{a}	ns
ASN	4.25±0.02^{b}	7.26±0.10^{a}	****	10.83±0.26^{b}	13.25±0.44^{a}	**	15.66±0.31^{b}	41.42±0.18^{a}	****
ASP	1.93±0.32^{b}	9.09±0.17^{a}	****	10.05±0.84^{b}	13.60±0.21^{a}	**	12.08±0.46^{b}	18.48±0.60^{a}	***
GLU	3.51±0.04^{b}	5.89±0.01^{a}	****	7.34±0.13^{b}	11.18±0.25^{a}	****	24.35±0.86^{b}	33.06±0.27^{a}	***
GLN	45.44±0.35^{b}	63.16±0.41^{a}	****	58.10±0.08^{b}	78.91±0.24^{a}	****	144.81±0.37^{b}	178.05±0.48^{a}	****
ORN	2.48±0.32^{b}	4.56±0.40^{a}	**	3.39±0.90^{b}	6.16±0.28^{a}	*	5.76±0.10^{b}	6.65±0.11^{a}	**
TYR	4.51±0.41^{b}	6.23±0.05^{a}	**	13.05±0.28^{b}	15.19±0.12^{a}	**	234.22±0.40^{b}	262.86±0.35^{a}	****
SER	9.52±0.18^{b}	26.95±0.44^{a}	****	20.81±0.22^{b}	43.02±0.58^{a}	****	20.59±0.25^{b}	30.49±0.33^{a}	****
PRO	14.42±0.24^{b}	18.10±0.48^{a}	**	12.35±0.79^{b}	19.66±1.13^{a}	**	16.97±0.46^{a}	18.73±0.86^{a}	ns
Total FAAs	205.66±1.19^{b}	304.63±0.76^{a}	****	348.38±3.05^{b}	466.07±2.01^{a}	****	1845.93±1.89^{b}	2247.09±2.9^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 24. Concentration of FAAs (mg/100g) in New Zealand lamb (PEF shanks) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

Cuts	Shank (PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	5.23±0.44^{b}	12.53±0.91^{a}	**	11.95±0.56^{b}	15.40±0.22^{a}	**	48.62±1.32^{b}	52.98±0.32^{a}	*
LEU	9.51±0.58^{b}	16.65±1.33^{a}	**	38.28±1.26^{b}	49.76±0.07^{a}	***	337.73±0.46^{b}	422.96±0.41^{a}	****
ILE	7.26±0.24^{b}	11.34±0.21^{a}	****	9.95±0.50^{b}	21.82±0.72^{a}	****	105.58±0.64^{b}	200.42±0.20^{a}	****
MET	2.50±0.11^{b}	5.41±0.37^{a}	***	13.95±0.63^{b}	18.38±1.49^{a}	*	93.76±0.79^{b}	136.38±1.52^{a}	****
PHE	6.70±0.51^{b}	11.17±0.10^{a}	***	33.11±0.95^{b}	42.67±0.29^{a}	***	302.06±0.43^{b}	437.27±1.01^{a}	****
LYS	5.18±0.35^{b}	12.62±0.31^{a}	****	15.62±0.95^{b}	23.80±0.42^{a}	***	243.24±0.56^{b}	312.01±0.56^{a}	****
HIS	7.79±0.49^{b}	14.24±0.77^{a}	**	15.46±0.18^{b}	18.64±0.24^{a}	***	162.60±1.06^{b}	221.96±0.66^{a}	****
THR	10.69±0.35^{b}	16.74±1.47^{a}	**	12.63±0.66^{b}	19.23±0.89^{a}	**	38.40±0.36^{b}	44.22±0.74^{a}	**
TRP	1.18±0.06^{a}	1.28±0.06^{a}	ns	6.85±0.43^{b}	6.84±0.45^{a}	ns	99.47±1.01^{b}	171.95±0.36^{a}	****
NEAAs									
ALA	36.82±0.39^{b}	44.96±0.24^{a}	****	42.77±1.49^{b}	52.81±1.53^{a}	**	72.15±0.51^{b}	82.62±0.49^{a}	****
GLY	17.62±0.30^{b}	28.69±0.53^{a}	****	26.37±0.76^{b}	40.51±0.32^{a}	****	71.00±0.58^{b}	75.81±0.55^{a}	**
ASN	6.77±0.13^{b}	13.65±0.15^{a}	****	22.32±0.39^{b}	20.19±0.70^{a}	*	25.76±0.97^{b}	43.69±0.38^{a}	****
ASP	1.80±0.14^{b}	7.03±0.33^{a}	****	12.52±0.13^{a}	13.05±0.56^{a}	ns	12.15±0.45^{a}	11.23±0.92^{a}	ns
GLU	4.53±0.04^{b}	7.87±0.02^{a}	****	8.46±0.71^{b}	15.32±0.22^{a}	***	15.47±0.12^{b}	71.02±0.47^{a}	****
GLN	73.54±0.85^{b}	76.37±0.29^{a}	*	90.49±0.25^{b}	93.60±0.46^{a}	**	161.09±0.55^{b}	221.20±0.65^{a}	****
ORN	2.56±0.23^{b}	4.44±0.27^{a}	**	5.42±0.35^{a}	6.14±0.43^{a}	ns	6.34±0.34^{b}	14.16±1.10^{a}	**
TYR	4.59±0.34^{b}	6.75±0.39^{a}	**	15.56±0.15^{b}	17.37±0.19^{a}	***	273.68±1.33^{b}	386.97±0.68^{a}	****
SER	11.01±0.18^{b}	32.95±0.28^{a}	****	24.48±0.23^{b}	31.36±0.14^{a}	****	33.18±1.27^{b}	51.73±0.57^{a}	****
PRO	5.87±0.57^{b}	17.71±0.51^{a}	****	9.84±0.55^{b}	19.42±0.23^{a}	****	19.77±1.14^{b}	27.73±0.86^{a}	**
Total FAAs	221.16±0.73^{b}	342.40±3.82^{a}	****	416.00±2.77^{b}	526.29±2.58^{a}	****	2122.04±0.56^{b}	2986.31±1.95^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 25. Concentration of FAAs (mg/100g) in New Zealand lamb (bolar) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

	Bolar (non-PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	4.77±0.16^{b}	7.11±0.27^{a}	***	10.79±0.51^{b}	12.78±0.39^{a}	*	38.53±0.25^{a}	39.09±0.45^{a}	ns
LEU	6.42±0.35^{b}	11.51±0.36^{a}	***	40.91±1.20^{b}	52.22±0.49^{a}	***	275.76±0.38^{b}	333.25±0.76^{a}	****
ILE	5.12±0.90^{a}	7.27±1.79^{a}	ns	14.26±0.97^{a}	15.65±0.30^{a}	ns	41.96±0.40^{b}	68.22±0.45^{a}	****
MET	1.79±0.10^{b}	4.05±0.31^{a}	**	15.22±0.25^{b}	18.60±0.37^{a}	***	91.92±0.76^{b}	78.28±0.44^{a}	****
PHE	5.48±0.16^{b}	11.10±0.43^{a}	****	35.43±0.38^{b}	47.05±0.97^{a}	****	311.97±0.69^{b}	334.57±0.39^{a}	****
LYS	5.77±0.16^{b}	7.88±0.43^{a}	**	15.43±0.84^{b}	19.94±0.44^{a}	**	281.56±0.34^{b}	292.50±0.43^{a}	****
HIS	8.41±0.04^{b}	10.41±0.02^{a}	****	14.13±0.33^{b}	20.02±0.51^{a}	***	129.96±0.55^{b}	114.07±0.59^{a}	****
THR	6.03±0.15^{b}	10.02±0.15^{a}	****	14.06±0.88^{b}	18.51±0.34^{a}	**	21.08±0.68^{b}	23.28±0.49^{a}	*
TRP	1.37±0.07^{b}	1.69±0.02^{a}	**	5.84±0.34^{b}	7.02±0.32^{a}	*	101.69±0.55^{a}	102.95±1.23^{a}	ns
NEAAs									
AlA	32.00±0.62^{b}	47.82±0.03^{a}	****	35.60±0.67^{b}	51.80±0.90^{a}	****	54.69±0.41^{a}	53.62±0.56^{a}	ns
GLY	15.62±0.72^{b}	15.57±0.33^{a}	ns	26.54±0.35^{b}	29.60±0.36^{a}	**	49.64±0.57^{b}	53.63±0.31^{a}	**
ASN	4.44±0.01^{b}	9.66±0.12^{a}	****	12.73±0.96^{a}	13.35±0.39^{a}	ns	20.48±0.21^{b}	45.30±0.01^{a}	****
ASP	1.64±0.05^{b}	2.27±0.13^{a}	**	10.92±0.03^{b}	9.99±0.45^{a}	*	28.81±0.57^{b}	33.08±0.23^{a}	**
GLU	3.57±0.04^{b}	6.45±0.13^{a}	****	4.98±0.02^{b}	9.48±1.07^{a}	**	24.01±0.47^{b}	32.62±0.35^{a}	****
GLN	76.74±0.57^{a}	76.39±0.45^{a}	ns	76.00±0.89^{b}	71.97±0.35^{a}	**	126.14±0.76^{b}	173.45±0.50^{a}	****
ORN	3.29±0.17^{b}	6.63±0.10^{a}	****	5.25±0.23^{b}	10.79±0.46^{a}	***	7.17±0.97^{a}	8.63±0.13^{a}	ns
TYR	3.28±0.17^{a}	3.32±0.24^{a}	ns	14.25±0.73^{b}	17.49±0.56^{a}	**	262.74±0.42^{b}	323.13±0.59^{a}	****
SER	11.49±0.8^{b}	25.35±0.09^{a}	****	29.07±0.69^{b}	51.01±0.11^{a}	****	25.68±0.07^{b}	24.41±0.29^{a}	**
PRO	4.43±0.05^{b}	6.36±0.48^{a}	**	10.02±0.80^{b}	14.52±0.27^{a}	**	10.00±0.64^{b}	13.94±0.34^{a}	**
Total FAAs	201.64±1.03^{b}	270.87±1.05^{a}	****	391.43±2.89^{b}	491.78±1.59^{a}	****	1903.78±1.40^{b}	2148.04±1.81^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 26. Concentration of FAAs (mg/100g) in New Zealand lamb (PEF bolar) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

Cuts	Bolar (PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	6.78±0.30^{b}	12.45±0.35^{a}	****	8.75±0.30^{b}	12.97±0.25^{a}	***	41.79±0.23^{b}	62.94±0.54^{a}	****
LEU	6.00±0.57^{b}	12.91±0.20^{a}	****	28.23±0.56^{b}	41.27±0.68^{a}	****	341.76±0.36^{b}	475.97±0.15^{a}	****
ILE	7.35±2.33^{a}	10.87±5.54^{a}	ns	11.89±0.62^{b}	15.17±0.78^{a}	**	39.72±0.78^{b}	72.36±0.31^{a}	****
MET	1.75±0.12^{b}	3.33±0.04^{a}	****	12.19±0.47^{b}	17.49±0.15^{a}	***	89.15±1.38^{b}	127.45±0.55^{a}	****
PHE	4.99±0.26^{b}	9.68±0.05^{a}	****	23.70±0.38^{b}	38.60±0.23^{a}	****	352.14±0.67^{b}	448.07±0.50^{a}	****
LYS	2.38±0.35^{b}	3.91±0.03^{a}	**	13.58±0.65^{a}	15.07±0.53^{a}	ns	308.04±0.66^{b}	319.55±0.40^{a}	****
HIS	6.41±0.21^{b}	8.92±0.22^{a}	***	14.31±0.21^{b}	18.55±0.27^{a}	****	121.53±0.6^{b}	192.30±0.49^{a}	****
THR	7.32±0.11^{b}	17.44±0.16^{a}	****	12.38±0.94^{b}	17.61±0.14^{a}	**	31.11±0.39^{b}	62.26±0.41^{a}	****
TRP	0.96±0.03^{b}	1.91±0.11^{a}	***	5.10±0.42^{a}	6.31±0.65^{a}	ns	99.88±0.82^{b}	148.87±0.32^{a}	****
NEAAs									
ALA	30.23±0.52^{b}	44.58±0.08^{a}	****	33.09±1.05^{b}	53.70±0.05^{a}	****	68.21±0.45^{b}	76.16±0.15^{a}	****
GLY	15.06±0.59^{b}	19.12±0.23^{a}	**	22.18±1.06^{b}	35.14±0.25^{a}	****	67.07±1.21^{b}	76.10±0.31^{a}	**
ASN	2.77±0.06^{b}	3.30±0.13^{a}	**	13.46±0.26^{b}	17.18±0.34^{a}	***	23.33±0.99^{b}	57.83±0.29^{a}	****
ASP	3.82±0.08^{b}	3.59±0.06^{a}	*	12.25±0.55^{a}	11.11±0.52^{a}	ns	30.54±0.36^{b}	38.87±0.54^{a}	****
GLU	4.78±0.04^{b}	7.36±0.12^{a}	****	6.52±0.05^{b}	13.13±0.54^{a}	****	25.96±0.69^{b}	37.75±0.37^{a}	****
GLN	68.92±0.35^{b}	94.01±0.11^{a}	****	68.52±0.36^{b}	102.07±0.56^{a}	****	204.96±0.54^{b}	249.82±0.64^{a}	****
ORN	4.21±0.35^{b}	5.41±0.34^{a}	**	5.08±0.63^{b}	10.03±0.56^{a}	**	9.69±0.18^{b}	14.10±0.62^{a}	**
TYR	2.82±0.22^{b}	3.95±0.15^{a}	**	10.80±0.13^{b}	12.62±0.38^{a}	**	333.79±0.34^{b}	398.21±0.45^{a}	****
SER	7.64±0.78^{b}	22.50±0.34^{a}	****	24.92±0.38^{b}	48.17±0.25^{a}	****	40.10±1.34^{b}	47.77±0.67^{a}	**
PRO	6.93±0.32^{b}	11.31±0.36^{a}	****	7.24±0.03^{b}	15.42±0.48^{a}	****	16.37±0.21^{b}	29.86±0.74^{a}	****
Total FAAs	191.14±2.06^{b}	296.57±2.86^{a}	****	334.19±1.73^{b}	501.62±0.99^{a}	****	2245.17±0.84^{b}	2936.24±2.04^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 27. Concentration of FAAs (mg/100g) in New Zealand lamb (full back) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

	Full back (non-PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	8.39±0.42^{b}	9.54±0.23^{a}	*	13.60±0.28^{b}	11.02±0.66^{a}	**	34.83±0.78^{a}	35.25±0.77^{a}	ns
LEU	12.55±0.21^{b}	21.78±0.60^{a}	****	34.06±0.27^{a}	34.69±0.28^{a}	ns	325.42±1.05^{a}	327.83±1.15^{a}	ns
ILE	18.70±0.19^{b}	19.77±0.04^{a}	**	20.26±0.15^{b}	10.80±0.67^{a}	****	35.84±0.79^{a}	36.69±0.40^{a}	ns
MET	3.37±0.12^{b}	4.79±0.08^{a}	***	15.13±0.07^{a}	15.40±0.34^{a}	ns	76.62±0.53^{b}	82.83±0.33^{a}	***
PHE	6.79±0.39^{b}	15.14±0.41^{a}	****	28.85±0.32^{b}	38.59±1.10^{a}	***	330.87±0.72^{b}	335.42±0.52^{a}	**
LYS	3.47±0.05^{b}	10.08±0.41^{a}	****	11.57±0.14^{b}	12.99±0.64^{a}	*	285.53±1.03^{b}	318.84±0.55^{a}	****
HIS	4.40±0.23^{b}	8.50±0.68^{a}	**	12.86±0.22^{a}	13.15±0.07^{a}	ns	119.49±1.36^{a}	120.03±0.80^{a}	ns
THR	9.34±0.21^{b}	13.69±0.20^{a}	****	9.99±0.25^{b}	13.07±0.31^{a}	***	25.95±0.52^{a}	27.05±0.55^{a}	ns
TRP	1.90±0.29^{b}	3.03±0.22^{a}	*	7.00±0.60^{a}	8.02±0.60^{a}	ns	117.03±0.32^{a}	117.52±1.10^{a}	ns
NEAAs									
ALA	28.04±0.02^{b}	33.87±0.16^{a}	****	25.55±0.61^{b}	29.37±0.50^{a}	**	47.48±0.32^{b}	48.74±0.50^{a}	*
GLY	18.81±0.24^{b}	17.36±0.19^{a}	**	18.50±0.42^{b}	19.45±0.17^{a}	*	51.91±0.07^{b}	53.10±0.14^{a}	***
ASN	7.81±0.13^{b}	12.55±0.41^{a}	***	7.09±0.66^{b}	4.09±0.64^{a}	*	18.47±0.20^{b}	42.63±0.31^{a}	****
ASP	5.71±0.10^{b}	6.47±0.14^{a}	**	6.44±0.05^{a}	6.83±0.47^{a}	ns	25.78±0.45^{b}	28.53±0.05^{a}	**
GLU	8.19±0.29^{b}	17.71±0.11^{a}	****	7.93±0.24^{b}	5.98±0.06^{a}	***	37.28±0.42^{a}	36.00±0.61^{a}	ns
GLN	41.31±0.53^{b}	52.67±0.51^{a}	****	27.73±0.50^{b}	36.15±0.51^{a}	****	115.5±0.94^{b}	148.01±0.72^{a}	****
ORN	3.86±0.12^{b}	5.54±0.26^{a}	**	2.93±0.71^{a}	2.84±0.61^{a}	ns	8.49±0.27^{a}	8.73±0.18^{a}	ns
TYR	5.35±0.25^{a}	5.20±0.26^{a}	ns	12.52±0.34^{b}	14.70±0.33^{a}	**	320.30±0.98^{b}	342.71±0.40^{a}	****
SER	14.06±0.33^{b}	34.96±0.08^{a}	****	14.62±0.59^{b}	27.66±0.52^{a}	****	23.28±0.58^{a}	24.34±0.54^{a}	ns
PRO	4.82±0.35^{b}	6.52±0.21^{a}	**	8.07±0.56^{a}	7.36±0.16^{a}	ns	9.07±0.60^{a}	9.56±0.39^{a}	ns
Total FAAs	206.88±0.82^{b}	299.18±0.89^{a}	****	284.71±2.03^{b}	312.18±2.69^{a}	***	2009.14±2.79^{b}	2143.81±2.91^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 28: Concentration of FAAs (mg/100g) in New Zealand lamb (PEF full back) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

Cuts	Full back (PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	13.81±0.15^{b}	18.34±0.78^{a}	**	9.29±0.51^{b}	14.97±0.27^{a}	***	37.99±0.35^{b}	40.75±0.67^{a}	**
LEU	14.79±0.26^{b}	26.42±0.40^{a}	****	34.69±0.44^{b}	46.9±0.50^{a}	****	321.76±0.41^{b}	355.41±0.68^{a}	****
ILE	14.97±0.09^{b}	19.48±0.21^{a}	****	11.02±0.45^{b}	17.22±0.22^{a}	****	42.06±1.05^{b}	61.98±0.02^{a}	****
MET	5.10±0.19^{b}	9.67±0.09^{a}	****	14.39±0.83^{b}	18.47±0.45^{a}	**	85.87±0.36^{a}	85.30±0.83^{a}	ns
PHE	13.93±0.33^{a}	15.07±0.70^{a}	ns	30.01±0.98^{b}	40.57±0.67^{a}	***	312.13±1.47^{b}	403.17±0.62^{a}	****
LYS	4.74±0.03^{b}	7.81±0.07^{a}	****	12.58±0.88^{b}	20.86±0.58^{a}	***	291.23±0.59^{b}	347.83±0.67^{a}	****
HIS	16.04±0.24^{b}	11.28±0.49^{a}	***	11.92±0.05^{b}	21.91±0.34^{a}	****	114.41±0.80^{b}	138.48±0.43^{a}	****
THR	15.28±0.54^{b}	30.04±0.28^{a}	****	22.54±0.45^{a}	22.28±0.76^{a}	ns	26.79±0.38^{a}	25.81±0.52^{a}	ns
TRP	2.06±0.30^{b}	4.64±0.08^{a}	***	5.26±0.24^{b}	7.90±0.26^{a}	***	98.99±0.41^{b}	152.86±0.37^{a}	****
NEAAs									
ALA	37.00±0.29^{b}	42.30±0.20^{a}	****	34.17±0.50^{b}	45.51±0.26^{a}	****	51.22±0.43^{b}	54.93±1.22^{a}	*
GLY	19.37±0.50^{b}	36.38±0.17^{a}	****	20.98±0.64^{b}	44.55±0.23^{a}	****	53.97±0.46^{b}	65.5±0.52^{a}	****
ASN	12.86±0.25^{b}	21.38±0.19^{a}	****	19.98±0.29^{b}	12.89±0.44^{a}	****	20.75±0.35^{b}	54.64±0.58^{a}	****
ASP	2.49±0.16^{b}	3.18±0.28^{a}	*	12.65±0.36^{b}	8.77±0.41^{a}	**	32.14±0.17^{b}	40.89±0.25^{a}	****
GLU	8.57±0.05^{b}	14.44±0.74^{a}	***	11.17±0.90^{b}	19.09±0.44^{a}	***	30.95±0.58^{b}	38.16±0.30^{a}	****
GLN	47.60±0.26^{b}	68.04±0.21^{a}	****	54.37±0.72^{b}	49.68±0.73^{a}	**	132.82±0.48^{b}	142.95±0.22^{a}	****
ORN	2.50±0.01^{b}	4.22±0.16^{a}	****	3.79±0.19^{a}	4.16±0.11^{a}	ns	7.45±0.27^{b}	10.04±0.40^{a}	**
TYR	10.94±0.53^{b}	7.60±0.24^{a}	**	15.20±0.65^{b}	22.75±0.57^{a}	***	292.19±0.39^{b}	408.68±0.30^{a}	****
SER	21.25±0.19^{b}	53.20±0.50^{a}	****	31.23±0.61^{a}	30.80±0.47^{a}	ns	25.99±0.49^{b}	35.72±0.40^{a}	****
PRO	8.05±0.37^{b}	26.92±0.15^{a}	****	6.87±0.73^{b}	19.18±0.82^{a}	****	10.88±1.47^{b}	17.59±0.77^{a}	**
Total FAAs	271.36±1.09^{b}	420.41±2.53^{a}	****	362.12±2.31^{b}	468.45±3.02^{a}	****	1989.61±2.7^{b}	2480.67±3.13^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 29: Concentration of FAAs (mg/100g) in New Zealand lamb (cube roll) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

Cube roll (non-PEF)									
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	5.17±0.39^{b}	10.11±0.32^{a}	***	12.70±0.70^{b}	16.32±0.20^{a}	**	35.71±0.23^{a}	36.07±0.40^{a}	ns
LEU	7.69±0.23^{b}	10.26±0.52^{a}	**	32.86±0.54^{b}	34.57±0.32^{a}	*	351.09±0.39^{b}	371.68±0.79^{a}	****
ILE	7.71±0.20^{b}	9.54±0.19^{a}	**	11.05±0.74^{b}	24.11±0.32^{a}	****	46.06±0.62^{a}	45.15±0.61^{a}	ns
MET	2.67±0.48^{a}	3.46±0.20^{a}	ns	8.74±0.30^{b}	14.31±0.57^{a}	***	91.12±0.42^{b}	96.12±0.58^{a}	**
PHE	4.20±0.24^{b}	9.1±0.31^{a}	****	33.99±0.42^{a}	33.88±0.68^{a}	ns	384.72±0.22^{b}	421.26±0.13^{a}	****
LYS	7.16±0.28^{b}	5.09±0.38^{a}	**	12.79±0.54^{b}	15.64±0.36^{a}	**	308.77±0.40^{b}	339.01±0.53^{a}	****
HIS	7.99±0.26^{b}	14.21±0.66^{a}	***	12.83±0.31^{a}	11.52±0.68^{a}	ns	145.56±0.37^{b}	141.13±0.54^{a}	**
THR	16.08±0.62^{b}	17.76±0.25^{a}	*	10.76±0.43^{b}	13.13±0.28^{a}	**	30.42±0.75^{b}	26.7±0.56^{a}	**
TRP	3.05±0.19^{b}	2.59±0.01^{a}	*	7.48±0.17^{b}	6.23±0.35^{a}	*	137.68±0.42^{b}	150.16±0.77^{a}	****
NEAAs									
ALA	28.20±0.79^{b}	45.26±0.49^{a}	****	23.66±0.18^{b}	34.47±0.52^{a}	****	57.14±0.08^{b}	63.67±0.37^{a}	****
GLY	18.82±0.15^{b}	26.93±0.34^{a}	****	25.63±0.33^{b}	27.30±0.20^{a}	**	58.20±0.49^{b}	69.7±0.21^{a}	****
ASN	5.38±0.38^{b}	7.49±0.02^{a}	**	7.16±0.06^{b}	11.77±0.08^{a}	****	15.57±0.24^{b}	21.39±0.25^{a}	****
ASP	5.37±0.15^{b}	5.99±0.17^{a}	*	10.09±0.62^{a}	9.93±0.64^{a}	ns	15.78±0.78^{b}	39.85±0.56^{a}	****
GLU	4.65±0.27^{b}	14.13±0.23^{a}	****	7.52±0.03^{b}	22.75±0.13^{a}	****	21.63±0.15^{b}	35.45±0.48^{a}	****
GLN	51.11±0.47^{b}	62.37±0.83^{a}	****	92.76±0.52^{b}	112.46±0.22^{a}	****	176.03±1.28^{a}	187.43±0.75^{a}	ns
ORN	3.27±0.13^{b}	5.15±0.23^{a}	**	4.15±0.18^{b}	9.03±0.54^{a}	***	10.50±0.05^{b}	14.56±0.42^{a}	***
TYR	5.16±0.38^{b}	2.34±0.11^{a}	**	14.76±0.13^{b}	12.39±0.75^{a}	*	338.50±0.22^{b}	369.8±0.68^{a}	****
SER	15.75±0.34^{b}	34.46±0.05^{a}	****	24.61±0.22^{b}	34.46±0.23^{a}	****	37.26±0.28^{b}	46.76±0.10^{a}	****
PRO	9.99±0.23^{b}	15.59±0.27^{a}	****	12.53±0.56^{a}	11.32±0.79^{a}	ns	14.44±0.05^{b}	17.51±0.35^{a}	***
Total FAAs	209.42±2.97^{b}	301.82±1.67^{a}	****	366.06±1.32^{b}	455.59±3.04^{a}	****	2276.19±2.37^{b}	2493.39±2.27^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant.

Table 30. Concentration of FAAs (mg/100g) in New Zealand lamb (PEF cube roll) different phases (S1, S2 and S3), for 0 day and 7 days of storage. Values are presented as Mean±S.D.

	Cube roll (PEF)								
Phase	S1			S2			S3		
Storage (days)	0 day	7 days	P-values	0 day	7 days	P-values	0 day	7 days	P-values
EAAs									
VAL	7.27±0.13^{b}	10.23±0.71^{a}	**	12.99±0.61^{a}	11.84±0.28^{a}	ns	35.37±0.45^{b}	40.76±0.50^{a}	***
LEU	5.65±0.34^{b}	15.58±0.03^{a}	****	41.88±0.54^{b}	43.51±0.32^{a}	*	348.96±0.57^{b}	364.71±0.73^{a}	****
ILE	5.35±0.42^{b}	9.98±0.35^{a}	***	11.49±0.26^{b}	15.97±0.68^{a}	**	54.01±0.59^{b}	52.51±0.15^{a}	*
MET	2.05±0.03^{b}	6.96±0.38^{a}	****	16.86±0.74^{a}	17.82±0.61^{a}	ns	86.39±0.15^{b}	90.50±0.15^{a}	****
PHE	3.84±0.34^{b}	10.28±0.24^{a}	****	32.79±0.41^{b}	39.73±1.04^{a}	**	357.30±0.51^{b}	418.98±0.75^{a}	****
LYS	3.95±0.33^{b}	6.71±0.12^{a}	***	11.77±0.94^{b}	15.73±0.26^{a}	**	329.15±0.38^{b}	320.25±0.65^{a}	****
HIS	8.19±0.14^{b}	15.14±0.22^{a}	****	12.40±0.26^{b}	21.15±0.46^{a}	****	135.64±0.22^{b}	141.03±0.44^{a}	***
THR	8.88±0.18^{b}	9.70±0.21^{a}	*	16.49±0.41^{b}	19.64±0.11^{a}	***	25.79±0.49^{a}	25.97±0.53^{a}	ns
TRP	1.29±0.11^{b}	1.62±0.07^{a}	*	6.26±0.14^{b}	6.99±0.30^{a}	*	134.05±0.52^{b}	150.73±0.39^{a}	****
NEAAs									
ALA	26.54±0.32^{b}	42.62±0.26^{a}	****	38.02±0.68^{a}	38.99±0.31^{a}	ns	51.35±0.66^{b}	58.36±0.41^{a}	***
GLY	17.18±0.15^{a}	17.11±0.16^{a}	ns	23.19±0.26^{b}	27.21±0.77^{a}	**	59.76±0.74^{b}	70.35±0.17^{a}	****
ASN	7.87±0.04^{b}	6.67±0.11^{a}	***	8.61±0.29^{b}	15.82±0.22^{a}	****	17.63±0.18^{b}	23.78±0.21^{a}	****
ASP	2.79±0.15^{b}	3.79±0.31^{a}	*	8.71±0.47^{b}	10.91±0.11^{a}	**	21.22±0.54^{b}	52.05±0.05^{a}	****
GLU	8.61±0.04^{b}	15.61±0.04^{a}	****	12.7±0.04^{b}	22.15±0.52^{a}	****	23.55±0.05^{b}	37.26±0.23^{a}	****
GLN	66.09±0.46^{b}	73.38±0.37^{a}	****	88.87±0.06^{b}	103.52±0.37^{a}	****	218.2±0.48^{b}	254.35±0.31^{a}	****
ORN	3.33±0.07^{b}	3.79±0.11^{a}	**	4.16±0.70^{b}	7.86±0.41^{a}	**	11.77±0.42^{b}	15.01±0.26^{a}	**
TYR	3.39±0.11^{a}	3.53±0.06^{a}	ns	12.58±0.94^{a}	14.39±0.55^{a}	ns	364.96±0.70^{b}	389.32±0.37^{a}	****
SER	7.80±0.12^{b}	17.48±0.14^{a}	****	25.87±0.49^{b}	37.25±0.38^{a}	****	39.56±0.08^{b}	48.17±0.14^{a}	****
PRO	6.23±0.17^{b}	9.48±0.16^{a}	****	11.48±0.57^{a}	10.30±0.29^{a}	ns	12.24±0.73^{b}	14.29±0.31^{a}	*
Total FAAs	196.29±0.93^{b}	279.67±1.43^{a}	****	397.10±3.17^{b}	480.77±2.36^{a}	****	2326.92±2.29^{b}	2568.39±1.25^{a}	****

Values with different superscripts (^{a,b,c,d,e}) in the same row differ significantly for the storage time. EAA stands for essential FAAs; NEAA stands for non-essential FAAs. 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 stands for not statistically significant

4.2.4. Physical properties of PEF treated lamb

The pH of non-PEF and PEF treated lamb were unable to be tested due to the limitation in the sample amount (25g). Moisture and color have been examined.

4.2.4.1. Moisture

Table 31. Moisture content of non-PEF and PEF treated cooked lamb.

	non-PEF			PEF		
Moisture %	0 day	7 days	P-values	0 day	7 days	P-values
Knuckle	70 ± 0.06^{cA}	65 ± 0.01^{dA}	*	87 ± 0.17^{aE}	84 ± 0.04^{bA}	*
Rump	65 ± 0.33^{bB}	87 ± 0.02^{aD}	***	58 ± 0.22^{cA}	57 ± 0.13^{dB}	ns
Flat	66 ± 0.03^{aBC}	66 ± 0.32^{aAB}	ns	65 ± 0.03^{aB}	62 ± 0.23^{bC}	*
Shanks	67 ± 0.22^{bcC}	69 ± 0.01^{aC}	*	68 ± 0.02^{abC}	66 ± 0.04^{cE}	*
Bolar	66 ± 0.01^{abBC}	67 ± 0.03^{aB}	ns	65 ± 0.01^{aB}	66 ± 0.32^{bE}	ns
Full back	65 ± 0.32^{bB}	65 ± 0.35^{bA}	ns	67 ± 0.54^{aBC}	64 ± 0.44^{cD}	**
Cube roll	62 ± 0.45^{cD}	64 ± 0.28^{A}	*	69 ± 0.04^{bCD}	62 ± 0.51^{cC}	**
P-values	****	****		****	****	

Values with different superscripts (^{abcde}) in the same row differ significantly for the storage time. Values with different superscripts (^{ABCDE}) in the same column differ significantly for different 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 31 shows the moisture content of cooked PEF and non-PEF treated samples. For the non-PEF, knuckle, rump, shanks and cube roll showed significant difference ($P < 0.05$) in 0 day and 7 days storage. However, storage time did not have an effect on the flat, bolar and full back. For the non-PEF, the significant difference ($P < 0.05$) was observed in all cuts in 0 day and 7 days, respectively.

For the PEF treated lamb cuts, the knuckle, flat, shanks, full back and cube roll showed significant difference in the 0 day and 7 days storage time except rump and bolar. All

PEF treated cuts showed significant difference ($P < 0.05$) in 0 day and 7 days separately. Data on the moisture content of lamb are absent in the literature. However higher moisture content was observed in PEF-treated lamb at 0 day compared to the non-PEF. PEF must have contributed towards denaturation of proteins, allowing moisture to be trapped. There was not a distinctive pattern in change in moisture content for both PEF and non-PEF treated lamb during the storage. Overall, knuckle contained the highest amount of moisture at 0 day, for both PEF and non-PEF treated lambs.

4.2.4.2. Color

Table 32 shows the mean values of the color attributes measured as lightness (L*), redness (a*) and yellowness (b*) during the storage (0 day and 7 days) for both non-PEF and PEF treated lamb. As shown by the non-PEF data, the lightness increased, but the redness and yellowness decreased from 0 day to 7 days. The same conclusion was found by the study of Arroyo et al. (2015) who analyzed the raw beef muscles. For the PEF treated beef, an increase in lightness (L*), redness (a*) and yellowness (b*) decreased from 0 day to 7 days. However, a gradual increase of L* values with the ageing period has been also previously reported for vacuum-packed beef *longissimus thoracis* muscle (Boakye & Mittal, 1996; Joseph & Connolly, 1977). These authors attributed this increase in lightness throughout ageing to increased retention of oxygen in the outer layers of freshly cut meat since the oxygen-utilizing enzymes in the deeper tissues became progressively inactivated with the passing of ageing time (Boakye & Mittal, 1996; Joseph & Connolly, 1977).

Table 32. The color of non-PEF and PEF treated lamb cuts at 0 day and 7 days of storage

			L	a	b
Knuckle	non-PEF	0 day	31.67±0.05^{b}	6.7±0.23^{a}	13.42±0.51^{a}
		7 days	35.09±0.11^{a}	4.71±0.18^{c}	12.83±0.18^{a}
	PEF	0 day	26.31±0.1^{d}	5.3±0.27^{b}	11.96±0.08^{b}
		7 days	28.04±0.07^{c}	4.79±0.17^{c}	10.07±0.33^{c}
		P-values	****	****	****
Rump	non-PEF	0 day	27.84±0.45^{b}	5.4±0.26^{b}	12.03±0.11^{a}
		7 days	32.05±1.77^{a}	4.25±0.12^{c}	11.47±0.51^{b}
	PEF	0 day	24.79±0.06^{c}	8.73±0.29^{a}	10.05±0.26^{c}
		7 days	27.55±0.1^{b}	4.43±0.23^{c}	7.5±0.12^{d}
		P-values	****	****	****
Flat	non-PEF	0 day	29.45±0.12^{b}	8.08±0.23^{a}	13.49±0.58^{a}
		7 days	35.46±0.05^{a}	5.78±0.1^{c}	13.67±0.24^{a}
	PEF	0 day	25.62±0.06^{c}	6.41±0.29^{b}	13.74±0.16^{a}
		7 days	35.6±0.18^{a}	5.9±0.3^{c}	12.56±0.61^{b}
		P-values	****	****	*
Shanks	non-PEF	0 day	28.21±0.02^{b}	5.95±0.14^{bc}	12.32±0.23^{a}
		7 days	29.42±0.07^{a}	5.75±0.06^{c}	11.92±0.49^{a}
	PEF	0 day	25.6±0.03^{c}	7.13±0.09^{a}	10.85±0.38^{b}
		7 days	28.23±0.05^{b}	6.12±0.16^{b}	10.32±0.49^{b}
		P-values	****	****	***
Bolar	non-PEF	0 day	36.75±0.04^{b}	8.65±0.22^{a}	18.05±0.31^{a}
		7 days	38.65±0.13^{a}	6.6±0.13^{b}	16.22±0.26^{b}
	PEF	0 day	32.06±0.06^{d}	6.5±0.23^{b}	14.8±0.3^{c}
		7 days	35.16±0.14^{c}	6.56±0.2^{b}	13.87±0.49^{d}
		P-values	****	****	****
Full back	non-PEF	0 day	26.42±0.14^{c}	7.48±0.24^{b}	12.96±0.62^{a}
		7 days	34.03±0.11^{a}	4.71±0.21^{d}	13.77±1.55^{a}
	PEF	0 day	24±0.16^{d}	8.51±0.58^{a}	13.02±0.54^{a}
		7 days	29.08±0.38^{b}	6.13±0.05^{c}	11.05±0.42^{b}
		P-values	****	****	*
Cube roll	non-PEF	0 day	29±0.16^{d}	4.08±0.25^{c}	14.42±0.4^{b}
		7 days	35.63±0.13^{b}	2.38±0.05^{d}	16.06±0.44^{a}
	PEF	0 day	33.44±0.05^{c}	6.12±0.31^{a}	11.19±0.61^{d}
		7 days	39.59±0.09^{a}	4.65±0.24^{b}	12.28±0.19^{c}
		P-values	****	****	****

Values with different superscripts (^{a,b,c,d,e}) in the same column differ significantly for the storage time. 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.

Chapter 5. Conclusions

In the present study, HPP (rump and knuckle) and PEF treated lamb cuts (bolar, rump, knuckle, flat, shanks, cube roll and full back) were evaluated in terms digestibility of different lamb cuts at different phases of simulated digestion (S1, S2 and S3); the effect of HPP and PEF treatments on digestibility of lamb; storage effect for PEF treated lamb on digestibility have been investigated. Protein digestibility was measured in the form of FAAs. Nineteen FAAs, including 9 essential and 10 non-essential amino acids were identified in the both HPP and PEF lamb samples. Studies examining the digestibility of meat, practically lamb, and treated (HPP and PEF) lambs, are missing in the literature. This was the first study to investigate the FAAs of HPP and PEF treated lamb cuts after the *in vitro* digestion. In this project, examinations of the physical properties of lamb samples such as color, moisture and pH, were also studied.

For the HPP treated lamb, the total FAAs found in the three different digestion phases of two lamb cuts (rump and knuckle) were significantly different from one another ($P < 0.05$). The high pressure treatments affected the lamb cuts significantly ($P < 0.05$), in terms of digestibility. The total FAAs of rump and knuckle increased from 200 MPa to 300 MPa, and decreased from 300 MPa to 600 MPa for all of the digestion phases. The same pattern was observed with most of the nineteen individual FAAs as well. The different cuts also affected both total FAAs and individual FAAs significantly ($P < 0.05$). The high pressure condition affected the pH significantly ($P < 0.05$) for both cooked rump and knuckle lamb cuts. Lamb samples processed at higher pressure levels (600 MPa) had higher L* and b * values. Moisture content of the lamb samples differed largely ($P < 0.05$) between the high pressure treatments.

For PEF treated lamb, the total FAAs found in the three digestion phases of the seven lamb cuts (bolar, rump, knuckle, full back, cube roll, shanks and flat) differed significantly ($P < 0.05$) from each other. The storage time of 0 day where the samples were stored at -20°C immediately after the slaughter and 7 days of storage at 4°C and then stored at -20°C until further analysis, had a significant effect ($P < 0.05$) on the amount of FAAs present at all digestion phases (S1, S2 and S3) for both non-PEF and PEF treated lamb. The amount of total FAAs differed depending on the lamb cuts, for all three phases of digestion significantly ($P < 0.05$) as well. For the color, the lightness

increased, and redness and yellowness decreased from 0 day to 7 days of storage for both non-PEF and PEF treated lamb. Moisture for most of the cuts did not show significant difference with respect to the storage time (0 day and 7 days).

In this study, the HPP and PEF were shown to be effective treatments to increase the high digestibility of lamb, particularly for 300 MPa (HPP) and 7 days storage time (PEF) This would mean that more FAAs were available to be absorbed from the same amount of lamb. The shortcomings of the study were that the total protein content was not quantified due to a small amount of samples. The amount of FAAs in the lamb cuts is different. Some lamb cuts are ignored by the consumers though they are still nutritionally valuable to contribute to human health. Further work can be adopted to determine the protein contents of HPP and PEF treated lamb. Measuring the protein structure using X-ray or NMR and determine dipeptide or small fragment of peptide, which are related to the digestibility can be further investigated. The data from this study will be useful to the New Zealand meat industry to increase the value of New Zealand lamb by adopting HPP and PEF treatments to the meat products. With continuous development of the HPP and PEF treated meat, sensory and shelf life testing of processed meat should be studied to evaluate the acceptability of the meat quality by the consumers.

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A diagram showing the seven different cuts of lamb used in the project (modified from http://www.aussiermb.org.au/images/lamb.gif. Last access date: 07/09/2016

A picture of a High Pressure Processing equipment (Hiperbaric 55) (http://www.hiperbaric.com/en/hiperbaric55) Late access date: 07/09/2016

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Appendices

Appendice 1. Enzyme activity protocol for α-Amylase (EC 3.2.1.1)

Based from SSSTAR01. Refer to CR SOP DEK ENZ

1. OBJECTIVE

To standardize a procedure for determining the enzymatic activity of α-Amylase.

2. SCOPE

This procedure applies to all products that have a specification for α-amylase.

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3. DEFINITIONS

3.1 3.1 Purified Water - water from a deionizing system, resistivity > or = 18MΩ•cm @ 25ºC

3.2. Unit Definition - One unit will liberate 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20°C.

3.3. STD = Maltose Standard

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4. DISCUSSION

Starch + H_2O $\xrightarrow{\text{α-Amylase}}$ > Reducing Groups (Maltose)

5. RESPONSIBILITIES

It is the responsibility of trained Analytical Services laboratory personnel to follow this procedure as written.

6. SAFETY

Refer to Material Safety Data Sheets (MSDS) for hazards and appropriate handling precautions.

7. PROCEDURE

7.1 CONDITIONS:

T = 20°C, pH = 6.9, A_{540nm}, Light path = 1 cm

7.2 METHOD:

Spectrophotometric Stop Reaction

7.3 REAGENTS:

7.3.1 20 mM Sodium Phosphate Buffer with 6.7 mM Sodium Chloride, pH 6.9 at 20°C (**Buffer**)

Prepare 100 ml in purified water using Sodium Phosphate, Monobasic, Anhydrous, Sigma-Aldrich Product Number S0751and Sodium Chloride, Sigma Product No S9888. Adjust to pH 6.9 at 20°C with 1 M NaOH.

7.3.2 1.0% (w/v) Soluble Starch Solution (**Starch**)

Prepare 25 ml in Reagent 7.3.1 using Starch Potato Soluble, Sigma-Aldrich Product Number S2630. Facilitate solubilization by heating the starch solution in a glass beaker directly on a heating/stir plate using constant stirring. Bring to boil and maintain the solution at this temperature for 15 minutes. Allow the starch solution to cool to room

temperature with stirring. Return the starch solution to its original volume (25 ml) by the addition of purified water and dispense aliquots for assay with stirring.

7.3.3 Sodium Potassium Tartrate Solution
Dissolve 12.0 g of Sodium Potassium Tartrate, Tetrahydrate, Sigma-Aldrich Product Number S2377, in previously heated 8.0 ml of 2 M NaOH, 50°C - 70°C. Heat directly on a heating/stir plate with constant stirring to dissolve. **DO NOT BOIL**.

7.3.4 96 mM 3,5-Dinitrosalicylic Acid Solution
Prepare 20 ml in purified water, 50°C - 70°C, using 3,5-Dinitrosalicylic Acid, Sigma-Aldrich Product Number D0550. Heat directly on a heating/stir plate with constant stirring to dissolve. **DO NOT BOIL**.

7.3.5. Color Reagent Solution (**Clr Rgt Soln**)
To 12 ml of purified water, 50°C - 70°C, slowly add Reagent 7.3.3 followed by Reagent 7.3.4. If not completely dissolved, the reagents should dissolve when mixed. The solution should be stored in an amber bottle at room temperature. The Color Reagent Solution is stable for 6 months.

7.3.6 0.2% (w/v) Maltose Standard (**STD**)
Prepare 10 ml in purified water using Maltose, Monohydrate, Sigma-Aldrich Product Number M5885 .

7.3.7 α-Amylase Solution (**Enzyme**)

7.3.7.1 Immediately before use, prepare a solution containing 1 unit/ml of α- Amylase in 20°C purified water.

7.3.7.2 If test enzyme requires a dilution scheme, the first dilution and subsequent dilutions should be in cold purified water until the last dilution, and the last dilution should be in 20°C purified water.

7.4 ASSAY

7.4.1 Pipette (in milliliters) the following reagents into suitable containers:

	Test1	**Test2**	**Test3**	**Blank**
Reagent 7.3.2 (**Starch**)	1.00	1.00	1.00	1.00

7.4.2 Mix by swirling and equilibrate to 20°C. Then add:

Reagent 7.3.7 (**Enzyme**)	0.50	0.70	1.00	----

7.4.3 Mix by swirling and incubate for exactly 3.0 minutes at 20°C. Then add:

Reagent 7.3.5 (**Clr Rgt Soln**)	1.00	1.00	1.00	1.00

7.4.4 Cap with a vented cap and place in a boiling water bath. Then add:

Reagent 7.3.7 (**Enzyme**)	0.50	0.30	-----	1.00

7.4.5 Boil for exactly 15 minutes, then cool on ice to room temperature, approximately 3 minutes, and add:

7.4.6

Purified water	9.00	9.00	9.00	9.00

7.4.7 Mix by inversion and record the A_{540nm} for both the Test and Blank using a suitable spectrophotometer.

7.4.8 Due to the short enzymatic incubation time of three minutes, each test lot must be run one at a time.

7.5. Standard Curve:

7.5.1 A standard curve is made by pipetting (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std Blank
Reagent 7.3.6 (**STD**)	0.05	0.20	0.40	0.60	0.80	1.00	2.00	-----
Purified Water	1.95	1.80	1.60	1.40	1.20	1.00	-----	2.00
Reagent 7.3.5 (**Clr Rgt Soln**)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

7.5.2 Place in a boiling water bath for exactly 15 minutes, then cool on ice to room temperature and add:

Purified water	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00

7.5.3 Mix by inversion and record the A540nm for the Standards and Standard Blank using a suitable spectrophotometer.

7.6 CALCULATIONS

7.6.1 Standard Curve

ΔA_{540nm} Standard = A_{540nm} Std - A_{540nm} Std Blank

Plot the ΔA_{540nm} of the Standards vs milligrams of Maltose. Calculate and record the slope, y-intercept, and linear regression(r-square).

7.6.2 Sample Determination

ΔA_{540nm} Sample = A_{540nm} Test - A_{540nm} Test Blank

Determine the milligrams of Maltose liberated using the Standard Curve.

7.6.3	Units/Ml enzyme =	mg of Maltose released(df) / (1)

df = Dilution Factor
1 = Volume (in milliliter) of enzyme used

7.6.4	Units/mg solid =	units/mL enzyme / mg solid/mL enzyme

7.6.5	Units/mg protein =	units/mL enzyme / mg protein/mL enzyme

7.7 FINAL ASSAY CONCENTRATION
In a 2.00 ml reaction mix, the final concentrations are 8 mM sodium acetate, 0.50% (w/v) starch and 1 unit β-amylase.

Appendice 2. Enzyme activity protocol for Pepsin (EC 3.4.23.1)
Description
This procedure may be used for determination of Pepsin activity using hemoglobin as the substrate. It is a spectrophotometric stop rate determination.
Unit Definition: One unit of Pepsin will produce a ΔA_{280} of 0.001 per minute at pH 2.0 at 37 °C measured as trichloroacetic acid (TCA)-soluble products using hemoglobin as the substrate. (Final volume = 16 ml, light path = 1 cm.)
Precautions
Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Reagents and Equipment Required
1.0 M Hydrochloric Acid (Catalog No. 318949)
Hemoglobin from bovine blood (Catalog No. H2625)
6.1 N [~100% (w/v)] Trichloroacetic acid solution (Catalog No. T0699)

Preparation Instructions

Use ultrapure water (≥18 MΩxcm resistivity at 25 °C) for the preparation of reagents.

10 mM HCl (Hydrochloric Acid)

– Dilute 1.0 M Hydrochloric Acid (Catalog No. 318949) 100-fold with ultrapure water.

2.5% (w/v) Hemoglobin Stock Solution

– Prepare a 25 mg/ml solution in ultrapure water using Hemoglobin from bovine blood (Catalog No. H2625). Stir vigorously, creating a vortex, for a minimum of 10 minutes at 37 °C. Then filter through a polypropylene column with a coarse filter (90–130 µm).

Substrate [2.0% (w/v) Hemoglobin solution]

– Transfer 80 ml of the filtered 2.5% (w/v) Hemoglobin Stock Solution into a suitable container. Adjust the pH of this solution to 2.0 at 37 °C using 5 M HCl. Bring the final volume to 100 ml with ultrapure water.

TCA Solution [5% (w/v) Trichloroacetic Acid]

– Dilute 6.1 N [~100% (w/v)] 6.1 N [~100% (w/v)] Trichloroacetic acid solution (Catalog No. T0699) 20-fold with ultrapure water.

Enzyme Solution (Pepsin)

– Prepare a 1 mg/ml stock solution in cold (2–8 °C) 10 mM HCl. If insoluble material is present, allow the stock solution to sit on ice until dissolved. Allow up to 1 hour for the Pepsin to completely dissolve. When the Pepsin has dissolved or after 1 hour, whichever occurs first, dilute the 1 mg/ml stock solution to 0.01–0.05 mg/ml in cold 10 mM HCl.

Procedure

1. Pipette the Substrate into suitable glass vials.

Reagent	Blank (ml)	Test (ml)
Substrate	5.00	5.00

2. Place vials in a thermostatted water bath and equilibrate to 37 °C for ~10 minutes, then add:

Reagent	Blank (ml)	Test (ml)
Enzyme Solution	–	1.00

3. Mix by swirling and incubate at 37 °C for *exactly* 10 minutes, then add:

Reagent	Blank (ml)	Test (ml)
TCA Solution	10.0	10.0
Enzyme Solution	1.00	–

4. Mix by swirling and incubate at 37 °C for an additional 5 minutes.

5. Filter the Blank and Test mixtures through a 0.45 µm syringe filter. Record the A_{280} versus air for each filtered solution for each vial.

Results

Calculations

$$\text{Units/ml enzyme} = \frac{(A_{280}\ \text{Test} - A_{280}\ \text{Blank})\ (\text{df})}{(10)\ (1.0)\ (0.001)}$$

where:
df = Dilution factor
10 = Assay incubation time in minutes
1.0 = Volume of Enzyme Solution (ml) added
0.001 = ΔA_{280} per unit of Pepsin per unit definition

Appendice 3. Enzyme activity protocol for Trypsin (EC 3.4.21.4)
Description

This procedure is for products with a specification for Trypsin activity using N_α-Benzoyl-L-arginine ethyl ester (BAEE) as a substrate. The procedure is a continuous spectrophotometric rate determination (A_{253}, Light path = 1 cm) based on the following reaction:

BAEE $\xrightarrow{\text{Trypsin}}$ + H2O Nα-Benzoyl-L-arginine + ethanol

where:
BAEE – N_α-Benzoyl-L-arginine ethyl ester
Unit Definition – One BAEE unit of trypsin activity will produce a ΔA_{253} of 0.001 per minute with BAEE as substrate at pH 7.6 at 25 °C in a reaction volume of 3.20 ml.

Precautions
Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagents and Equipment Required
Sodium phosphate, monobasic (Catalog No. S0751)
N_α-Benzoyl-L-arginine ethyl ester (BAEE, Catalog No. B4500)
1 M Hydrochloric acid (Catalog No. 318949)

Preparation Instructions
Use ultrapure water (≥18 MΩxcm resistivity at 25 °C) for the preparation of reagents.
Buffer (67 mM Sodium Phosphate Buffer, pH 7.6 at 25 °C) – Prepare a 8.04 mg/ml solution using sodium phosphate, monobasic (Catalog No. S0751) in ultrapure water. Adjust to pH 7.6 at 25 °C with 1 M NaOH solution.
Substrate Solution (0.25 mM N_α-Benzoyl-L-arginine ethyl ester) – Prepare a 0.086 mg/ml solution using N_α-Benzoyl-L-arginine ethyl ester (BAEE, Catalog No. B4500) in Buffer.
HCl Solution (1 mM Hydrochloric Acid) – Prepare a 1,000-fold dilution of 1 M Hydrochloric acid solution (Catalog No. 318949) in ultrapure water.
Enzyme Solution (Trypsin) – Immediately before use, prepare a solution containing 425-575 units/ml of Trypsin in cold (2-8 °C) HCl Solution.

Procedure
In a 3.20 ml reaction mix, the final concentrations are 62.8 mM sodium phosphate, 0.23 mM N_α-Benzoyl- L-arginine ethyl ester, 0.031-0.063 mM hydrochloric acid, 42.5-115.0 units of trypsin.
1. Pipette the following reagents into suitable quartz cuvettes:

Reagent	Blank (ml)	Test (ml)
Substrate Solution	3.00	3.00
HCl Solution	0.200	0.125

2. Mix by inversion and equilibrate to 25 °C using a suitably thermostatted spectrophotometer. Then add:

Reagent	Blank (ml)	Test (ml)
Enzyme Solution	–	0.075

Note: Final volume in each cuvette must be 3.2 ml per unit definition.
3. Immediately mix by inversion and record the increase in A_{253} for 5 minutes. Using a 1 minute time period and a minimum of 4 data points, obtain the ΔA_{253}/minute using the maximum linear rate for both the Blank and Test.

Results
Calculations
1.

$$\text{BAEE units/ml enzyme} = \frac{(\Delta A_{253}/\text{minute Test} - \Delta A_{253}/\text{minute Blank}) \times (df)}{(0.001) \times (0.075)}$$

Where:
df = dilution factor
0.001 = The change in A_{253}/minute based on unit definition
0.075 = volume (ml) of test sample used in assay
Note: The total volume in the cuvette is not used in the calculation since the unit definition is based on 3.2 ml.
2.

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

Appendice 4. Enzyme activity protocol for Chymotrypsin
Description

This procedure is for products with a specification for Chymotrypsin activity. It is not to be used to assay Insoluble Chymotrypsin such as Catalog No. C9134. The procedure is a continuous spectrophotometric rate determination (A_{256}, Light path = 1 cm) based on the following reaction:

$$\text{BTEE} + H_2O \xrightarrow{\text{Chymotrypsin}} \textit{N}\text{-Benzoyl-L-tyrosine} + \text{ethanol}$$

where:
BTEE – *N*-Benzoyl-L-tyrosine ethyl ester
Unit Definition – One unit of chymotrypsin will hydrolyze 1.0 µmole of BTEE per minute at pH 7.8 at 25 °C.
Precautions
Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Reagents and Equipment Required
Trizma® Base (Catalog No. T1503)

N-Benzoyl-L-Tyrosine Ethyl Ester (Catalog No. B6125)
Methanol (Catalog No. M1775)
Calcium chloride, dihydrate (Catalog No. C3881)
Hydrochloric acid solution (Catalog No. 318949)

Preparation Instructions

Use ultrapure water (≥18 MΩxcm resistivity at 25 °C) for the preparation of reagents.

Buffer (80 mM Tris HCl Buffer, pH 7.8 at 25 °C)
– Prepare a 9.69 mg/ml solution in ultrapure water using Trizma® Base (Catalog No. T1503). Adjust the pH of this solution to 7.8 at 25 °C.

BTEE Solution (1.18 mM *N*-Benzoyl-L-Tyrosine Ethyl Ester)
– Weigh 37 mg of *N*-Benzoyl-L-Tyrosine Ethyl Ester (Catalog No. B6125) into a 100 ml Class A volumetric flask. Add 63.4 ml of Methanol (Catalog No. M1775) and mix by swirling. Bring the final volume of the solution to 100 ml using ultrapure water. Invert the flask several times to ensure complete mixing.

$CaCl_2$ Solution (2 M Calcium Chloride)
– Prepare a 294 mg/ml solution in ultrapure water using Calcium chloride, dihydrate (Catalog No. C3881).

HCl Solution (1 mM Hydrochloric Acid)
– Prepare a solution by diluting 0.10 ml of 1.0 M Hydrochloric acid solution (Catalog No. 318949) to 100 ml with ultrapure water in a 100 ml Class A volumetric flask. Mix by inversion and place on ice.

Enzyme Solution (Chymotrypsin)
– Immediately before use, prepare a solution containing 2-5 chymotrypsin units per milliliter in cold (2-8 °C) HCl Solution.

Procedure

In a 3.00 ml reaction mix, the final concentrations are 38 mM Tris, 0.55 mM *N*-Benzoyl-L-Tyrosine Ethyl Ester, 30% (v/v) Methanol, 53 mM Calcium Chloride, 0.03 mM Hydrochloric Acid, and 0.2-0.5 units of Chymotrypsin.

1. Pipette the following reagents into suitable quartz cuvettes:

Reagent	Blank (ml)	Test (ml)
Buffer	1.42	1.42
BTEE Solution	1.40	1.40
$CaCl_2$ Solution	0.08	0.08

2. Mix by inversion and equilibrate to 25 °C using a suitably thermostatted spectrophotometer.
3. Add the following to the cuvettes:

Reagent	Blank (ml)	Test (ml)
HCl Solution	0.10	–
Enzyme Solution	–	0.10

4. Immediately mix by inversion and record the increase in A_{256} for 3-5 minutes.
5. Obtain the ΔA_{256}/minute for both the blank and test reactions using the maximum linear rate over a one minute interval using at least 4 points.

Results

Calculations

1.

$$\text{Units/ml enzyme} = \frac{(\Delta A_{256}/\text{minute Test} - \Delta A_{256}/\text{minute Blank}) \times (3) \times (df)}{(0.964) \times (0.10)}$$

Where:

3 = volume (ml) of reaction mix

df = dilution factor

0.964 = millimolar extinction coefficient of BTEE at 256 nm

0.10 = volume (ml) of test sample used in assay

2.

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

Appendice 5. Reagents and procedures of EZ: Faast amino acids kit (GC-FID)

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.

Figure 1a

Figure 1b

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.

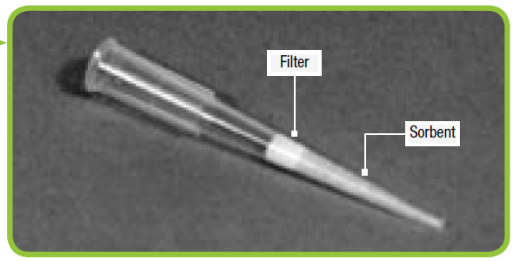
3.4 Optimizing Sample Preparation Time

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	ßAIB	HIS	LYS	PRO	TYR
alLE	C-C	HYP	MET	SAR	VAL
ALA	GLU	ILE	ORN	SER	

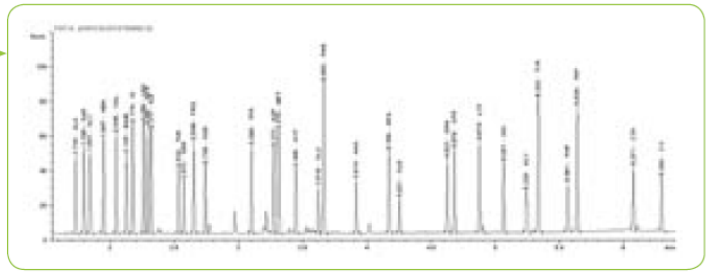
SD2: Complementary amino acids not stable in acidic solution, 200 nmoles/mL each, as follows:

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.