Effects of Pulsed Electric Field (PEF) processing on physicochemical and flavour characteristics of fresh and frozen-thawed beef muscles

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Effects of Pulsed Electric Field (PEF) processing on physicochemical and flavour characteristics of fresh and frozen-thawed beef muscles

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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, 'Effects of Pulsed Electric Field (PEF) processing on physiochemical and flavour characteristics of fresh and frozen-thawed beef muscles' 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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Abstract

PEF treatment is a new non-thermal food processing technique, which can improve food quality and extend shelf-life. This study investigated the effects of pulsed electric field (PEF) treatment on the sensory attributes, physicochemical properties and flavour of beef. Two types of beef muscles, *biceps femoris* (BF) and *semitendinosus* (ST) either as fresh or as frozen-thawed muscles were used. The muscles were treated at electric field strength of 0.8-1.1 kV/cm, pulse width of 20 μs, frequency of 50 Hz and energy of 130 kJ/kg. Temporal dominance of sensations (TDS) analysis were carried out to determine the temporal changes in sensory attributes of PEF treated beef samples. Oxidized and brothy were the dominant sensory attributes during mastication. The Canonical Variate Analysis (CVA) results showed that the "oxidized" sensory attribute was positively affected by both storage and PEF processing.

PEF processing enhanced the lipid oxidation of both fresh and frozen-thawed beef samples. Values above 0.5 are considered critical since they indicate a level of lipid oxidation products, which produce a rancid odour and taste that can be detected by consumers. Values of thiobarbituric acid reactive substances (TBARS) in our study with beef muscles before and after PEF treatment at 0 and 7 days storage was well below 0.5. In general fatty acids decreased with PEF processing. The oxidation of lipid and fatty acids is an important contributor of cooked beef flavour. Ten common volatile compounds found in beef were investigated in this study. PEF processed samples stored for 7 days were associated with more flavour compounds.

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Abbreviations

ANOVA Analysis of Variance

BF *bicep femoris*

BHT butylated hydroxytoluene

CLA conjugated linoleic acid

CVA Canonical Variate Analysis

DDW deionised distilled water

FAME Fatty Acid Methyl Ester

FID Flame Ionisation Detector

GC Gas Chromatography

GC-FID Gas Chromatography - Flame Ionisation Detector

GC-MS Gas Chromatography - Mass Spectrometry

GM gluteus medius

GMP guanosine monophosphate

IMP inosinic acid

LD *longissimus dorsi*

LL *longissimus lumborum*

LTL *longissimus thoracis et lumborum*

MANOVA Multiple Analysis of Variance

MDA malondialdehyde

MSG monosodium glutamate

MUFA monounsaturated fatty acid

PCA Principal Component analysis

PEF pulsed electric field

PUFA polyunsaturated fatty acid

QDA Quantitative Descriptive Analysis

RI retention index

SFA saturated fatty acid

SM *semimembranosus*

SPME Solid Phase Microextraction

ST *semitendinosus*

TBA thiobarbituric acid

TBARS thiobarbituric acid reactive substances

TCA trichloroacetic acid

TDS temporal dominance of sensations

TEP tetraethoxypropane

TI time intensity

TPA texture profile analysis

TVA trans vaccenic acid

WBS warner-Bratzler shear force

Chapter 1 Introduction

Meat and meat products are high in protein and contain several essential amino acids (Biesalski, 2005). Beef is a common meat food, which contains high biological value protein and micronutrients (Scollan et al., 2006). Beef colour and texture are the main attributes that influence the quality of beef. Colour provides consumers the first impression of foods and affects their purchasing decisions (Wulf & Wise, 1999). Texture on the other hand is directly related to consumer satisfaction of meat and meat products (Bekhit, van de Ven, Fahri, & Hopkins, 2014). Hence the improvement of meat texture and colour would be a significant achievement for the meat processing industry.

During the processing and storage, lipid oxidation can seriously reduce the quality of fresh or frozen stored meat and meat products. Meat lipid oxidation and its relative interaction can cause several negative effects on meat colour, flavour, nutrition and safety during the ageing and processing periods (Frankel, 1984). In order to improve the quality or extend the shelf life of meat and meat products, there are many processing methods applied on meat and meat products. Traditionally thermal treatments have been widely used to extend the shelf life of most foods. However, thermal treatment can reduce food quality by damaging nutritional components and flavour compounds in foods (Noci et al., 2008). Therefore, a non-thermal treatment technique applied on foods, pulsed electric field (PEF) processing may solve this problem by extending food shelf without reducing food quality (Barbosa-Cánovas & Altunakar, 2006).

The PEF system comprises a high voltage pulse generator, a treatment chamber and an operation system with a monitor and controlling devices (Barbosa-Cánovas & Altunakar, 2006). High voltage electrical pulses (μs) that passes through a food sample can damage the structure of cell membrane due to the electroporation effect (Angersbach, Heinz, & Knorr, 2000). These changes in cell membrane can therefore influence the viscoelastic properties of food tissues (Lebovka, Bazhal, & Vorobiev, 2002).

Traditional sensory evaluation is based on the intensity judgement of one single sensory attribute (Cliff & Heymann, 1993). However, with the increased requirements of food

quality, it is necessary to investigate all the temporal sensory information during the whole eating process (Piggott, 2000). Temporal dominance of sensations (TDS) was developed as a new sensory analytical method to analyse complex sensory properties in real time. In order to gain a better result, TDS requires highly trained panellists who are familiar with dominant sensory attributes in food samples.

The aim of this research is to study the effects of PEF and storage on the physical, chemical and sensory characteristics of *semitendinosus* (ST) and *biceps femoris* (BF) beef muscles. Both fresh and frozen-thawed beef samples were evaluated in this study as consumers often purchase chilled meat and freeze it at home. This thesis will cover the research background, experimental methods used in this study, and experimental results and discussion. The results of this study focuses on the sensory attributes, initial tenderness, colour, lipid oxidation, fatty acid composition, and flavour volatile compounds in both control and PEF treated beef samples. In the results and discussion section, fresh and frozen beef meat are discussed separately.

Chapter 2 Literature Review

2.1 Beef Nutritional Components

Meat and meat products provide nutritional components like high quality protein, long-chain fatty acids, vitamins and highly bio-available minerals, which are essential in the human diet. The micronutrients such as vitamins A, B6, B12, D, E, iron, zinc, selenium and folic acid, which can be provided by plants are limited (Biesalski, 2005). Thus, beef is normally considered an important source of high biological value protein and micronutrients (Scollan et al., 2006; Williamson, Foster, Stanner, & Buttriss, 2005). In addition, as beef is high in protein and low in carbohydrate, it helps contribute to a low glycemic index and reduce the risk of obesity and diabetes (Biesalski, 2005). The evaluation of beef is complex, and beef quality is commonly determined by its tenderness, colour, juiciness, flavour, and nutritive value (Listrat & Hocquette, 2004).

2.1.1 Protein and Amino Acids

Meat contains about 10 to 40% protein (Jiménez-Colmenero, Carballo, & Cofrades, 2001). Protein is an important nutrient for the human body. Proteins consist of different amino acids, which are the building blocks of proteins, and have important functions for human body. During digestion, active enzymes digest protein molecules into free amino acids, which are absorbed through the gastro-intestinal wall (Robinson & Kreis, 1992; Whitney, DeBruyne, Pinna, & Rolfes, 2010). Therefore, the nutritional value of a protein is determined by the absorbed proportion of dietary amino acid after ingestion in the human body (Hall & Schönfeldt, 2013). In animals, collagen represents 30% of total protein, and both glycine and proline account for about 50% of the amino acids in collagen (Bolboaca & Jäntschi, 2007).

Protein content can be determined by measuring total nitrogen (N) using the Kjeldahl or Dumas method. In both methods, a specific factor of 6.25 is multiplied, based on the assumption that all proteins contain 16% nitrogen (Hall & Schönfeldt, 2013). However, nitrogen content may vary in proteins of different foods. To calculate total protein in different foods, different nitrogen factors may need to be used. The factors used are 6.37 for human milk, 5.55 for gelatin, 5.18 for almonds, and normally 6.25 for meat and fish (Greenfield & Southgate, 2003). However, since amino acids produced from proteins by

digestive enzymes are directly related to bioavailability of protein, it is considered to be more suitable to estimate protein content based on amino acid content. In addition, free amino acids should be considered to ensure the accuracy of using amino acid content in determining total protein content (Hall & Schönfeldt, 2013).

2.1.2 Fat and Fatty Acids

Fat in meat is important in the human diet. Previous studies have indicated that the fatty acid composition of ruminant meat is different from that of non-ruminants (Enser, Hallett, Hewitt, Fursey, & Wood, 1996). Fats from animal sources such as milk, dairy products, and beef, usually contain between 2% to 4.5% trans-fatty acids of total fat content., These acids are generated during rumination and subsequently absorbed and stored in animal tissues (Yılmaz & Geçgel, 2007).

Fat composition of beef can be affected by many factors such as feeding time, finishing diet, animal age and breed type, which can in turn influence fatty acid composition (De Smet, Raes, & Demeyer, 2004). The content of mono-unsaturated fatty acids (MUFA) in grain-fed and pasture-fed cattle usually increase with prolonged feed time. In addition, the changes in the ruminal environment may also increase MUFA in cattle fed high-grain diets. Generally, when comparing at a similar age range, beef from pasture-finished animals is leaner than animals finished on high-concentrate diets (Duckett, Pratt, & Pavan, 2009; Fincham et al., 2009). Fat from pasture-fed animals contained greater proportions of polyunsaturated fatty acid (PUFA) n-3, trans vaccenic acid (TVA), and conjugated linoleic acid (CLA) cis-9, trans-11 (Daley, Abbott, Doyle, Nader, & Larson, 2010)

High intake of saturated fatty acids can increase the risk of cardiovascular diseases and some type of cancer (Slattery, Benson, Ma, Schaffer, & Potter, 2001). However natural fats and oils that are present in the human diet contain only small amounts of trans fatty acids. Smith, Gill, Lunt, and Brooks (2009) collected data from a large number of studies on cattle of different ages, breed types, and finishing diets, and showed the large variation in fatty acid composition across different varieties of steers and cows (Table 1).

The main unsaturated fatty acids in meat lipids are oleic, linoleic, linolenic and

arachidonic acids. Autoxidation of these unsaturated fatty acids produces different hydroperoxides, which lead to the production of a large number of volatile compounds (D. Mottram, 1987). The oxidation of linoleic acid can form volatile hexanal or 2, 4-decadienal, which are the indicators of lipid oxidation because of their off-flavours in oxidised meat and meat products (Shahidi & Zhong, 2010).

Table 1 Fatty acid concentrations (g/100 g total fatty acids) in subcutaneous adipose tissue of different varieties of steers and cows (Smith et al., 2009)

Item	Cattle group/diet					
	Brahman	Hereford	Angus	Australian	J. Black	Hanwoo
Age (months)	54	54	16	22	27	28
14: 0	4.3	4.0	3.0	1.5	1.3	3.2
14: 1n-5	3.2	2.4	1.1	0.1	1.3	1.0
16: 0	22.7	26.0	27.4	24.2	24.2	27.9
16: 1n-7	10.7	9.4	5.6	1.6	5.2	4.6
18: 0	7.6	8.9	8.8	26.1	7.6	9.6
18: 1trans-11	NR	NR	1.6	2.3	0.7	NR
18: 1n-9	49.6	47.8	41.3	39.8	52.9	47.3
18: 1n-7	NR	NR	2.0	1.0	3.0	NR
18: 2n-6	4.3	1.7	1.9	1.6	2.0	4.2
18: 3n-3	0.9	0.7	0.1	0.5	0.2	0.4
16:1:18:0	1.41	1.06	0.19	0.06	0.68	0.48
MUFA: SFA	1.85	1.59	1.26	0.77	1.86	1.28

NR = Not reported.

MUFA = monounsaturated fatty acids (14:1n-5, 16:1n-7, 17:1n-8, 18:1n-9, and 18:2cis-9, trans11). SFA = saturated fatty acids (14:0, 16:0, 17:0, 18:0, and 18:1trans-11).

2.2 Beef Physical Attributes

2.2.1 Beef Texture

Meat texture is an important attribute for eating quality. It is directly related to consumer satisfaction of meat and meat products (Bekhit et al., 2014). The evaluation of beef texture is complex. Tenderness is an important attribute that can be determined in terms of initial tenderness, which is decided by the first bite, and overall tenderness which is considered the whole chewing process. To describe the texture of beef, sensory attributes of chewing and mouthfeel should be included as well, such as cohesiveness, friability, mushiness, hardness, softness, rubberiness and so on (Juarez et al., 2012).

There are three major methods to evaluate meat texture. Firstly, the sensory method is a direct way to understand if the texture is acceptable for consumers. Secondly, instrumental methods can quantify different parameters, such as hardness, factorability,

adhesiveness, springiness and cohesiveness. Finally an indirect method can be applied determine the collagen content or amount of dry matter in meat to describe the product texture (Kamdem & Hardy, 1995). For beef analysis, hardness, cohesiveness, springiness, gumminess and chewiness are the most common attributes should be considered (Chen & Trout, 1991). In a previous study, the hardness and chewiness characteristics of meat using TPA and Warner-Bratzler shear force (WBS) were found to have a positive relationship (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003). However, WBS had a negative correlation with initial tenderness, and chewiness that was calculated using hardness as a factor. This suggests that resistance to compression force was probably the main textural property determining tenderness characteristics.

Beef texture can be influenced by many factors, such as muscle structure, chemical composition, different treatments, sample shapes and so on (Szczesniak, 2002). O'Dowd, Arimi, Noci, Cronin, and Lyng (2013) reported that when low strength 1.1-2.8 kV cm^{-1} of pulsed electric field (PEF) (total energy 12.7-226 kJ/kg) processing was carried out on beef semitendinosus (ST) muscles, it was not enough to break the structure of muscle fibres, which influenced the tenderisation of beef. However, Carne, van de Ven, Bekhit, and Hopkins (2015b) found that there was a significant decrease in shear force of hot-boned beef samples during four different storage periods (3, 7, 14, and 21 days) under two different voltages (5 and 10 kV cm^{-1}) used during PEF treatment.

There are limited studies discussing the effects of PEF processing on cohesiveness, resilience and springiness. These effects however might not have much influence on mouthfeel. The change in hardness can be due to thermal processing caused by an increase in temperature during PEF treatment. With increasing temperature, the moisture will loss is faster resulting in increased hardness of meat. In addition, electric field processing may cause contraction of muscle fibres, which can also increase hardness of meat. O'Dowd et al. (2013) mentioned that there was no significant increase in shear force of beef samples that were subjected to PEF treatment (1.9 kV cm^{-1}, 65 Hz, 250 pulses) and water bath treatment (ΔT 22 °C). It was believed that the tenderness in beef could be affected by the freezing-thawing process as intracellular ice crystal formation in beef muscle cells could make physically disrupt muscle cells (Faridnia et al., 2015).

2.2.2 Beef Colour

Colour is another important criterion for meat quality as it usually gives consumers a first impression of foods. It is probably one of the most important visual factors in determining purchasing decisions of meat and meat products (Wulf & Wise, 1999). The colour of beef can be affected by many factors, such as cattle breeds, aging periods, storage environment, packaging methods, and other steps during the process of beef production (Kropf, 1993).

The colour of myoglobin is deep purplish red, which has been reported to be the main factor that influenced the colour of beef muscle (Faridnia, Bekhit, Niven, & Oey, 2014; Insausti et al., 1999; MacDougall, 1977). The concentration of myoglobin and the degree of its oxidation, as well as meat structure may influence meat colour (De Huidobro, Miguel, Onega, & Blázquez, 2003). Concentration of myoglobin in animals vary with species, age, diet and other factors. Hence the colour of different meats would not be the same (Livingston & Brown, 1981). Boles and Pegg (2010) reported that the beef meat contained higher concentrations of myoglobin than lamb and pork, and its concentration increased with the age of animals.

When displaying meat for sale, oxygen is considered an important factor that influences meat colour. Oxygen can bind to the ferrous ion in the porphyrin structure of myoglobin and form oxymyoglobin, which provides a lighter red colour in meat. However, after further oxidation, the ferrous ion will be oxidized to ferric ion and changing myoglobin to metmyoglobin, which then decreases the lightness of meat colour (Kannan, Kouakou, & Gelaye, 2001). Insausti et al. (1999) also stated that limited oxygen in vacuum packaging could further penetrate into the beef meat and result in a lighter colour.

2.3 Beef Lipid Oxidation

Lipids are important constituents in meat and meat products, and are essential for improving the tenderness and juiciness of meat, and the flavour characteristics of meat products. Lipids exist in both intramuscular and intracellular spaces in meat and meat products. Most intermuscular lipids are stored in connective tissues and distributed throughout the muscle tissues (Kono & Colowick, 1961). Intracellular lipids on the other hand are associated with protein and contain phospholipids (Love & Pearson,

1971). Although phospholipids only comprise a small percentage in meat tissues (about 1% of total weight), they are considered an important factor that influences meat quality, as it is easily oxidized (I Hornstein, Crowe, & Heimberg, 1961).

Lipid oxidation is a major cause of fresh or stored meat and meat product deterioration. Meat lipid oxidation and its interaction with other constituents can induce changes in meat colour, flavour, nutritive value, safety, biological damage, ageing, and functional property (Frankel, 1984). Lipid oxidation is a complex process during which polyunsaturated fatty acids (PUFA) of meat react with molecular oxygen via a free radical chain mechanism to form fatty acyl hydroperoxides and other primary products of oxidation (Gray, 1978; Shahidi & Zhong, 2010). Primary auto-oxidation is then followed by a series of secondary reactions, which lead to the degradation of the lipid and the development of oxidative rancidity (Roldan, Antequera, Armenteros, & Ruiz, 2014). During this reaction, compounds formed include thiobarbituric acid reactive substances (TBARS) and some volatile compounds.

2.4 Flavour of Beef

Taste and aroma are the two most important sensory characteristics of meat. The flavour compounds formed in the process of heat treatment such as frying and boiling are complicated. Normally there are three main types of flavour compounds: (1) the aroma formed from volatile compounds; (2) the taste from non-volatile compounds and water soluble compounds with tactile properties; (3) flavour enhancer and synergist. However, the main factor that influences meat flavour is volatile compounds. Meat flavour has been extensively studied by many researchers, and there are currently more than 1000 types of volatile compounds identified in cooked meat (Hall & Schönfeldt, 2013; Glesni MacLeod, Seyyedain-Ardebili, & Chang, 1981).

2.4.1 Taste Contributing Compounds

The most important taste contributing compounds in meat include: inorganic salt (salty), hypoxanthine (bitter), sugar (sweet), organic acids (sour), nucleotides, amino acids and peptides (e.g., anserine, carnosine), etc. The concept of umami by Japanese scholars describes the flavour characteristic of MSG and 5'- nucleotide. Umami substances can increase the overall intensity of flavour and improve texture and softness to enhance

product quality. So far, creatine (sarcosine) and creatine phosphate content are highest in muscles, and account for about 0.5% of fresh muscle. Although creatinine does not have any taste, it can give obvious bitterness when its concentration levels increase to twice the natural concentration in the meat. Meat taste contributing compounds are summarized in Table 2 (Melton, 1999).

Table 2 Meat taste contributing compounds (Melton, 1999)

Tastes	Taste contributing compounds
Sweetness	Sugar: RNA, glucose and fructose Amino acids: hydroxyproline, proline, alanine, glycine, serine, threonine, lysine, cysteine and methionine, asparagine, l-glutamine
Sour	Amino acids: aspartic acid, glutamic acid, histidine, arginine Acids: lactic acid, inosinic acid, succinic acid, four hydrogenated pyrrole carboxylic acid, orthophosphoric acid
Salty	Inorganic salt, glutamic acid, aspartic acid salt
Bitterness	Hypoxanthine, carnosine and anserine, other bitter peptides, histidine, arginine, lysine, methionine, valine, leucine, isoleucine, Phenylalanine and tyrosine
Umami	Glutamic acid, monosodium glutamate (MSG), inosinic acid (IMP), guanosine monophosphate (GMP) and some peptides

2.4.2 Flavour Enhancers

There are two types of flavour enhancers: 1) L-amino acids contain five carbon atoms, and 2) 5'-nucleotide containing 6-hydroxyl purine, such as 5'-GMP, 5'-IMP and 5' and 5'-XMP. The flavour enhancement effect of 5'- GMP is about 4 times higher than that of 5'-IMP, but it usually has a lower effect on flavour because of its low concentration in raw meat. In meat, the most important flavour enhancers are glutamate, MSG and 5'-IMP. Studies have shown that the molecular structure characteristic or requirement of flavour enhancers is that the two negative charges have to be separated by 1 to7 carbon atoms (G MacLeod, 1994; Glesni MacLeod et al., 1981).

Taste threshold is the minimum concentration of compounds at which they can be tasted. The range of taste threshold of MSG has been reported to be from 4×10^{-5} M to 4×10^{-3} M (G MacLeod, 1994). Melton (1999) further reported that the absolute taste threshold of MSG was 6.25×10^{-4} M, which is higher than bitterness (quinine sulfate)

and sour (tartaric acid), lower than sucrose, and similar to salt (NaCl). The threshold of 5'- IMP is reported to be between 0.01 - 0.025%, and 5'- GMP is 0.0035-0.02%.

2.4.3 Volatile Flavour Compounds

Aromatic compounds are mainly volatile compounds, of which some tend to be water-soluble, and some lipid soluble. The concentration of volatile components is very low, but they may produce a variety of different flavours. Unlike non-volatile taste compounds, most volatile flavour compounds originate from carbohydrate, lipid and protein (Fisk, Boyer, & Linforth, 2012).

The composition of different kinds of meat is similar, and only has small differences. Undoubtedly, volatile compounds are mainly responsible for meat flavour. More than 1000 types of volatile compounds from cooked beef, pork, chicken, and lamb meat have been reported (G MacLeod, 1994; Glesni MacLeod et al., 1981). These compounds mainly include: hydrocarbons, alcohols, aldehydes, acids, esters, lactones, ethers, heterocyclic compounds and other sulfur compounds (Shahidi, Rubin, D'Souza, Teranishi, & Buttery, 1986). The distribution of different types of volatile compounds in meat is shown in Table 3.

Table 3 Classification of volatile compounds found in meat (Shahidi et al., 1986)

Compound category	Beef		Chicken (%)		Pork (%)		Lamb (%)	
	amount	%	amount	%	amount	%	amount	%
Hydrocarbons	123	18.1	71	20.5	45	14.3	26	11.5
Aldehydes	66	9.7	3	21	35	11.2	41	18.1
Ketones	59	8.7	31	8.9	38	12.1	23	10.2
Alcohols	61	9.0	28	8.1	24	7.6	11	4.9
Phenols	3	0.4	4	1.2	9	2.9	3	1.3
Acids	20	2.9	9	2.6	5	1.6	46	20.4
Esters	33	4.8	7	2.0	20	6.4	5	2.2
Lactones	33	4.8	2	0.6	2	0.6	14	6.2
Furans	40	5.9	13	3.8	29	9.2	6	2.7
Miazines	10	1.5	10	2.9	5	1.6	16	7.1
Pyrazines	48	7.0	21	6.1	36	11.5	15	6.6
Nitrogen compounds	37	5.4	33	9.5	24	7.6	8	3.5
Sulfo compounds	126	18.6	33	9.5	31	9.9	12	5.3

Halogenated compound	6	0.9	6	1.7	4	1.3	-	-
Other compounds	16	2.3	6	1.7	7	2.2	-	-

D. S. Mottram (1985) indicated that the main source of different meat flavour compounds originate from lipids, mainly phospholipids. Early research found that water extraction of beef, pork and lamb had very similar flavour after heating, but had significantly different flavour characteristics when heated with their distinctive fats (Irwin Hornstein & Crowe, 1960). G MacLeod (1994) reported 25 compounds with actual meat flavour from the 880 volatile components identified in cooked beef (Figure 1).

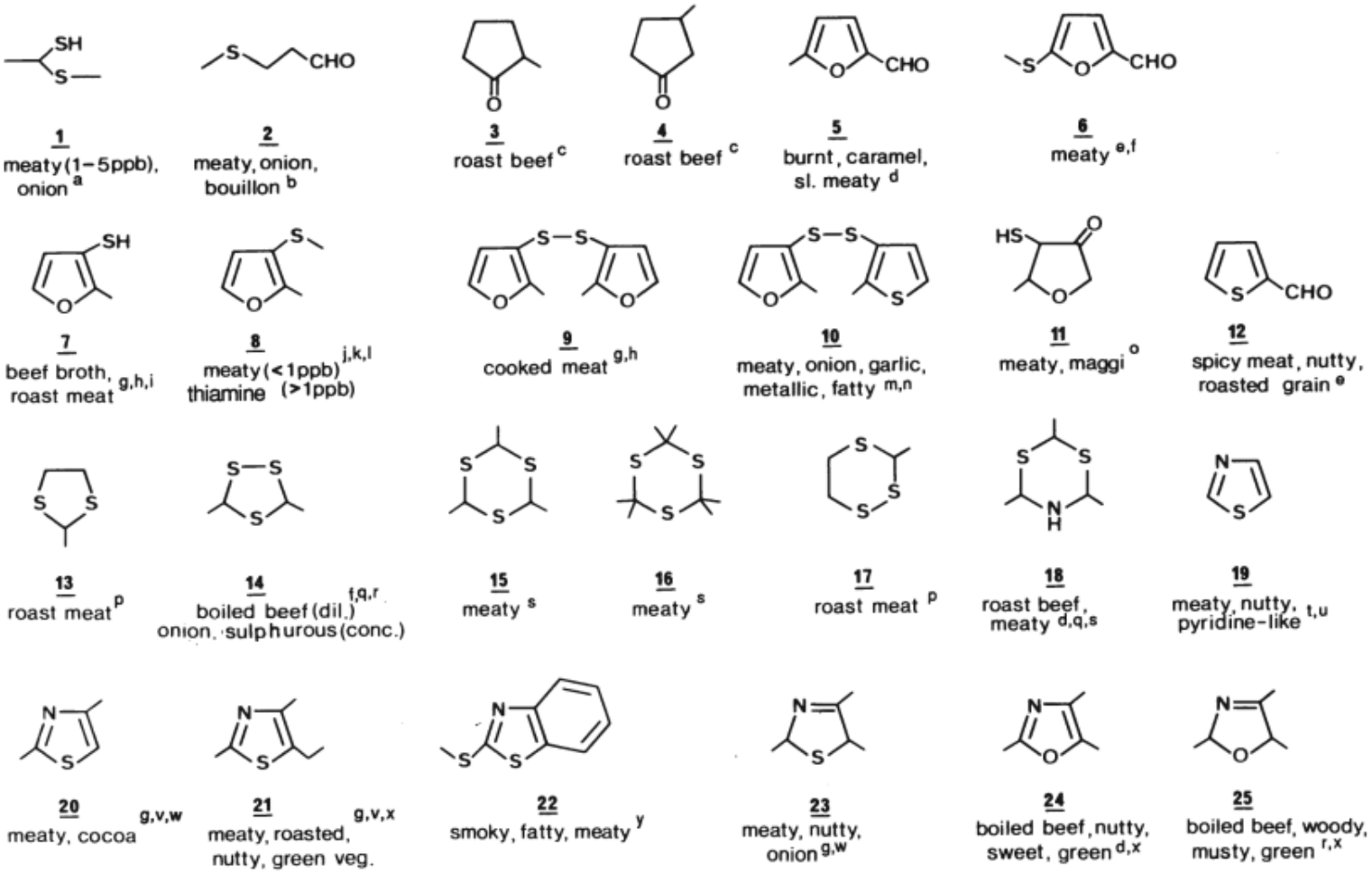

Figure 1 Structures of meaty flavour compounds identified from beef (MacLeod, 1994)

2.5 Effect of PEF Processing on Meat

Pulsed electric field (PEF) has been applied in food processing since the 1950s (Allen & Soike, 1966). Barbosa-Cánovas and Altunakar (2006) reported that PEF was one of the most appealing non-thermal technologies, because of its short processing time and its less damaging effects on processed food. This method has been widely used in the processing of liquid foods, such as fruit juices and milk for decades (Aronsson, Borch, Stenlöf, & Rönner, 2004; Grimi, Mamouni, Lebovka, Vorobiev, & Vaxelaire, 2011; Mosqueda-Melgar, Elez-Martínez, Raybaudi-Massilia, & Martín-Belloso, 2008) and recently there are increasing researches focused on the application of PEF on solid food such as meat (Barbosa-Cánovas & Altunakar, 2006; Bekhit et al., 2014; Faridnia et al., 2014; O'Dowd et al., 2013).

2.5.1 PEF Components and Principle

The PEF processing equipment comprises a high voltage pulse generator, a treatment chamber and an operation system with a monitor and controlling devices (Barbosa-Cánovas & Altunakar, 2006). The treatment chamber constitutes two stainless steel electrodes insulated with a nonconductive material to avoid electrical flow between them. The food sample is placed in the treatment chamber which is set between the two electrodes. High voltage electrical pulses (μs) passing through the electrodes, conduct the high intensity electrical pulse to the treatment chamber, which provides the food sample a force per unit charge (Barbosa-Cánovas & Altunakar, 2006; Puértolas, Luengo, Álvarez, & Raso, 2012).

The application of PEF processing on foods is based on a pulsing power applied to the food sample between the two electrodes (Barbosa-Cánovas & Altunakar, 2006). The transportation of substances in and out of the cell is controlled by the pores in cellular membranes. Electrical potential from the electric field can move across the cell membrane, which may change the structure of the cell membrane and result in a rapid electrical breakdown of the membrane (Angersbach et al., 2000). PEF destroys cell membranes by removing the cellular turgor component of the texture, which in turn affects the viscoelastic properties of food tissues (Lebovka et al., 2002).

2.5.2 Application of PEF on Meat and Meat Products

The application of PEF processing can induce several changes in the structure and texture of meat, potentially improving its functional properties or aiding in the development of new products (Toepfl, Heinz, & Knorr, 2007). PEF technology can be applied as a relatively new method for cell disintegration (Knorr et al., 2013). Studies on the effect of electro permeabilization of protein-based foods such as fish and meat are limited, and the different experimental setups and processing parameters make them difficult to be compared (Töpfl, 2006). Table 4 summarizes several applications of PEF on beef meat in the past three years. The electric field strengths applied on beef samples normally range from 1.1-10.0 kV cm-1 (Bekhit et al., 2014; Carne, van de Ven, Bekhit, & Hopkins, 2015a; O'Dowd et al., 2013). It has been reported that PEF processing can decrease the shear force in beef muscles (Bekhit et al., 2014; Faridnia et al., 2014). Arroyo et al. (2015) also reported that PEF treatment could further improve the sensory texture and odour of beef longissimus lumborum muscle. In addition lipid oxidation was reported to increase with PEF processing (Faridnia et al., 2014).

Table 4 Summary of recent studies on applications of PEF used on meat and meat products

Type of meat	Processing parameters	Results	Reference
Beef ST muscle from Limousin cross heifers	Electric field strength: 1.1-2.8kV/cm; frequency: 5-200Hz; pulse number: 152-300; total energy input: 12.7-226kj/kg.	This study compared conventional and PEF treated beef muscles. PEF treatment significantly decreased the weight loss of beef muscles, as well as a and b colour values. There was no difference in quality and other physiochemical characteristics between conventional and PEF treated beef samples.	O'Dowd et al. (2013)
Beef loins (*longissimus lumborum*, LL) and topsides (*semimembranosus*, SM) muscles	Electric field strength: 5 and 10 kV/cm; frequencies: 20, 50 and 90 Hz.	This study indicated that PEF decreased 19 % shear force in both LL and SM beef muscles. The tenderness in SM beef muscles was not influenced by the muscle post-mortem time. Cooking loss was improved by PEF treatment, and might contribute to positive sensory attributes such as juiciness.	Bekhit et al. (2014)
Fresh and frozen-thawed beef ST muscles from 9 animals	Electric field strength: 1.4 kV/cm; frequency: 50 Hz, pulse number: 1032; total energy input: 250 kJ/kg.	PEF processing affected microstructure, texture and water holding ability of beef muscle tissues. The shear force in frozen–thawed beef samples was decreased by PEF treatment but the case for fresh samples. The authors suggested that both application of PEF and pre-treatment (fresh or frozen-thawed) are important factors to consider that influences meat tenderization. In addition, PEF also increased lipid oxidation of beef muscles, and the volatile profiles of beef samples showed some differences after PEF processing, with little change in fatty acid composition.	Faridnia et al. (2014)

Beef *longissimus thoracis et lumborum* (LTL) muscles from steers under 30 months	Electric field strength: 1.4 kV/cm; frequency: 10 Hz; pulse width: 20μs; pulse number: 300 and 600; total energy input: 25 kJ/kg and 50 kJ/kg.	This study indicated that the PEF did not negatively affect cooking loss, storage loss and colour regardless of the ageing period before and after PEF application. PEF treatment also reduced the toughness of beef muscles at different post-mortem times, but the tenderization process provided by ageing was not affected. PEF treatment resulted in a better texture sensory, but had similar odour to non-PEF treated beef samples.	Arroyo et al. (2015)
Beef Loins (LL) and topsides (SM) muscles	Electric field strength: 5 and 10 kV/cm; frequencies: 20, 50 and 90 Hz.	LL and SM beef muscles were influenced by PEF treatments. The shear force in SM muscles was related to the frequency of PEF treatment, and the post-mortem time did not affect the tenderness of SM muscles. However the shear force in LL muscles did not depend on PEF frequency.	Carne et al. (2015a)
Beef loins (LL) and topsides (SM) muscles	Electric field strength: 5 and 10 kV/cm; frequencies: 20, 50 and 90 Hz.	This study indicated that PEF decreased shear force of SM beef muscles by 21.6% but resulted in tougher LL beef muscles with higher PEF frequency. The water holding capacity of both LL and SM muscles were significantly affected. After PEF processing, the cooking loss decreased with ageing and purge loss was significantly increased in SM beef muscles. LL muscle had an increased cooking loss and similar purge loss after PEF processing.	Carne et al. (2015b)

O'Dowd et al. (2013) stated that PEF could lead to several changes in beef muscle fibres and affect the attributes of beef quality in terms of colour, tenderness and sensory characteristics. Their work showed that PEF treatment significantly induced weight loss but had no impact on Hunter colour values compared to a comparable ΔT induced by the use of a water bath, which reduced both a and b colour values. Other quality and physicochemical characteristics were not affected. The lack of impact on tenderness was attributed to the low electric fields applied (1.36 kV cm^{-1}) that however influenced the microstructure and texture of chicken and fish (Gudmundsson & Hafsteinsson, 2001). In addition, post-mortem proteolysis showed an increase in both troponin and desmin degradation in beef LL treated with low intensity PEF treatment (20 Hz) compared to non-treated samples (Carne et al., 2015b).

The effects of PEF on the quality of different meat muscles are variable. Beef *longissimus lumborum* (LL) muscles were found to be tougher with increasing treatment frequency, whereas beef *semimembranosus* (SM) muscle was found to have up to 21.6% reduction in shear force with pulsed electric field treatment (Carne et al., 2015b). Faridnia et al., (2015) further investigated that relationship between freezing prior to PEF and changes in beef tissue microstructure that influenced storage stability and safety. Results revealed that combined freeze-thawing and PEF resulted in improved tenderness as indicated by reduced shear force, compared to PEF processing alone.

The tissue microstructure of beef muscles can influence tenderness and water holding ability of beef muscles. The SEM micrographs of PEF treated beef muscles indicated that myofibrils of PEF treated beef muscles were ruptured along with the z-lines (Faridnia et al., 2015). As a result, the activity of proteolytic enzymes in the muscle tissues increased due to ruptures in myofibrils resulting in breakdown of relative microstructure in beef cells. Faridnia et al. (2015) also mentioned that PEF significantly increased purge loss, but did not affect cooking loss. A two log-unit increase in aerobic microbial counts during log phase of frozen-thawed PEF-treated samples was positively associated with increased purge loss. PEF however did not affect the ratios of polyunsaturated/saturated fatty acids and omega 6/omega 3 free fatty acid profiles.

2.6 Temporal Dominance of Sensations (TDS)

Traditional descriptive sensory techniques are widely applied to judge the total sensations of foods. This method can evaluate the average intensity of a sensory attribute, but as changes in sensory characteristics can change over time during the eating process, important temporal sensory information may be amiss (Cliff & Heymann, 1993). Normally it takes time for food to be savoured and reach maximum intensity since flavour decreases and disappears after the food has been swallowed (Halpern, 1991). Therefore, temporal sensory methodologies are becoming increasingly important in understanding how food products are perceived by consumers (Piggott, 2000).

In order to decrease the effect of halo-dumping and sensory evaluation in real time, Temporal dominance of sensations (TDS) was developed as a new sensory analytical method at the "Centre Européen des Sciences du Goût" in the LIRIS lab in 1999, and was first presented at the Pangborn Symposium by Pineau, Cordell and Schlich in 2003 (Di Monaco, Su, Masi, & Cavella, 2014; Pineau et al., 2009).

2.6.1 TDS Procedures

TDS requires that all panellists have good understanding of conventional descriptive methods (Pineau et al., 2012). After several training sessions, the qualified panellists are familiarised with all the definitions of dominance of sensations perceived (Meyners, 2010). However, over-training is not necessary, because it may cause individual type responses that may lead to all products being described in the same order (S Meillon, Urbano, & Schlich, 2009). In order to improve the list of dominant attributes, TDS training is more oriented to sample description (Pineau et al., 2012). As a result, consensus must be reached among panellists on the definition of each attribute (Di Monaco et al., 2014).

The attribute list is a selected list of all the attributes that describes the examined product. The most common method to select sensory attributes is to provide panellists samples with different flavour properties, get them to discuss all the sensations perceived, and then classify different sensory descriptions and combine similar characteristics, leaving only a few major attributes that are selected for further TDS

analysis (Albert, Salvador, Schlich, & Fiszman, 2012; Lenfant, Loret, Pineau, Hartmann, & Martin, 2009; Paulsen, Næs, Ueland, Rukke, & Hersleth, 2013).

The evaluation using TDS starts when the samples are placed into panellists' mouths. All the selected attributes are shown on the same computer screen using the FIZZ software with scales ranging from "weak" to "strong". The panellists record all the changes in sensations of selected attributes during the whole chewing process. Panellists are free to choose the same attribute several times as long as they think it is dominant (Di Monaco et al., 2014). It must be pointed out that the attribute is registered as dominant until another attribute is chosen. Only one attribute can be selected at each time, but not all attributes shown on the screen have to be used (Pineau et al., 2009).

2.6.2 Application of TDS

Due to the complexity of sensory properties in various food products, TDS is a very useful method to analyse the changes in sensory attributes during consumption (Le Révérend, Hidrio, Fernandes, & Aubry, 2008). Many researches compared the difference between TDS and other sensory analysis methods (Albert et al., 2012; Sokolowsky & Fischer, 2012; Vázquez-Araújo, Parker, & Woods, 2013). TDS was found to have particular advantages but the other methods were also suitable to describe the sensory attributes of food.

TDS has been widely applied to describe sensory characteristics of many different types of food. Most researches gained benefits from TDS analysis, which explained flavour perception of consumers better (Bruzzone, Ares, & Giménez, 2013). This methodology effectively provide abundant temporal sensory profiles for many sensory attributes (S Meillon et al., 2009; Pineau et al., 2009). It can also be used to analyse the influence of a single ingredient on the whole food product, such as the impact of gel structure on salt release in cheese (De Loubens et al., 2011). Table 5 summarizes the studies carried out using TDS on different types of food products over the past five years. Most of these studies compared TDS with other descriptive methods.

Table 5 Summary of TDS on different types of foods

Food products	Panellists	Compared methods	References
	Liquid		
Commercial 2006 South Australian Syrah wine	8 trained panellists (2 males, 6 females, 30 - 56 years)	None	Sophie Meillon et al. (2010)
Bottled waters and de-chlorinated tap waters	16 selected and trained panellists	Sensory Profiling	Teillet, Schlich, Urbano, Cordelle, and Guichard (2010)
Commercial flavoured vodka	10 panellists (7 males, 3 females, 19 - 45 years)	None	Déléris et al. (2011)
Commercial dry white wines	18 panellists (9 males, 9 females, 24 - 56 years)	Quantitative Descriptive Analysis; Time Intensity	Sokolowsky and Fischer (2012)
Commercial blackcurrant squashes	11 trained panellists (1 males, 10 females, 30 - 55 years)	Quantitative Descriptive Analysis	Ng et al. (2012)
Espresso coffee	12 trained panellists	Headspace; Nose-space	Barron et al. (2012)
Commercial coffee	13 panellists, (6 males, 7 females, 20 - 29 years)	None	Dinnella, Masi, Naes, and Monteleone (2013)
Traditional English ale beer, American-style lager, European-style lager	12 highly trained panellists	Time Intensity; Drinking Profile	Vázquez-Araújo et al. (2013)

	Semi-solid		
Cheeses	16 trained panellists, volunteer and motivated	None	De Loubens et al. (2011)
Yoghurt	10 trained panellists, 20 – 50 years, with a minimum of 200 h of experience in discrimination and descriptive tests and at least 30 h of experience in the evaluation of dairy products	Quantitative Descriptive Analysis	Bruzzone et al. (2013)
Ice-cream	14 panellists had previous experience in sensory descriptive analysis, but no specific training on ice-cream description	None	Varela, Pintor, and Fiszman (2014)
	Solid		
Wheat flakes	25 panellists (12 males, 13 females, 20 - 35 years)	None	Lenfant et al. (2009)
Commercial frozen precooked breaded fish sticks	9 trained panellists were familiar with sensory evaluation	Key Attribute Sensory Profiling	Albert et al. (2012)
Biscuits	13 trained panellists with previous experience in quantitative descriptive analysis	None	Laguna, Varela, Salvador, and Fiszman (2013)
Different types of nuts (blanched almonds, roasted cashews, raw macadamia nuts, and roasted peanuts)	40 panellists (20 males, 20 females, 21 -29 years, 55 -70 years)	None	Hutchings, Foster, Grigor, Bronlund, and Morgenstern (2014)
Berliner and Ardenner sausages	23 trained panellists	None	de Lavergne, Derks, Ketel, de Wijk, and Stieger (2015)

The TDS method has been widely used on different types of foods which are either liquid, semi-solid or solid. Most panellists are trained and were familiar with the sensory attributes of the tested food products. To date, TDS analysis on beef and beef product has not been carried out. Only limited studies on TDS analysis of fish sticks and sausages have been carried which may share some common sensory characteristics of beef and beef products. Albert et al. (2012) examined attributes of crunchiness, juiciness, oiliness, fried flavour, different texture, bolus and swallowing in fried fish sticks using TDS. The dominance rates represented sensorial changes during consumption. The sensory attributes in sausages are different from fish sticks, and de Lavergne et al. (2015) investigated the attributes of smooth, hard, fatty, sticky, juicy and grainy in sausages. Due to the normalised or standardized time line, the dominance rates of all selected attributes in different samples were examined at real time.

Many researches not only use TDS for sensory analysis but also compares it with other methods. Time intensity (TI) is a well-known method to characterize specific organoleptic properties. Le Révérend et al. (2008) stated that both TI and TDS showed similar results in the sensory evaluation of dairy products. Although TI was good at determining the kinetic of one specific attribute, TDS could describe the sensory changes of food products over time, and showed additional multi-sensory attributes information and their interaction. Quantitative Descriptive Analysis (QDA) is the most common descriptive analysis method used to evaluate the sensory characteristics of a food product, and TDS is the most used method to determine the dominant sensory properties during consumption. Although the advantages of TDS is clear, it does not mean that the other methods should be abandoned. In fact, TDS should be seen as a method that complements other descriptive sensory methods as it provides the perception of aroma, taste, flavour and texture changes during consumption.

Chapter 3 Materials and Methods

3.1 Raw Materials and Sampling Procedure

Semitendinosus (ST) and *bicep femoris* (BF) (Figure 2) were used in this research as they were the two most common beef cuts consumed. Both were sampled from fresh and frozen beef cuts separately.

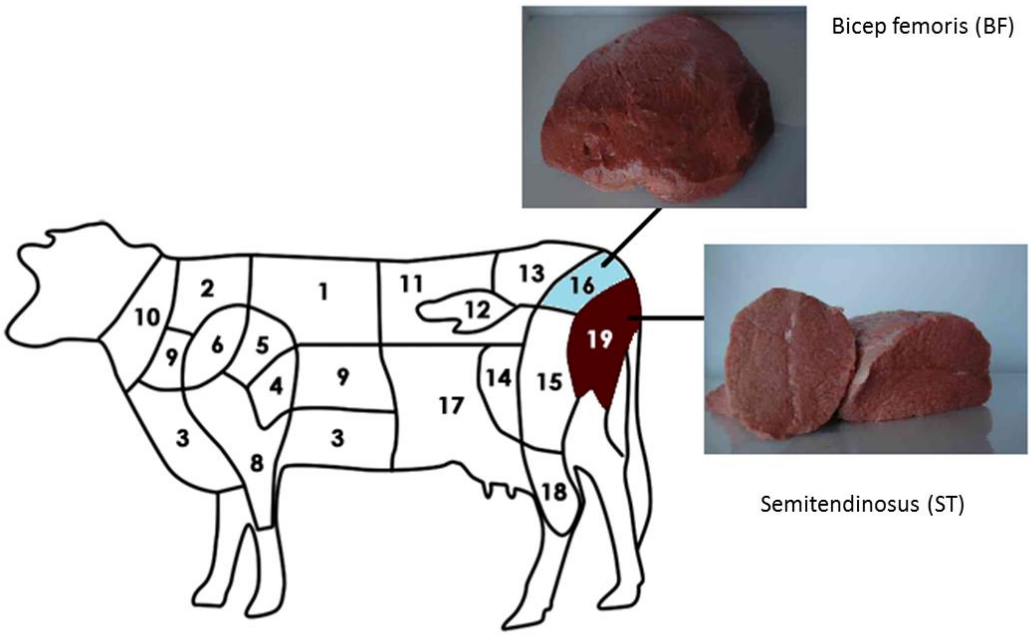

Figure 2 ST and BF beef muscles in cattle

Three animals (mean carcasses weight of 260~285 kg) were used in this study. ST and BF muscles were gained at 24 h post-mortem from a commercial slaughter-house in Dunedin (Silver Fern Farms Ltd., Finegand Plant, Balclutha, New Zealand), and then vacuum packed. Upon arrival, half portion of the muscles was used as fresh sample and the other half portion was stored at -20°C for about 3 months until use. Prior to further PEF processing, the frozen samples were thawed overnight at 4 °C.

3.2 Pulsed Electric Field (PEF) Treatments

Three PEF batch treatment chambers of different sizes were available (Figure 3). The middle chamber in Figure 3 (6 cm height × 4 cm width × 6 cm length) was used for

further PEF treatment to reduce the occurrence of gaps in meat samples and avoid flashover during PEF processing.

Both fresh and frozen-thawed samples (both ST and BF cuts) were further divided into two groups separately. One of which was subjected to pulsed electric field (PEF) treatment (‘fresh-ST-PEF’ (FSP), ‘fresh-BF-PEF’ (FBP), ‘frozen-ST-PEF’ (SP) and ‘frozen-BF-PEF’ (BP)) and another retained as ‘control’ sample (‘fresh-ST-control’ (FSC), ‘fresh-BF-control’ (FBC), ‘frozen-ST-control’ (SC) and ‘frozen-BF-control’ (BC)). In order to fit the samples in a chamber with a triangular groove (Figure 3-b) which has been shown in the middle of Figure 3, muscles for both control and PEF samples were cut parallel to the fibre direction into triangular pieces to fill the selected PEF batch treatment chamber. The weight of sample was approximately 65 g per piece and the fibre direction was arranged in such a way that the fibre direction was perpendicular to the electric current, which makes pulsed electric directly pass through muscle fibres without flashing out caused by the gaps in meat.

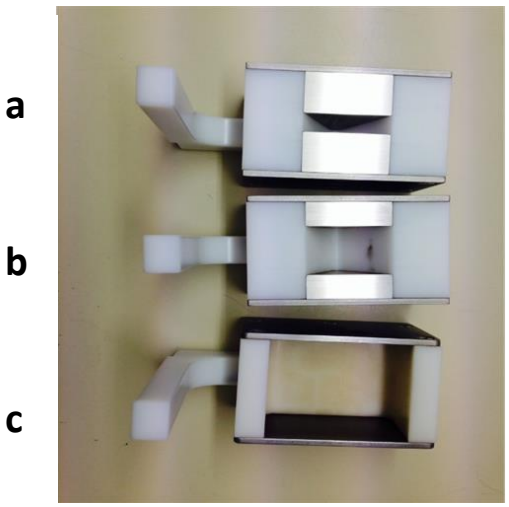

Figure 3 PEF batch treatment chambers

The beef samples were processed in a pilot plant scale PEF system (Elcrack-HVP 5, DIL Quakenbruck, Germany) (Figure 4) using a batch mode configuration. In order to determine the parameters of PEF processing, there were several different levels of electric field strength tested in preliminary trials. By assessing visual quality of beef samples and stability of PEF processing, the electric field strength was targeted at 0.8-1.1 kV/cm, pulse width 20 μs, frequency 50 Hz, which provided a stable and effective electric field that ensured meat samples were not overcooked. Colour of meat was

observed and if there was a change from red to grey, this indicated protein breakdown by electric field processing.

Figure 4 PEF system (Elcrack-HVP 5, DIL Quakenbruck, Germany)

Pulse shape (square wave bipolar) was monitored on-line with an oscilloscope (Model UT2025C, Uni-Trend Group Ltd., Hong Kong, China) during PEF processing. The specific input energy (W_{spec}) applied to meat samples at square-wave pulse was calculated using Equation (1) (Faridnia et al., 2015).

$$W_{spec}(kj/kg) = \frac{V^2 \times (N\tau)}{R \times W} \qquad (1)$$

V is the pulse peak voltage (kV), N is the number of pulses (dimensionless), τ is the pulse width of square pulses (µs), R is the effective load resistance (ohm) and W is the weight of sample (kg) in the PEF treatment chamber. All PEF conditions and processing parameters impact on the changes in temperature and conductivity. These changes are summarized in Table 6.

Table 6 Effects of PEF processing parameters on the changes in temperature and conductivity

		Fresh		Frozen	
		ST	BF	ST	BF
Weight of sample (g)		63.98±3.94	63.78±4.46	62.15±4.05	63.68±4.86
pH	before	4.71±0.94	4.80±1.12	6.14±0.35	5.44±0.20
	after	5.16±0.14	4.92±0.45	6.16±0.23	5.53±0.09
Conductivity (S/m)	before	10.55±0.78	9.45±0.64	8.30±3.25	6.85±3.46
	after	13.05±0.07	12.20±0.42	12.75±0.35	12.90±0.00
Temperature (°C)	before	15.15±3.75	13.55±0.49	13.95±0.64	11.70±1.41
	after	31.10±2.40	28.67±5.14	26.70±0.99	24.90±3.68
Pulse voltage (kV)		3.17±0.12	3.26±0.10	3.32±0.12	3.33±0.13
Pulse current (A)		81.61±1.61	80.45±1.33	79.42±1.53	79.59±1.79
Pulse power (kW)		258.2±4.53	261.72±3.52	264.65±3.85	264.17±4.30
Pulse energy (J)		4.39±0.09	4.45±0.068	4.49±0.07	4.49±0.07
Pulsed electric strength (kV/cm)		0.8-1.1			
Pulse number		1843.96±0.45	1844.15±0.63	1844.23±0.75	1844.50±0.62
Pulse resistance (ohm)		38.84±2.17	40.59±1.98	42.04±2.12	41.74±2.52
Calculated energy (kJ/kg)		127.05±10.11	129.53±11.01	133.95±10.42	130.96±11.69

The temperature (°C), pH and electrical conductivity (σ) of all the samples were measured before and after PEF treatment. The initial temperature of beef samples was maintained at 4 °C, using a temperature logger (Grant Squirrel SQ800, Cambridge, UK). The pH was measured by inserting a calibrated pH probe (HANNA HI 98140, Woonsocket, USA). The electrical conductivity of beef samples was determined by a hand held meat conductivity meter (LF-STAR, R. Mathäus, Germany). For all measurements, the probes were directly inserted into the meat samples at three different positions.

After PEF treatment, samples were vacuum packed in polyamide polyethylene bags separately (approximate 70g each sample). For fresh samples, half of them was stored at -20 °C immediately as 0 day aging samples (fresh sample 0), and another half was stored at 4 °C for 7 days followed by storage at -20 °C as 7 days stored sample (fresh sample 7). Frozen samples were divided into two half as well, one was immediately stored at -20 °C (frozen sample 0), and another was stored at 4 °C for 7 days before being stored at -20 °C (frozen sample 7). In total, 16 independent samples were used (n= 16). All the samples and their corresponding codes are summarized in Table 7.

Table 7 Summary of samples used in this experiment

Refrigeration	Beef cuts	PEF processing	Storage days	Sample code
Fresh	ST	PEF	0	FSP0
			7	FSP7
		Control	0	FSC0
			7	FSC7
	BF	PEF	0	FBP0
			7	FBP7
		Control	0	FBC0
			7	FBC7
Frozen	ST	PEF	0	SP0
			7	SP7
		Control	0	SC0
			7	SC7
	BF	PEF	0	BP0
			7	BP7
		Control	0	BC0
			7	BC7

3.3 Temporal Dominance of Sensations (TDS) Sensory Profiling

3.3.1 Ethics Statement and Test Conditions

All the panellists were informed about this study in advance and they consented. This study obtained ethics approval (AUTEC 13/317) from the Auckland University of Technology Ethics Committee.

Sensory testing took place at the Auckland University of Technology sensory testing facility in Auckland, New Zealand. Each test session lasted about 2 hours. As compensation, participants received gift vouchers. The presentation design of the samples utilized balanced position and order effects. Products were coded with three-digit random numbers and placed into a 100 ml container served at room temperature (22 °C). Panellists rinsed between each product evaluation with water and unsalted crackers during a 45-second break. Tests were carried out in individual booths under white light at 18 °C.

3.3.2 Sample Preparation

Before cooking, the samples stored at -20 °C in vacuum bags were thawed at 4 °C for 24 h. The sous vide method of cooking according to Charley and Weaver (1998) was employed. Vacuum packed samples were placed into a water bath (Model 360, Contherm, New Zealand) at 60 °C for 2 hours. Samples were removed from the packaging and then seared using a griller (Breville BGR200BSS Healthsmart Grill, Australia). The grill plate was pre-heated to a temperature of 180 °C to ensure heat was equally distributed between bottom and top plates. The beef samples were then placed on the bottom plate and cooked for 10 s before the top plate was lowered and further cooked for 2 min that resulted in an internal temperature of 73±2.5 °C. The samples were cut into equal small pieces (approximate 5g) and placed into small containers prior to serving. The serving temperature (approximate 70 °C) was strictly monitored to maintain consistency. Samples were coded with a 3-digit number, randomized, and counter balanced (MacFie, Bratchell, Greenhoff, & Vallis, 1989).

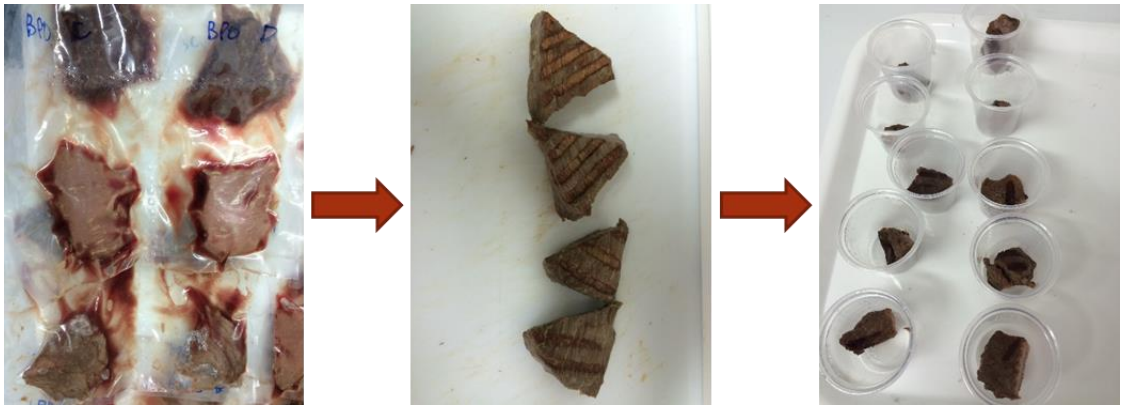

Figure 5 The preparation of samples for sensory testing

3.3.3 Panellists and Panel Training

Ten trained panellists aged between 21 and 29 years old participated in this study. They were recruited online and received a voucher for their participation. The participants were non-smokers, and did not suffer from any eating disorders and health problems associated with food. Informed written consent was obtained prior to participation. The trials were carried out between 2:00 to 4:00 pm from Tuesday to Thursday (December, 2014), and replicated twice with the same panelists.

Panel training was carried out over three sessions (12 hours in total). In the first session, all panellists were informed about the definitions and temporal measurement principles of the TDS method according to Pineau et al. (2009). This helped panellists to understand the concept and measurement of dominant sensations in the TDS procedure. Panellists were trained to have a specific cognition of dominant attributes that were dominant at a given time (Pineau et al., 2009). They were also trained to use an unstructured 100 mm line scale, anchored with "None" at the left end and with "Extreme" at the right end (Pineau et al., 2009). Panellists were informed that dominance might change when a new sensation is perceived. These sensations were then rated using a line scale.

In the second session, panellists consumed cooked meat samples and listed the relevant sensory attributes of cooked meat. A total of 18 relevant sensory attributes were selected. These attributes were further refined by the panel. After group discussion, five sensory attributes of beef meat were confirmed with inter-agreement among all panellist. Definitions of attributes and reference food samples used to describe attributes are summarized in Table 8. Finally, the panellists were introduced to the FIZZ Acquisition Software (Biosystems, Counternon, France) to help them understand the TDS interface in actual operation. A dummy TDS trial was carried out by the panellists to assess cooked beef meat based on selected sensory attributes.

Table 8 Descriptions of sensory attributes used in the TDS trial

Attributes	Definition	Reference sample
Brothy	Flavours and aromatics associated with boiled meat or soup stock.	MSG 0.5% in water.
Browned	Flavours associated with meat that is cooked and charred on the outside.	Beef (same size as our samples) cooked at 70 °C, allowed to brown on each side (10 min).
Juicy	Amount of water retained in cooked meat that contribute to succulence.	Different cuts of beef with varying levels of juiciness.
Livery	Taste associated with animal organs.	Beef liver cooked at 70 °C for 10 min.
Oxidized/ warmed over flavour	Flavour of reheated meat.	Cooked beef that was refrigerated for at least 24 h before reheating.

3.3.4 TDS Procedure

Five selected sensory attributes (brothy, browned, juicy, livery and oxidized) were examined in the TDS process. On a computer screen, all attributes as well as detailed consumption instructions were displayed. The attribute labels were presented with 100 mm unstructured line scales in the computer screen, anchored at the extremities with 'not at all intense' and 'very intense'.

TDS data collection started when panellists clicked on a start button on the screen to begin the evaluation. During an evaluation, the panellist selected the dominant attribute and rated it on an unstructured line scale. If the dominant perception changed, the panellist changed to the on-screen scale that corresponded to the new flavour sensation and rated its intensity. The panellist was free to choose the same attribute several times or, conversely, to not select an attribute as being dominant. A duration parameter was also computed as the time that elapsed between the elicitation of the given attribute and the following attribute elicited. This means an attribute is considered as dominant until another attribute is scored as such. Data obtained were recorded using the FIZZ Acquisition software (FIZZ Network v2.46b, Biosystemes) as TDS ratings, at every second for up to 80 seconds. A compulsory 1 minute break in between samples was provided to allow panellists to drink and rinse the mouth with filtered water.

3.3.5 Panel Dominance Curves

Panel dominance curves for TDS were generated using the FIZZ Calculations software (FIZZ Calculations v2.46b, Biosystemes). The determination of chance and dominance levels on panel curves were carried out using the methods developed by Pineau et al. (2009), and Lenfant et al. (2009). Chance level, is the dominance rate that an attribute would obtain by chance. Its value, P_0, is equal to 1/p, where p is the number of attributes. Significance level is the minimum value this proportion should equal to be considered as significantly higher than P_0. This was calculated using the confidence interval of a binomial proportion based on a normal approximation. Temporal dominance curves depict the proportion of panellists who selected the attribute as dominant at a given time. The higher the dominance rate for the attribute, the better the agreement among panellists.

3.3.6 Statistical Analysis

Canonical Variate Analysis (CVA) was conducted (dominance duration as variable) to evaluate the differences between products based on sensory attributes. In addition, Hotelling Lawley Multiple Analysis of Variance (MANOVA) was carried out in order to observe significant differences between the samples differing in storage and treatments.

Significance level was set to 5%, all the univariate and multivariate analysis in this study was carried out using XLSTAT 2014 (Addinsoft, U.S.A)

3.4 Initial Tenderness

Initial tenderness was defined as the minimum force necessary (first bite) to bite the meat sample with incisors teeth. The panellists were severed 3 different cuts (tenderloin, shoulder, and leg) of grilled beef (70 °C for 5 min) to understand the initial tenderness.

At the beginning of TDS procedure, panellists were instructed to rate initial tenderness of meat after their first bite. The intensity of initial tenderness was measured using a scale from 0 (least tender) to 100 (most tender). Two-way Analysis of Variance (ANOVA) was carried out to observe the main effect of storage and treatment and interaction effect of storage and treatment on the initial tenderness of the meat sample. In addition, post hoc Tukey's analysis was carried out if significance was observed.

3.5 Colour Measurement

All the samples were thawed at 4 °C for 24 h, and then measured using a Hunter lab (45/0, Colour flex) colorimeter (Figure 6). The samples were cut into cubes (2cm *2cm* 2cm) and placed in a petri dish that was then covered and placed on top of the colorimeter lens. Three different colour measurements were made for each sample. Prior to use, the colorimeter was calibrated with a white tile and checked for recalibration in between measurements.

Colour values were recorded as *L**, *a** and *b**. *L** indicates lightness on a scale of 0 (all light absorbed) to 100 (all light reflected); a* spanned from +60 (red) to -60 (green); and b* spanned from +60 (yellow) to -60 (blue). Metric Chroma (C*) was calculated using Equation (2) (Hunter & Harold, 1987).

$$C* = \sqrt{a^{*2} + b^{*2}} \qquad (2)$$

Data collected in this study first separated by storage condition (fresh vs frozen) and different cuts (ST and BF). Paired student t-test was carried out in order to observe the significant differences between 0 and 7 days samples, and PEF treated samples. Significance level was set at 5% for all the univariate analysis carried out using XLSTAT 2014 (Addinsoft, U.S.A).

Figure 6 Hunter lab (45/0, Colour flex) colorimeter

3.6 Determination of Lipid Oxidation

Lipid oxidation was determined by the 2-thiobarbituric acid reactive substances (TBARS) method (Nam & Ahn, 2003). Minced meat sample (3.0 g) was homogenised in 9 ml of deionised distilled water (DDW) at 14,000 rpm for 30 seconds using a homogenizer mixer (Janke Kunkel IKA Labortechnik Ultra Turrax T25). The obtained beef homogenate (1 ml) was transferred to a disposable test tube. This was followed by addition of 50 µl of butylated hydroxytoluene (BHT) (7.2% w/v in ethanol) and 2 ml thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA and 15% (w/v) TCA). The mixture was vortexed and then incubated in a 90 °C water bath for 30 min to develop colour. Samples were then cooled down in an ice-water bath for 10 min, and centrifuged at 3500rpm for 15 min at 5 °C in homogenizer mixer (Eppendorf Centrifuge 5810 R). The absorbance of the resulting upper layer was measured at 531 nm using a spectrophotometer (Ultraspec 7000 Pro spectrophotometer, Biochrom Ltd, Cambridge, England, Figure 7) against a blank prepared with 1 ml deionised water and 2 ml TBA/TCA solution.

Figure 7 Ultraspec 7000 Pro spectrophotometer

The results are expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde (MDA) per kg of meat using a standard curve constructed using tetraethoxypropane (TEP). Mean values were obtained for triplicate samples (n = 3).

The data collected in this study was separated in terms of storage (fresh and frozen) and cuts (ST and BF). Separate Two-way ANOVA was carried out to observe the main effect of storage and treatment and interaction effect of storage and treatment on the lipid oxidation value of each meat cuts sample and each storage condition. In addition, post hoc Fisher's LSD analysis was carried out if significance was observed. Significance level was set at 5%. All univariate analysis was carried out using XLSTAT 2014 (Addinsoft, U.S.A).

3.7 Fatty Acid Analysis

Quantification of fatty acids was carried out by acid hydrolysis of lipids in lyophilized samples to release free fatty acids followed by in situ esterification to Fatty Acid Methyl Esters (FAMEs) and their extraction into toluene for analysis by Gas Chromatography (GC) (Juárez et al., 2008).

Samples were lyophilized for 48 hours and then approximately 20 mg samples were placed into 10 ml test tubes. A 10µl volume of 2 g/l tridecanoic acid in toluene was added as internal standard followed by further addition of 490 µl of toluene and 750 µl of freshly prepared 5% methanolic HCl. The mixture was mixed using a vortex and the headspace of each tube was filled with nitrogen. The tubes were then sealed and incubated in a water bath at 70 °C for 2 hours. After tubes were cooled down to room

temperature, 1 ml of 6% aqueous K_2CO_3 and 500 µl toluene were added. After vortexing gently to mix, the mixture was centrifuged at 2000 rpm for 5 min in homogenizer mixer (Eppendorf Centrifuge 5810 R). The organic phase was removed using a glass Pasteur pipette for analysis of FAME content.

For fatty acid analysis, a Shimadzu GC2010 GLC equipped with a Flame Ionisation Detector (FID) (Figure 8), a split injector and an AOC-20i auto-injector was used. The Phenomenex Zebron ZB-WAX capillary column (0.25mm x 30m x 0.25µm) was used with Nitrogen as a carrier gas. The pressure was set to 43 Pa, the flow rate was 7 ml/min, and the initial oven temperature was 140 °C, increased to 245 °C at a rate of 5 °C/min, and held for 15 minutes at this temperature. FAME peaks were identified and quantified by comparison with the retention times and peak areas of 37 FAME standards (Supelco product 47885-U, Sigma Aldrich, Sydney, Australia), which were serially diluted to five concentrations from 10 to 0.625 g /L.

Figure 8 Shimadzu GC2010 GLC equipped with a Flame Ionisation Detector

The data collected in this study was separated in terms of storage (fresh and frozen) and cuts (Semitendinosus and Biceps femoris). Separate Two-way ANOVA was carried out to observe the main effect of storage and treatment and interaction effect of storage and treatment on the free fatty acid value of each meat cuts sample and each storage condition. In addition, post hoc Fisher's LSD analysis was carried out if significance was observed.

Significance level was set at 5%. All univariate analysis was carried out using XLSTAT 2014 (Addinsoft, U.S.A)

3.8 Flavour Compounds Analysis

Flavour volatile analysis by Solid Phase Microextraction (SPME) and GC was carried out according to the previous study of Q. Ma, Hamid, Bekhit, Robertson, and Law (2012).

3.8.1 Extraction by SPME

Beef samples were lyophilized for 48 hours and then about 50 mg samples were placed into 20 ml flat bottom headspace vials. A 250 µl flat bottom insert was also placed in the headspace vial which was fitted with a PTFE/silicone septum and crimp cap (Supleco, USA). The head space vial was heated using a plate heater at 80 °C for 10 min. After cooling down, 2 µl of 1, 2-dichlorobenzene (1.3 ppm) solution was injected into the flat bottom insert as an internal standard. The SPME fibre was preconditioned prior to analysis at 250 °C for 30 min. After equilibration at 60 °C for 5 min, the volatile components in the samples were adsorbed onto a 50/30 µm layer of divinylbenzene-carboxen-polydimethylsiloxane (Supelco Co., Bellefonte, USA) fibre that was exposed to the sample headspace for 40 min.

3.8.2 GC analysis

After flavour extraction, volatiles entrapped in the SPME device was directly inserted into the injection port of the GC. The SPME fibre was immediately thermally desorbed for 3 min at 250 °C in the SPME-specific liner of the injector port of both the Gas Chromatography - Flame Ionisation Detector (GC-FID) and Gas Chromatography - Mass Spectrometry (GC-MS).

The Shimadzu GC-17A was equipped with a FID and a ZB-5 low bleed/MS fused-silica capillary column (5%-phenyl-95%- dimethylpolysiloxane phase, 30 m×0.53 mm×1.50 µm) (Phenomenex, Inc, USA). The carrier gas was nitrogen. The pressure was set to 43 Pa, flow rate was 7 ml/min, and the oven was held for 2 min at 40 °C, heated to 250 °C at 5 °C/min, and held for 3 min at this temperature.

The Trace GC Ultra (Thermo Scientific, USA, Figure 9) was equipped with a DSQ series mass spectrometer (Thermo Scientific, USA). The GC-MS was installed with a VF-5 ms low bleed/MS fused-silica capillary column (5%- phenyl–95%-dimethylpolysiloxane phase, 60 m×0.25 mm×0.25 µm) (Phenomenex). Helium was the carrier gas with a constant flow rate of 1.5 ml/min in the GC-MS. Chromatographic

conditions were as follows: the oven was held for 2 min at 40 °C, heated to 250 °C at 5 °C/min, and held 3 min at this temperature. The mass spectrometer operated in the electron impact mode with a source temperature of 200 °C, an ionising voltage of 70 eV, and the transfer line temperature was 250 °C. The mass spectrometer scanned masses from 48 to 400 m/z at a rate of 3.41 scan/s. Peak identification was carried out by comparison of their mass spectra with spectra in the NIST/EPA/NIH Mass Spectral Database (National Institute of Standards and Technology, Gaithersburg, MD, Version 2.0a, 2002, USA. To confirm the identity of volatile compounds, the retention index (RI) was calculated for each volatile compound using the retention times of a homologous series of C7 to C30 n-alkanes (1000 µg/ml in hexane from Supelco). The approximate quantities of the volatiles were estimated by comparison of their peak areas with that of the 1, 2-dichlorobenzene internal standard using a response factor.

Figure 9 The Trace GC Ultra (Thermo Scientific, USA) equipped with a DSQ series mass spectrometer

3.8.3 Statistical Analysis

Separate Two-way ANOVA was carried out to observe the main effect of storage and treatment and interaction effect of storage and treatment on the volatile compounds of each meat cuts sample and each storage condition. In addition, post hoc Fisher's LSD analysis was carried out if significance was observed. Significance level was set at 5%. All univariate analysis was carried out using XLSTAT 2014 (Addinsoft, U.S.A). The volatile compounds in control or PEF treated samples during 0 and 7 days storage were further analysed by Principal Component Analysis (PCA) using the XLSAT MX software release 2014.

Chapter 4 Results and Discussion

A New Zealand survey reported that while the majority of red meat (84.6%) purchased by consumers surveyed was fresh (rather than frozen), approximately 64% of the fresh meat they purchased was subsequently frozen in the home (Tropp & Gilbert, 2007). In this chapter, fresh and frozen beef meat will be discussed separately based on TDS, tenderness, colour, lipid oxidation, fatty acids and flavour compounds.

4.1 Fresh Beef Meat

4.1.1 Temporal Dominance of Sensations (TDS) Analysis of PEF Treated Fresh Beef Meat

The TDS methodology used yielded temporal dominance rates of different beef attributes (Albert et al., 2012; Pineau et al., 2009) immediately before and after PEF treatment, as well as after seven days after PEF treatment. The higher dominance rate indicate better agreement among panellists on a related attribute (Albert et al., 2012). In this study, the dominance rates of brothy, browned and oxidized attributes were above significant level representing main sensory sensations of samples in general.

The significance of TDS curves is to display the dominance rate of each sensation at different time during the eating period for a sample across the panel (Pineau et al., 2009). Figure 10 and figure 11 showed smoothed TDS curves using the spline equation for both ST and BF beef muscle samples immediately before and after PEF treatment, as well as after seven days after PEF treatment. In our study, with the use of trained panellists, the chance level was found to be 0.20, corresponding to a dominance rate of 20%. Therefore, taste attributes below 20% in our study were not considered dominant. The significance level was calculated considering the chance level and the 30 evaluations performed (10 panellists participated in triplicate trials). A significance level of 0.32 was obtained, corresponding to a dominance rate of 32%. TDS panel dominance curves afford identification of both dominance rate and time of evaluated attributes.

In general, brothy was the first dominant sensation in all samples, with the dominance rates starting at over 80% dominance rate and then decreasing to below chance level in less than 10 seconds. Dominance rate of the browned attribute increased from almost the start of mastication and reached a maximum in the first 3 seconds, then rapidly

decreased below chance level over the next 5 seconds. Starting from 10 seconds oxidized became the dominant attribute above significance level until the end of mastication. The attributes livery and juicy were occasionally above chance level, and only lasted a few seconds.

ST muscle

In ST beef muscles, brothy was the most significant at the start of mastication and then then decreased to below chance level in the first 10 seconds for all samples. Brothy however was the least dominant for sample FSC7 and FSP7 (Figure 10-D). For sample FSC7 and FSP7, brothy was dominant above chance level between 5 to 20 seconds, and 8 to 29 seconds, respectively. The oxidized attribute was dominant and above significance level from around 10 seconds until the end of mastication for all samples.

The dominance of livery did not reach significance level for all samples. It was only dominant above chance level for sample FSC7 between 20 and 50 seconds. The effects of aging on the livery intensity of Brown Swiss beef muscles over 21 days increased in the first 3 days, then decreased in the next 4 days, and finally increases from day 14 to 21 days of aging (Campo, Sañudo, Panea, Alberti, & Santolaria, 1999). They used a scale between 1 and 9 to measure the livery intensity of five breed type beef muscles, and most samples had an intensity of less than 2.

Only PEF treated samples had higher dominance of juiciness above chance level with FSP0 between 4 and 10 seconds (Figure 10-C). Juiciness however reached significance between 3 and 4 seconds for FSP7. The dominance of browned attribute increased significantly between 1 and 5 seconds for samples FSC0, FSC7 and FSP0 except for FSP7.

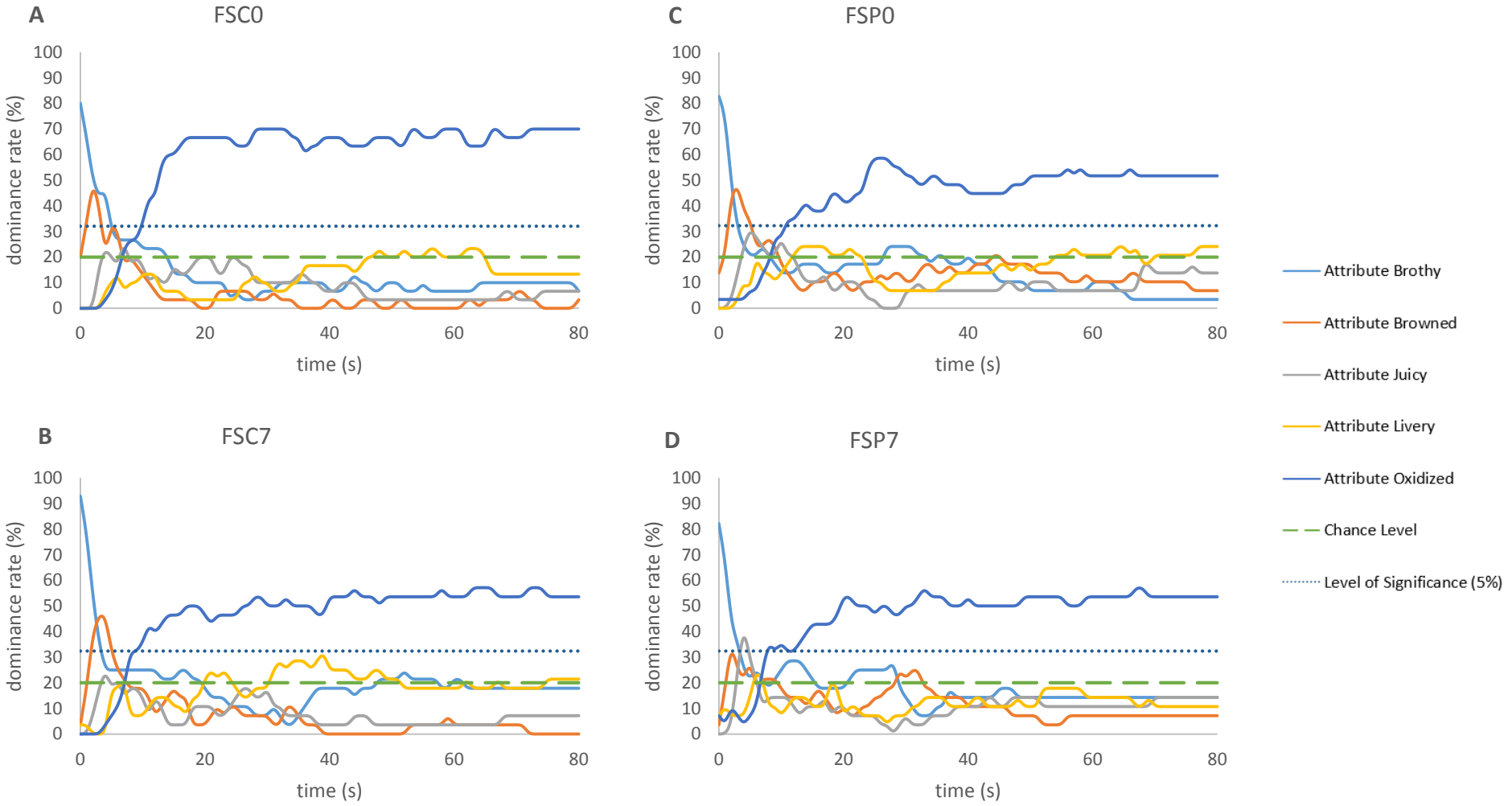

Figure 10 TDS curves for sensory attributes by fresh ST beef samples (FS, fresh semitendinosus beef samples; C, control samples; P, PEF treated samples; 0 and 7 days of aging period)

BF muscle

In BF beef muscles, brothy was the most significant attribute at the start of mastication and then then decreased to below chance level in the first 10 seconds for all samples. Brothy however was the least dominant for sample FBP7 (Figure 11-D). Oxidized also reached significance in all BF beef samples from about 10 seconds to the end. However there were no obvious differences in the dominance of oxidized between all samples.

The dominance of livery in all fresh BF beef samples was below significance level except for sample FBC7, which was livery above significance level between 9 and 12 seconds. In addition, for PEF treated samples (Figure 11-C and Figure 11-D) livery received a higher agreement of panels above chance level between 40 and 70 seconds. Juicy was only dominant at above chance level, in FBC0 and FBC7 samples at the start of mastication and then decreased to below chance level after 10 seconds. Browned on the other hand was only significant for samples FBP0 and FBC7 samples between 2 and 4 seconds, and 1 and 9 seconds respectively.

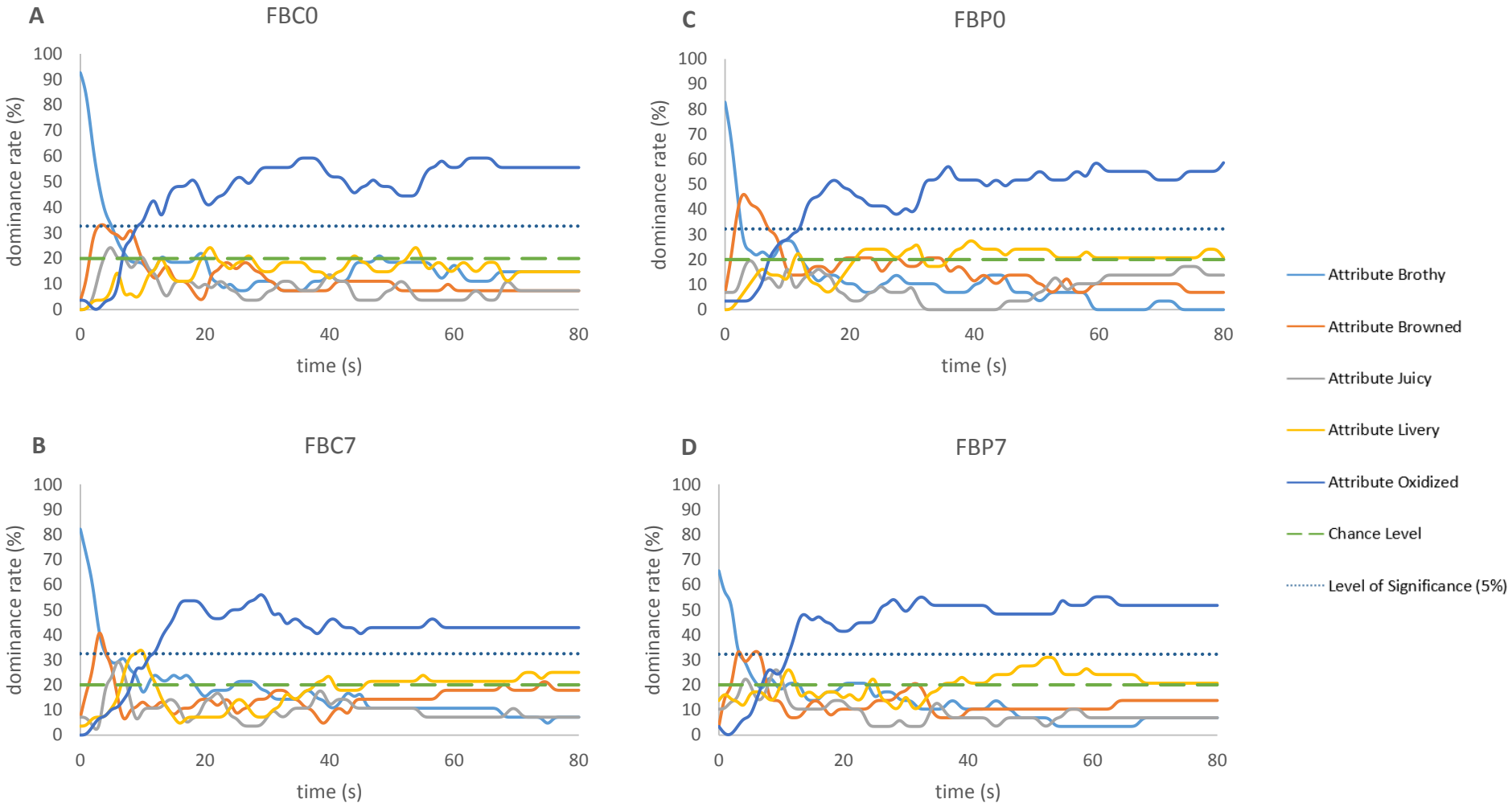

Figure 11 TDS curves for sensory attributes by fresh BF beef samples (FB, fresh Biceps femoris beef samples; C, control samples; P, PEF treated samples; 0 and 7 days of aging period)

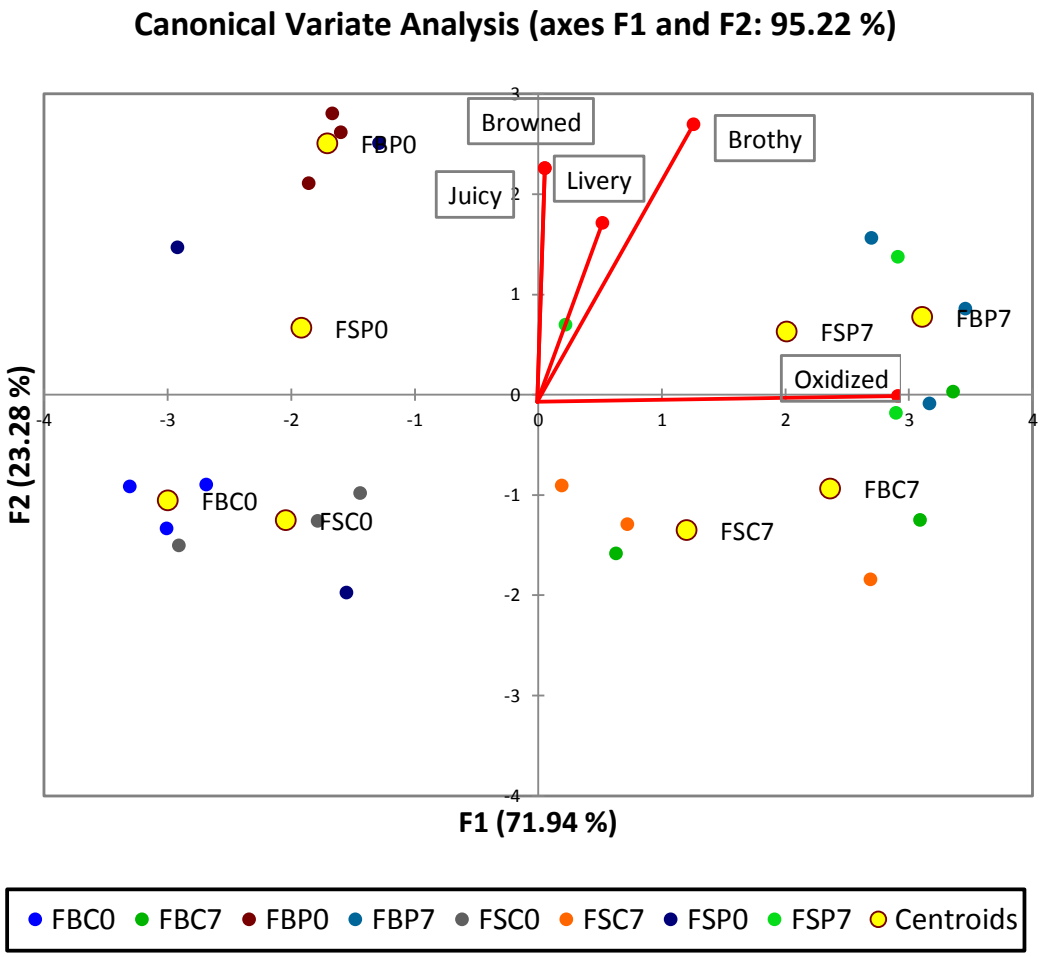

Figure 12 Canonical variate analysis of fresh meat cuts with different processing and storage time

Canonical Variate Analysis (CVA) was conducted with dominance duration as the dependent variable to evaluate the differences between muscle samples based on their sensory attributes as carried out by (Jager, Tijssen, Stieger, Schlich, & De Graaf, 2013). CVA was used in this study as it can maximize the distances between products, while minimizing residual variability (Monrozier & Danzart, 2001). Canonical Variate Analyses (CVA) was carried out on the duration of dominance per attribute (Figure 12). Sample discrimination was explained by the first two canonical variates that were high for sensory data (95.22%).

Hotelling Lawley MANOVA ($F_{(28,24)} = 4.521$; $p < .01$) showed significant differences between the samples in terms of the temporal flavour attributes measured by TDS. Samples differed mainly in terms of brothy ($F_{(7,16)} = 5.738$; $p < .01$) and oxidized flavour ($F_{(7,16)} = 16.909$; $p < .01$) attributes. Dimension 1 separated the samples in terms of storage, where negative loadings corresponded to 0 day storage samples, and positive loadings correlated to 7 days storage samples associated with the oxidized

attribute. Dimension 2 further separated the samples in terms of processing methods. Positive loadings of dimension 2 corresponded to PEF treated samples associated with juicy, brown, livery and brothy attributes, while negative loadings further separated non-PEF treated samples.

4.1.2 Initial Tenderness of Fresh Beef Muscles

The initial tenderness of fresh meat was evaluated on the first bite by panellists prior to carrying out TDS. Tenderness intensity was measured using a scale from 0 (least tender) to 100 (most tender). However many researchers used shear force as a measure of beef meat tenderness (Bekhit et al., 2014; Carne et al., 2015b; O'Dowd et al., 2013). Table 9 showed that PEF processing increased tenderness of fresh ST and BF beef samples. This finding is in agreement with findings by Bekhit et al. (2014) that reported a reduction in shear force with PEF treated cold-boned beef *longissimus lumborum* (LL) muscle compared to non-treated control samples.

The initial tenderness of both BF and ST beef muscles were significantly increased by PEF processing. This result was similar to Bekhit et al. (2014) who utilized *longissimus lumborum* and *Semimembranosus* (SM) muscles treated with PEF processing and reported that the shear force was decreased by PEF processing. O'Dowd et al. (2013) mentioned that myofibril fragmentation was significantly increase by PEF processing (1.9 kV cm-1, 65Hz, 250 pulses and 20μS pulse width), which might lead to the difference of beef tenderness.

Only the tenderness of PEF treated ST samples significantly increased after 7 days storage. Hence tenderness might be influenced by the structure of beef muscles. However no studies to date have compared initial tenderness of different PEF treated beef muscles of different types with storage. Different cuts and treatments have only been shown to affect the tenderness of meat with aging. Colle et al. (2015) analysed *gluteus medius* (GM) and LL beef muscles stored at 20 °C over 63 days and showed that the tenderness in GM and LL beef muscles increased from day 14 to 42, and day 2 to 14 of aging respectively.

Table 9 Initial tenderness of beef samples with different treatments, storage period, and cuts

Storage days	Control	PEF processing	F value		
			Storage	Processing	Storage*Treatment
		Semitendinosus			
0 day	38.50±4.39^{bx}	54.76+2.63^{ay}	43.185*	198.058*	27.434*
7 days	40.96±3.15^{bx}	76.48±2.12^{ax}			
		Biceps femoris			
0 day	38.25±1.39^{bx}	72.67±3.28^{ax}	4.525	304.106*	48.541*
7 days	49.13±1.41^{bx}	93.11±6.78^{ax}			

^{a,b} Means within a row of aging and processing for beef sample with different letters are different ($p<0.05$).
^{x,y} Means within a column of aging and processing for beef sample with different letters are different ($p<0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

4.1.3 Changes in Colour of PEF Processed Fresh Beef Muscles

Meat colour is an important character of meat quality, because it is the main factor affecting meat acceptability at point of sale (Sapp, Williams, & McCann, 1999). In this section, colour results of PEF processed fresh beef samples from two different cuts BF and ST muscles will discussed.

The colour of ST beef muscles without PEF treatment were darker after 7 days storage ($P<0.05$) (Table 10). Meanwhile a* and C values of the same beef muscle also increased significantly ($P<0.05$) with storage. On contrary, a* and C values of chevon (leg, shoulder/arm, and loin/rib) were reported to decrease with aging (Kannan et al., 2001). However, in our study, vacuum packaging might have restrained the effect from modified atmospheres (combinations of O_2, CO_2 and N_2) and protected the original colour. This is in agreement with Insausti et al. (1999) who also found that redness and Chroma of vacuum packed beef significantly increased in the first 5 days of storage. For PEF treated beef samples, only lightness of PEF treated ST beef muscles increased ($P<0.05$) after storage.

As for BF beef muscles, only a* value of non-PEF treated samples increased ($P<0.05$) by storage. Moreover, for PEF treated samples, the lightness and yellowness were decreased by storage ($P<0.05$). Similarly, Kadim et al. (2004) reported decreased lightness and yellowness of BF muscles from three goat breeds from 1 to 6 days of aging.

The effect of PEF processing on colour profiles was also highlighted in Table 10. The L value of ST beef muscle decreased ($P<0.05$) immediately after PEF treatment. The L value of 7 days storage samples increased ($P<0.05$) with PEF processing. The lightness of BF beef muscles on the other hand increased ($P<0.05$) immediately after PEF and then significantly decreased ($P<0.05$) after 7 days storage. It has been reported that over storage time, beef muscles normally turned darker (Faridnia et al., 2014). Only redness of both muscles increased ($P<0.05$) immediately after PEF processing. Similarly, Faridnia et al. (2014) reported that a* value decreased with PEF treatment, and stated that PEF did not increase metmyoglobin which is responsible for brownness in beef muscles. They concluded that colour would not be detrimentally affected by PEF processing.

Table 10 Mean values of colour profiles of fresh raw beef samples in different cuts, treatment and storage period

	Aging days	Treatment processing		F value		
		Control	PEF	Storage	Processing	Storage*Processing
			Semitendinosus			
Lightness[1] (L)	0	61.87±0.27^{ax}	54.18±1.89^{by}	0.354	1.267	71.271*
	7	54.61±1.97^{by}	60.49±0.47^{ax}			
Redness[2] (a*)	0	4.50±0.16^{by}	6.13±0.40^{ax}	58.260*	0.114	136.324*
	7	7.27±0.24^{ax}	5.55±0.08^{bx}			
Yellowness[3](b*)	0	15.49±0.08^{ax}	14.84±0.34^{bx}	5.569*	7.973*	0.232
	7	16.29±0.85^{ax}	15.36±0.33^{ax}			
Chroma[4](C)	0	16.13±0.03^{ay}	16.05±0.46^{ax}	13.420*	8.565*	6.904*
	7	17.84±0.76^{ax}	16.33±0.31^{bx}			
			Biceps femoris			
Lightness[1] (L)	0	51.06±0.39^{bx}	55.77±0.22^{ax}	45.398*	9.009*	38.873*
	7	50.80±0.86^{ax}	49.15±1.48^{ay}			
Redness[2] (a*)	0	5.96±0.06^{by}	6.78±0.34^{ax}	17.893*	4.793	0.125
	7	7.45±0.76^{bx}	8.04±0.76^{ax}			
Yellowness[3](b*)	0	14.98±0.14^{bx}	15.98±0.11^{ax}	8.161*	4.453	3.953
	7	14.76±0.70^{ax}	14.79±0.46^{ay}			
Chroma[4](C)	0	16.12±0.14^{bx}	17.37±0.09^{ax}	0.033	10.696*	4.174
	7	16.55±0.27^{ax}	16.84±0.75^{ax}			

^{a,b} Means within a row of L, a, b and Chroma for beef sample with different letters are different (P<0.05).
^{x,y} Means within a column of L, a, b and Chroma for beef sample with different letters are different (P<0.05).
[1]Measure of darkness to lightness (a greater value indicates a lighter colour).
[2]Greater value indicates redder colour.
[3]Greater value indicates more yellow colour.
[4]Chroma or saturation index is measure of the total colour/vividness of colour (greater value indicates greater total colour/more vivid colour).
*significant differences in student t values of processing and storage within 8 different samples at 5% level.

4.1.4 Effect of PEF on the Lipid Oxidation of Fresh Beef Muscles

Lipid oxidation is another important factor influencing meat quality. A standard test for lipid oxidative stability in foods is the measurement of thiobarbituric acid reacting substances (TBARS) as used in this study, which measures the oxidation product malondialdehyde. Values above about 0.5 are considered critical since they indicate a level of lipid oxidation products which produce a rancid odour and taste which can be detected by consumers (Wood et al., 2008). Values of TBARS in our study with beef muscles before and after PEF treatment at 0 and 7 days storage was well below 0.5. After 7 days of storage, the TBARS values of all fresh beef samples increased ($P<0.05$) 0.06-0.12 mg MDA/kg meat.

Table 11 Evolution of lipid oxidation marker (TBARS) in PEF treated and control fresh ST and BF beef samples during 0 and 7 days storage period

Samples				F value		
				Processing	Storage	Processing *Storage
Semitendinosus						
FSC0	FSC7	FSP0	FSP7			
0.117±0.004^{c}	0.170±0.010^{b}	0.167±0.001^{b}	0.231±0.001^{a}	229.402*	253.710*	2.604
Bicep femoris						
FBC0	FBC7	FBP0	FBP7			
0.208±0.000^{c}	0.279±0.007^{b}	0.209±0.002^{c}	0.325±0.015^{a}	17.135*	261.668*	14.978*

^{a,b,c} Mean of fatty acids with different treatments within the same row differs significantly using Fisher's least significant difference ($p < 0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

Rhee, Anderson, and Sams (1996) reported differences in lipid oxidation of *longissimus dorsi* (LD) and SM beef muscles. However, when the two beef muscles were stored at 4 °C, the level of lipid oxidation was similar over 6 days of storage period. As lipid content may vary in different beef muscles, the level of lipid oxidation may similarly vary as well. By parity of reasoning, the use of different beef muscles was not the only factor that can influence the level of lipid oxidation. Breeds, age, gender and any other factors that determine the content of fat in beef muscles would affect the oxidation of lipids. Moreover, the lipid oxidation of beef samples stored at 4°C was much higher (about 10 times) than -20°C (Rhee et al., 1996). Therefore, storage conditions especially

storage temperature is another important factor that influences lipid oxidation level in meat and meat products.

With all samples, lipid oxidation increased with or without PEF treatment (approximate 0.075 mg MDA/kg meat). TBARS values after PEF processing significantly increased ($P<0.05$) in all beef samples except for BF sample immediately after PEF. Similarly PEF processing significantly increased TBARS values of frozen-thawed beef muscles over 18 days of storage (Faridnia et al., 2015). PEF treatment can result in mechanical damages on the membrane of beef muscle cells, due to the changes in cell structure that can facilitate the exposure of pro-oxidants and accelerate lipid oxidation in beef muscles. This is supported by H. Ma, Ledward, Zamri, Frazier, and Zhou (2007) who studied the effects of high pressure and thermal treatment on lipid oxidation in beef and chicken muscle. In their study, minced beef and chicken samples processed by heating (20°C, 40°C, 60°C and 70°C) and high pressure processing (0.1 MPa, 200 MPa, 400 MPa, 600 MPa and 800 MPa) had increased TBARS values. They believed that lipid oxidation was related to the integrity of the cell membrane, similar to PEF processing.

However, Zeng, Han, and Zi (2010) claimed that PEF treatment could restrain the speed of lipid oxidation reaction, which is contrary to our findings. However peanut oil used in their study employed high electric field strengths of 20 kV cm^{-1}, 30 kV cm^{-1}, 40 kV cm^{-1}, and 50 kV cm^{-1}. In our study, the electric field strength employed was between 0.8-1.1 kV cm^{-1} to prevent over cooking of meat. Moreover, the lipid content of peanut oil was much higher than beef muscles, which could also affect the level of oxidation. Although Zeng et al. (2010) suggested that increase electric strength can decrease lipid oxidation to extend the shelf-life of lipid rich products, it is difficult to employ high electric strengths on meat and meat to avoid over cooking and flashing out during PEF processing. Hence further study on the effects of electric field strength that can provide an extended shelf-life without affecting the quality of meat is required.

4.1.5 Effects of PEF on the Fatty Acids of Fresh Beef Muscles

Fatty acids influence various quality aspects of meat. The ability of unsaturated fatty acids, especially those with more than two double bonds, to rapidly oxidise, is important in regulating the shelf life of meat (rancidity and colour deterioration). During processing, ageing and retail display, polyunsaturated fatty acids are not stable, and

their oxidative stability is affected by the composition of fatty acids (Wood et al., 2008). Oxidation is also important in flavour development during cooking.

The fatty acid composition of fresh beef samples with and without PEF processing is shown in table 12. The main fatty acids in beef samples were oleic acid (C18:1 (n-9)), palmitic acid (C16:0), stearic acid (C18:0) and linoleic acid (C18:2 (n-6)). Similarly, beef samples were reported to contain high amounts of C16:0 and C18:0 fatty acids before and after PEF treatment (Faridnia et al., 2015).

Most fatty acids were affected by both PEF treatment and storage period. In BF beef samples, most saturated fatty acids significantly decreased with PEF processing except for myristic acid (C14:0). All MUFAs increased with PEF treatment except for heptadecenoic (C17:1). As for PUFAs, linoleic (C18:2 (n-6)), arachidonic (C20:4 (n-6)), and docosadienoic (C22:6 (n-3)) acids were significantly reduced by PEF treatment except for arachidonic acid (C20:3 (n-6)), which increased significantly 7 days after PEF processing of fresh BF beef muscles. Most fatty acids significantly decreased in PEF treated BF samples after 7 days of storage compared to 0 day control samples. It has been demonstrated that the decrease in fatty acids can be a result of fatty acid oxidation and acidification (Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2008). This is supported by the significantly higher TBARS value of PEF treated BF samples after 7 days of storage compared to the non-PEF treated BF beef samples in section 4.1.4 of this study.

On the other hand, Table 12 showed that most fatty acids with less than 18 carbon atoms except for C14:0 and C17:0 in ST beef muscles were increased by PEF treatment. Zeng et al. (2010) claimed that during storage fatty acid, acid value, peroxide value, as well as carbonyl group value of various PEF-treated peanut oil samples were less than that of untreated oil. This indicated that PEF processing could restrain the oxidation and acidification speed of fatty acid. Application of high strength PEF processing (20 kV cm^{-1}, 30 kV cm^{-1}, 40 kV cm^{-1}, and 50 kV cm^{-1}) on peanut oil, increased the content of unsaturated fatty acid in peanut oil samples. The increase in fatty acids corresponded to lower TBARS value for PEF processed ST muscles compared to BF muscles in section 4.1.4 of this study.

The fatty acid composition of muscle affects its oxidative stability during processing

and retail display, with the PUFAs in phospholipid being liable to oxidative breakdown at this stage. A standard test for lipid oxidative stability in foods is the thiobarbituric acid reacting substances (TBARS) test, which measures the oxidation product malondialdehyde. PUFAs in BF muscle decreased significantly immediately after PEF processing (Table 12). However there were no significant differences in PUFAs in PEF treated ST muscles that corresponded to lower TBARS values in ST muscles compared to BF muscles for all treatments in section 4.1.4 of this study.

Table 12 The fatty acid composition of fresh beef with and without PEF treatments (mg/100 g dry meat) during 0 or 7 days of storage.

Fatty acids	Samples				F value		
					Processing	Storage	Processing*Storage
			Semitendinosus				
	FSC0	FSC7	FSP0	FSP7			
C14:0	36.47±4.45^{ab}	19.14±0.18^{c}	42.20±2.84^{a}	29.91±2.41^{b}	16.143*	51.998*	1.506
C16:0	300.907.00±^{b}	156.17±6.89^{c}	324.96±21.88^{a}	245.67±19.82^{b}	26.640*	103.660*	8.845*
C16:1 (n-7)	43.36±4.47^{ab}	31.58±9.27^{b}	47.90±3.23^{a}	36.65±2.96^{ab}	1.474	8.478*	0.005
C17:0	19.32±0.69	15.76±3.05	20.64±1.39	18.94±1.53	2.871	3.935	0.494
C17:1	20.78±0.84^{a}	12.98±3.55^{b}	21.55±1.45^{a}	19.28±1.56^{a}	5.611	11.373*	3.435
C18:0	230.842.18±^{a}	105.46±1.30^{c}	245.74±16.55^{a}	196.17±15.83^{b}	42.031*	115.327*	21.655*
C18:1 (n-9)	439.530.73^{a}	199.09±4.47^{c}	475.95±32.05^{a}	354.96±28.64^{b}	39.589*	139.872*	15.277*
C18:2 (n-6)	174.29±5.69^{a}	129.60±17.20^{b}	160.55±10.81^{ab}	171.73±13.86^{a}	2.531	3.525	9.806*
C18:3 (n-3)	58.39±2.13^{a}	47.58±0.38^{b}	54.03±3.64^{ab}	49.46±3.99^{b}	0.362	13.992*	2.300
C20:2 (n-6)	9.68±0.78	8.30±0.78	9.46±0.64	9.00±0.73	0.219	3.115	0.783
C20:3 (n-6)	17.31±1.63	15.01±2.33	17.29±1.16	15.84±1.28	0.118	2.536	0.132
C20:4 (n-6)	45.99±1.25	42.03±5.78	41.69±2.81	47.38±3.82	0.038	0.104	3.245
C20:5 (n-3)	39.81±0.08	41.34±6.75	41.31±2.78	38.60±3.11	0.048	0.044	0.572
C22:6 (n-3)	30.66±1.61^{a}	9.52±0.67^{c}	24.99±1.68^{b}	21.98±1.77^{b}	10.238*	129.422*	73.011*
SFA[1]	587.5314.32±^{a}	296.53±8.46^{c}	633.54±42.67^{a}	490.70±39.59^{b}	31.485*	102.733*	11.980*
MUFA[2]	503.684.58±^{a}	243.64±10.19^{c}	545.40±36.73^{a}	410.89±33.15^{b}	33.946*	121.009*	33.946*
PUFA[3]	376.121.47±^{a}	293.38±30.23^{b}	349.31±23.52^{ab}	354.00±28.56^{ab}	1.000	5.333	6.690
total n-3[4]	128.86±3.82^{a}	98.45±5.71^{b}	120.34±8.10^{a}	110.04±8.88^{ab}	0.099	17.288*	4.224
total n-6[5]	247.27±5.28^{a}	194.94±24.52^{b}	228.98±15.42^{ab}	243.95±19.68^{ab}	1.506	2.225	7.223
n-6:n-3	1.92±0.10^{b}	1.98±0.13^{b}	1.90±0.00^{b}	2.22±0.00^{a}	3.598	9.896*	4.820
PUFA: SFA	0.64±0.02^{b}	0.99±0.13^{a}	0.55±0.00^{b}	0.72±0.00^{b}	14.899*	31.394*	3.781
MUFA: SFA	0.86±0.01^{a}	0.82±0.01^{b}	0.86±0.00^{a}	0.84±0.00^{ab}	2.563	24.289*	1.065

	Bicep femoris						
	FBC0	FBC7	FBP0	FBP7			
C14:0	41.56±0.34[a]	43.33±3.87[a]	38.52±0.29[a]	41.00±2.26[a]	2.849	1.772	0.050
C16:0	365.63±7.95[b]	380.78±0.64[a]	373.25±0.87[ab]	322.78±3.08[c]	68.715*	33.764*	116.618*
C16:1 (n-7)	66.87±0.59[a]	57.99±0.05[b]	60.08±0.75[b]	60.38±1.85[b]	8.895*	33.715*	38.620*
C17:0	24.46±0.38[a]	22.66±1.09[a]	23.83±0.16[a]	19.68±0.79[b]	13.081*	35.477*	5.526
C17:1	29.17±0.60[ab]	25.43±0.94[b]	29.76±0.33[a]	27.93±2.58[ab]	2.397	0.050*	0.915
C18:0	313.237.53±[a]	311.67±10.65[a]	311.07±2.66[a]	268.03±11.32[b]	13.740*	13.037*	11.271*
C18:1 (n-9)	638.31±13.39[ab]	612.73±16.97[b]	652.52±5.79[a]	522.37±9.11[c]	19.851*	83.048*	37.449*
C18:2 (n-6)	322.25±7.54[a]	275.36±9.57[bc]	261.94±13.20[c]	293.96±10.07[ab]	8.203*	1.042	29.369*
C18:3 (n-3)	80.68±1.99	72.16±5.05	75.44±2.04	82.00±4.32	0.805	0.147	8.701*
C20:2 (n-6)	8.10±0.42	8.68±0.41	9.15±0.24	9.74±2.38	1.467	0.456	0.000
C20:3 (n-6)	21.46±0.53[ab]	18.30±0.40[b]	19.07±0.17[b]	24.03±2.26[a]	4.017	1.167	23.681*
C20:4 (n-6)	62.71±1.04[a]	51.33±0.65[b]	53.24±1.61[b]	65.37±2.50[a]	4.055	0.108	106.988*
C20:5 (n-3)	52.61±0.02[ab]	41.51±3.90[c]	46.63±1.05[bc]	59.09±2.61[a]	11.652*	0.162	48.099*
C22:6 (n-3)	26.33±1.61[a]	28.97±0.36[a]	28.90±1.07[a]	22.27±0.49[b]	8.289*	7.747*	41.623*
SFA[1]	744.88±14.76[a]	758.44±8.51[a]	746.67±1.66[a]	651.48±15.86[b]	40.605*	24.467*	43.411*
MUFA[2]	734.34±13.40[a]	696.15±16.08[b]	742.36±4.71[a]	610.68±4.68[c]	24.878*	119.699*	36.254*
PUFA[3]	574.14±7.10[a]	496.31±19.53[b]	494.36±14.66[b]	556.46±10.14[a]	1.029	0.661	52.246*
total n-3[4]	159.63±0.35[ab]	142.65±9.30[b]	150.96±0.08[a]	163.36±2.21[a]	3.171	0.460	18.845*
total n-6[5]	414.52±7.45[a]	353.67±10.22[b]	343.40±14.74[b]	393.10±7.94[a]	4.560	0.564	55.503*
n-6:n-3	2.60±0.05[a]	2.48±0.09[a]	2.27±0.10[b]	2.41±0.02[ab]	15.143*	0.028	5.790
PUFA: SFA	0.77±0.02[b]	0.65±0.02[c]	0.66±0.02[c]	0.85±0.01[a]	11.603*	7.956*	133.652*
MUFA: SFA	0.99±0.00[a]	0.92±0.01[b]	0.99±0.00[a]	0.94±0.02[b]	4.130	81.226*	0.677

[1]SFA = C14:0 + C16:0 + C17:0 +C18:0;
[2]MUFA = C16:1 (n-7) + C17:1 + C18:1 (n-9)
[3]PUFA = C18:2 (n-6) + C18:3 (n-3) + C20:2 (n-6) + C20:3 (n-6) + C20:4 (n-6) + C20:5 (n-3) + C22:6 (n-3)
[4]Total n-3 = C18:3 (n-3) + C20:5 (n-3) + C22:6 (n-3)
[5]Total n-6 = C18:2 (n-6) + C20:2 (n-6) + C20:3 (n-6) + C20:4 (n-6)
[a,b,c] Mean of fatty acids with different treatments within the same row differs significantly using Fisher's least significant difference ($p < 0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

4.1.6 Flavour Volatile Composition of PEF Treated Fresh Beef Muscles

The formation of secondary products in lipid and fatty acids oxidation can result in the release of off-flavour that can affect the original volatile compound composition in beef meat (Faridnia et al., 2015). The investigation on volatile compounds may help further understand the effects of PEF processing and storage on beef meat. There are several hundred volatile compounds that have been identified in cooked beef meat (Ruan, Aalhus, Juárez, & Sabik, 2015). Ten major beef volatile compounds identified in other studies (Faridnia et al., 2015; Q. Ma et al., 2012; Resconi et al., 2012; Ruan et al., 2015) were investigated in this study.

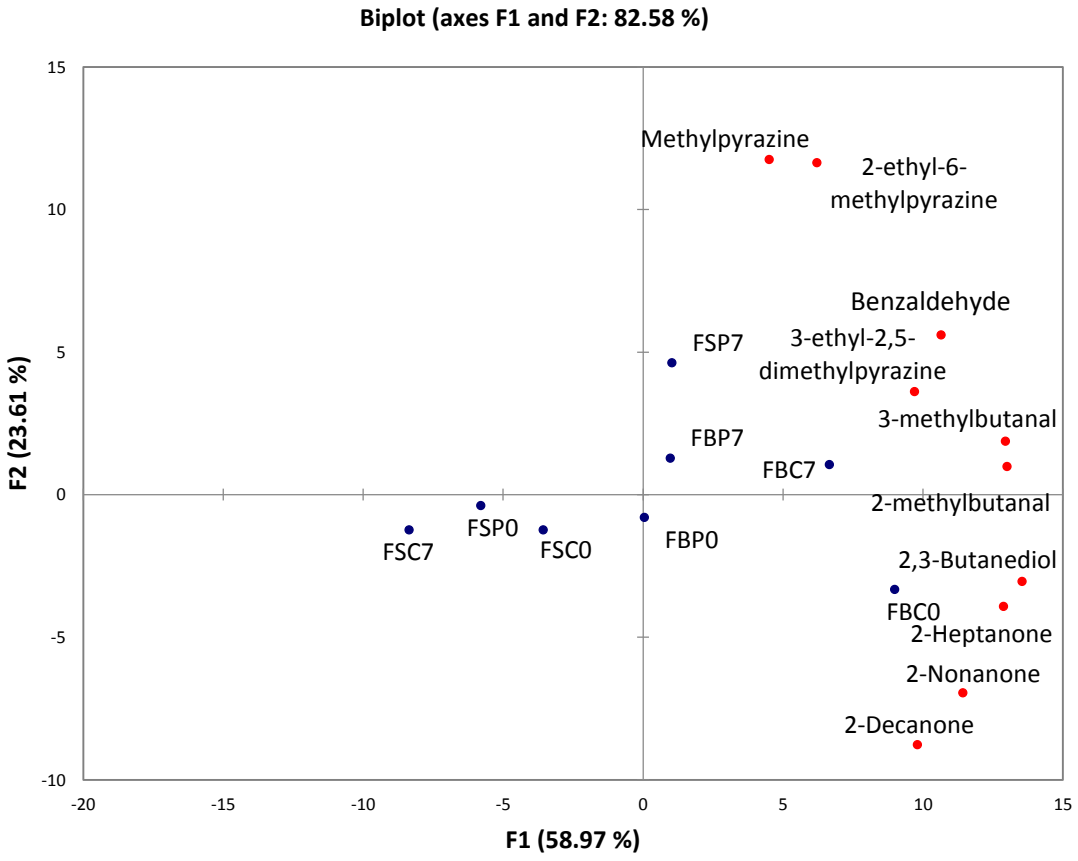

Figure 13 Bi-plot of volatile compounds in cooked fresh BF and ST beef muscles with or without PEF processing during 0 or 7 days storage

Principal Component Analysis (PCA) was carried out to illustrate the differences in volatile compounds between BF and ST beef samples with or without PEF processing on 0 and 7 days of storage on the basis of individual volatile compounds (Figure 13). The PCA described 58.97% and 23.61% of the total variation of factor 1 (F1) and factor 2 (F2), respectively. Dimension 1 separated the samples in terms of different cuts except

for FSP7 sample, where negative loadings of the PCA corresponded to most ST beef samples, and positive loadings corresponded to all BF samples. BF beef samples had a higher content of all volatile compounds. Dimension 2 separated the samples in terms of different storage days (except FSC7), where PEF treated 7 day stored ST sample had significantly higher content of methylpyrazine, 2-ethyl-6-methylpyrazine, benzaldehyde, 3-ethyl-2,5-dimethylpyrazine, and 2- and 3-methylbutanal (Table 13).

Table 13 Volatile compounds in cooked fresh ST and BF beef muscles with or without PEF processing during 0 or 7 days storage

Volatile compounds	Samples				F value		
					Processing	Storage	Processing*Storage
			Semitendinosus				
	FSC0	FSC7	FSP0	FSP7			
3-methylbutanal	0.17±0.01^{c}	0.25±0.01^{b}	0.15±0.02^{c}	0.42±0.00^{a}	143.954*	737.263*	212.973*
2-methylbutanal	0.27±0.01^{b}	0.30±0.01^{b}	0.27±0.00^{b}	0.41±0.03^{a}	16.529*	39.643*	17.277*
2,3-butanediol	0.48±0.00^{b}	0.18±0.00^{d}	0.35±0.02^{c}	0.64±0.01^{a}	33.695*	0.052	99.190*
methylpyrazine	2.66±0.24^{b}	3.37±0.14^{b}	2.93±0.08^{b}	4.64±0.45^{a}	16.757*	41.070*	0.500
2-heptanone	0.52±0.02^{a}	0.25±0.00^{c}	0.39±0.00^{b}	0.39±0.01^{b}	0.413	290.124*	296.688*
benzaldehyde	2.54±0.08^{b}	1.45±0.00^{c}	2.35±0.08^{b}	3.04±0.18^{a}	86.155*	6.642*	136.704*
2-ethyl-6-methylpyrazine	0.83±0.1^{b}	0.67±0.01^{b}	0.84±0.04^{b}	1.26±0.14^{a}	22.308*	4.397	20.885*
3-ethyl-2,5-dimethylpyrazine	7.27±0.7^{a}	4.01±0.22^{b}	5.95±0.69^{ab}	8.30±1.67^{a}	4.639	0.441	16.578*
2-nonanone	1.21±0.16^{a}	0.56±0.05^{b}	1.09±0.08^{a}	1.27±0.02^{a}	21.605*	13.894*	41.868*
2-decanone	1.16±0.00^{a}	0.50±0.05^{b}	0.40±0.02^{b}	0.42±0.08^{b}	160.169*	93.827*	104.486*
			Bicep femoris				
	FBC0	FBC7	FBP0	FBP7			
3-methylbutanal	0.57±0.04^{a}	0.60±0.14^{a}	0.32±0.06^{b}	0.40±0.03^{ab}	14.292*	0.932	0.190
2-methylbutanal	0.56±0.04^{ab}	0.64±0.12^{a}	0.43±0.08^{b}	0.47±0.03^{ab}	8.273*	1.260	0.158
2,3-butanediol	2.01±0.06	1.75±0.71	1.15±0.04	1.07±0.03	9.375*	0.470	0.134
methylpyrazine	3.03±0.42^{b}	4.23±0.54^{a}	2.90±0.19^{b}	3.56±0.38^{ab}	1.985	10.763*	0.907
2-heptanone	1.04±0.17^{a}	1.04±0.15^{a}	0.55±0.08^{b}	0.57±0.01^{b}	31.335*	0.022	0.019
benzaldehyde	2.72±0.02^{ab}	3.24±0.37^{a}	2.79±0.00^{ab}	2.43±0.28^{b}	5.065	0.218	7.330
2-ethyl-6-methylpyrazine	0.88±0.07^{b}	0.95±0.06^{ab}	0.87±0.03^{b}	1.09±0.05^{a}	2.551	13.451*	3.419
3-ethyl-2,5-dimethylpyrazine	8.32±0.15^{a}	6.48±1.07^{b}	6.77±0.51^{ab}	7.81±0.34^{ab}	0.056	0.840	10.856*
2-nonanone	4.88±0.29^{a}	2.16±0.2^{b}	2.32±0.02^{b}	1.13±0.07^{c}	196.667*	234.629*	35.691*
2-decanone	2.59±0.41^{a}	0.94±0.02^{b}	0.81±0.02^{b}	0.97±0.05^{b}	35.698*	26.160*	38.362*

^{a,b,c} Mean of fatty acids with different treatments within the same row differs significantly using Fisher's least significant difference ($p < 0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

4.2 Frozen-thawed Meat

4.2.1 Temporal Dominance of Sensations (TDS) Analysis of PEF Treated Frozen-thawed Beef Meat

Frozen meat was considered in this study as meat is often frozen for export purposes. Similar to unfrozen beef meat, temporal dominance of sensation analysis was carried out on ST and BF muscles of beef samples immediately before and after PEF treatment, as well as after seven days after PEF treatment. Similar to unfrozen beef meat, the chance level was at the dominance rate of 20%. The dominance rate of related attributes that was below 20% was not considered dominant. The significance level was at the dominance rate of 32%.

Figures 15 and 16 showed the dominance rates of five sensory attributes in both ST and BF frozen beef muscles separately. In general, brothy was the first dominant sensation in all samples, with the dominance rates starting at around 80% dominance rate and then decreasing to below chance level in less than 10 seconds except for samples BP7 and SP7. Dominance rate of the browned attribute increased almost from the start of mastication and reached a maximum above significance level for mainly Biceps femoris samples, then rapidly decreased below chance level over after 10 seconds. Starting at the 10^{th} seconds, oxidized became the dominant attribute above significance level until the end of mastication for all samples. The attributes livery and juicy were occasionally above chance level, and only lasted a few seconds.

ST muscle

Brothy was dominant from the start of mastication and then decreased to below chance level for all samples except for SP7 (Figure 14-A). The oxidized attribute was above significance level from around 10 seconds until the end of mastication for all samples but was least dominant for SC0 sample.

The dominance of livery did not reach significance level for all samples and hovered only above chance level for sample SC0 between 30 to 60 seconds. Similarly, previous research reported that the intensity of livery flavour in beef meat increased over 21 days aging time, but the changes were not obvious in the first 7 days (Campo et al., 1999).

Juiciness reached above chance level only between 3 and 5 seconds for all samples except for SP0. Juarez et al. (2012) stated that the cell membranes of beef meat are damaged during freezing, which might cause an increase in cooking loss and result in decreased juiciness. Browned attribute increased significantly between 1 and 5 seconds for all samples except for SP7.

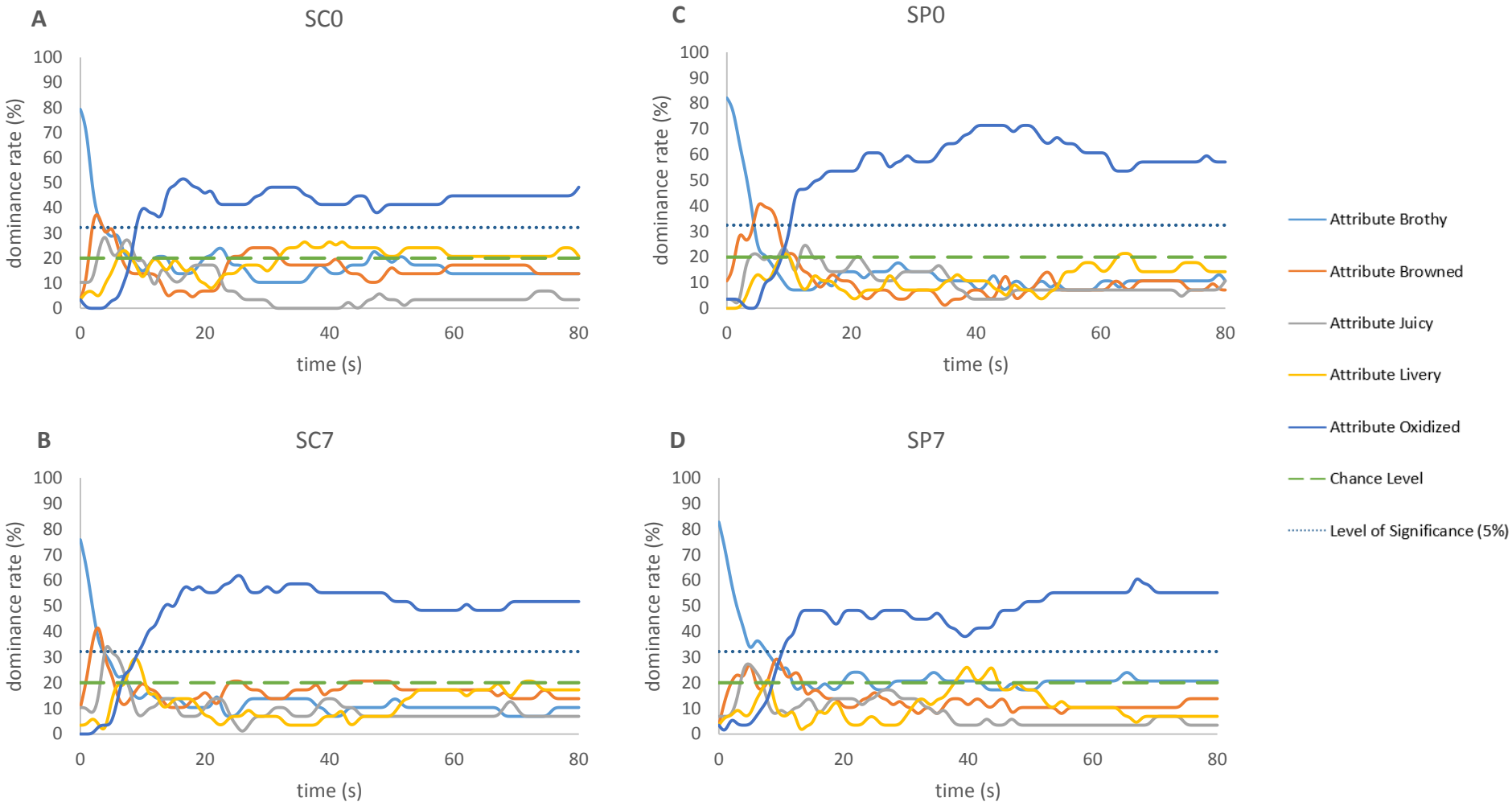

Figure 14 TDS curves for sensory attributes by frozen ST beef samples (S, frozen semitendinosus beef samples; C, control samples; P, PEF treated samples; 0 and 7 days, display days after thawing)

BF muscle

The influence of PEF treatment on flavour attributes of frozen BF beef samples were not much. In terms of brothy, all BF samples were similar with brothy being significant higher at the start of mastication and the decreasing to above chance level between 4 to 5 seconds. From about 8 seconds, oxidized was dominant in all the four samples.

The dominance of livery was below chance level for most frozen BF beef samples. Livery was dominant above chance level only in sample BP7 (frozen, BF muscle, PEF, 7 day) between 20 and 40 seconds.

Juicy was only above significance level for between 4 to 6 seconds in sample BC0 (frozen, BF muscle, control, 0 day) and 8 to 9 seconds in sample BP7. Browned attribute increased for all samples except for SP7 but was more dominant in PEF processed samples reaching significance between 1 and 7 seconds.

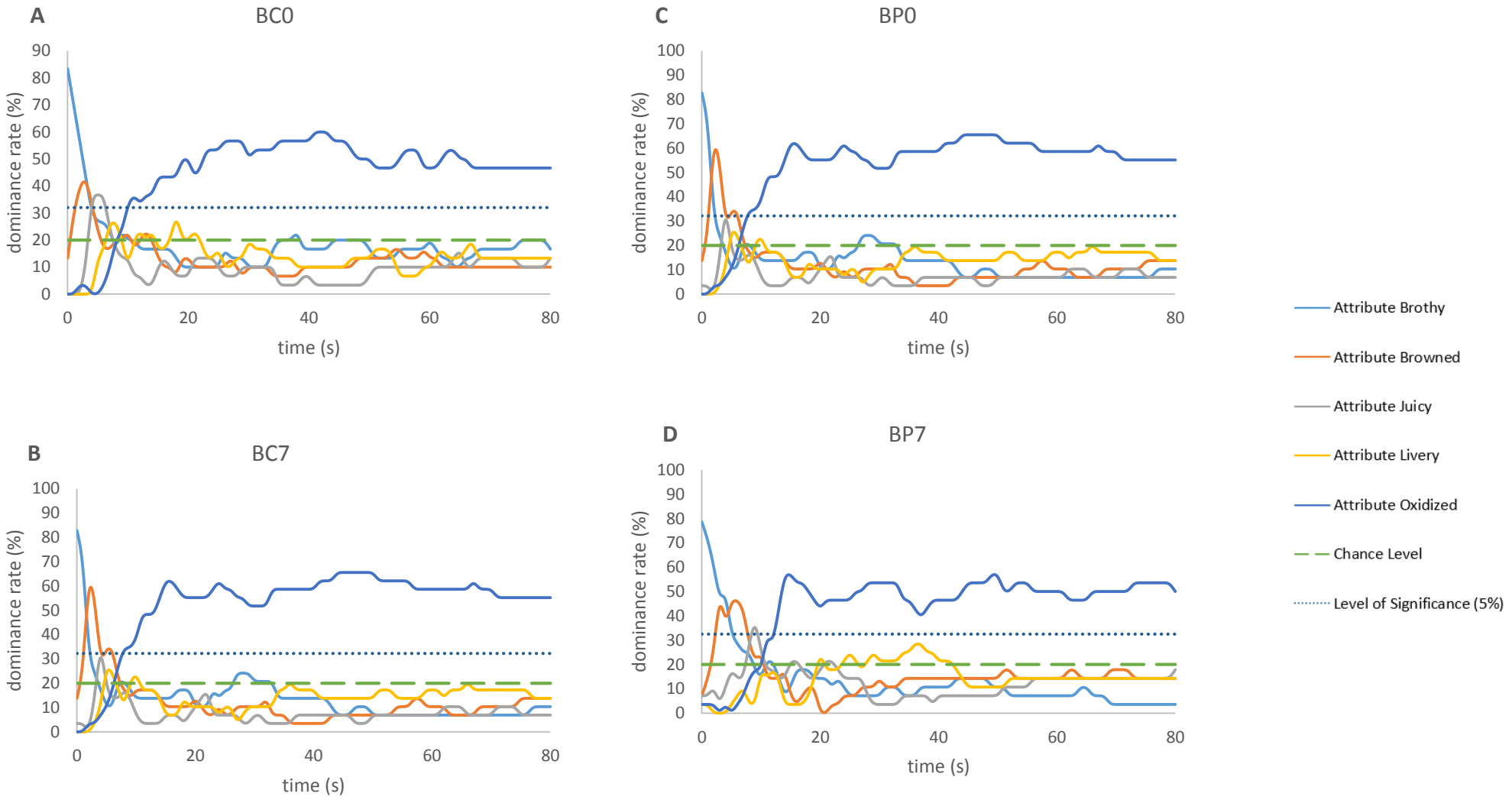

Figure 15 TDS curves for sensory attributes by frozen BF beef samples (B, frozen Biceps femoris beef samples; C, control samples; P, PEF treated samples; 0 and 7 days, display days after thawing)

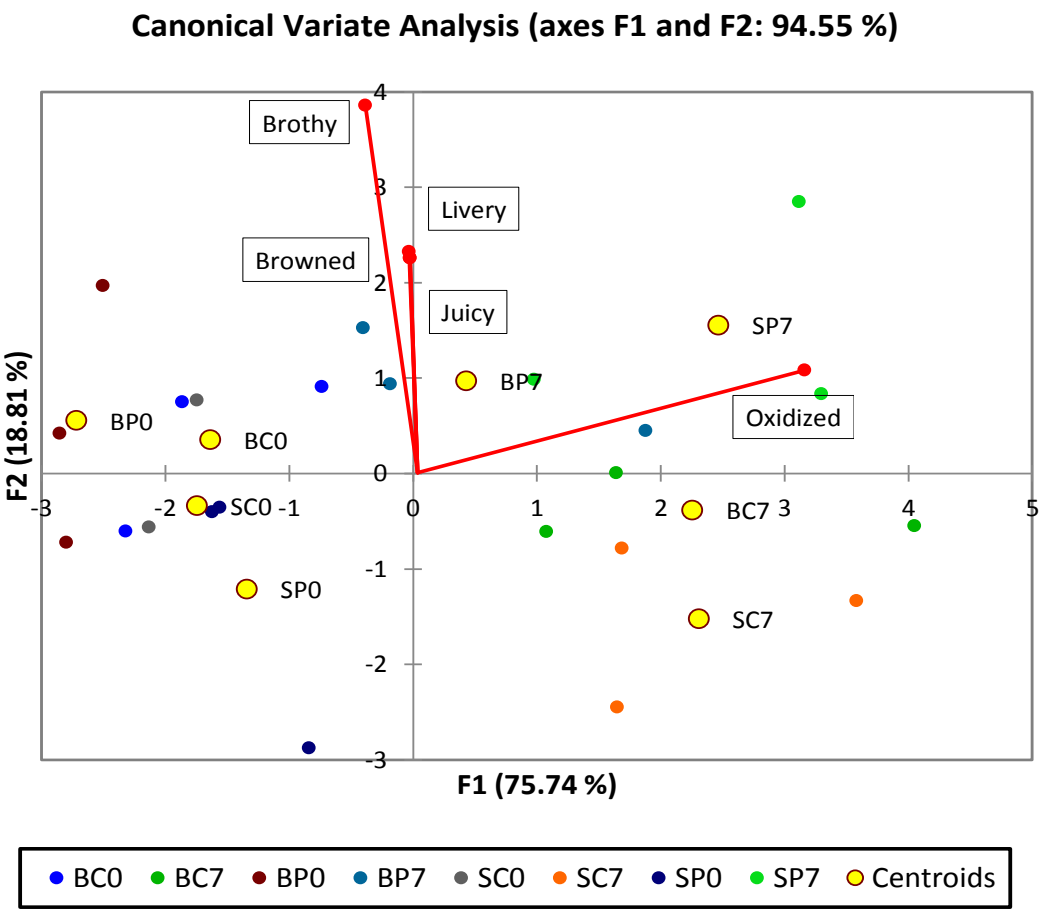

Figure 16 Canonical Variate Analysis of fresh meat cuts with different processing and storage time

Canonical Variate Analyses (CVA) was carried out on the duration of dominance per attribute (Figure 16). Sample discrimination was explained by the first two canonical variates that were high for sensory data (94.55%). Hotelling Lawley MANOVA ($F_{(28,24)} = 3.352$; $p < .01$) showed significant differences between the samples in terms of the temporal flavour attributes measured by TDS. Samples differed mainly in terms of brothy attribute ($F_{(7,16)} = 3.369$; $p < .05$) and oxidized flavour ($F_{(7,16)} = 9.020$; $p < .01$). Dimension 1 separated the samples in terms of storage, where negative loadings of the CVA corresponded to 0 day storage samples, and positive loadings corresponded to 7 days storage samples associated with oxidized flavour. The presence of oxidized flavour in FSP7, FBP7, and FBC7 samples are supported by the TBARS results decribed in section 4.2.4 below. Dimension 2 further separated the samples (except for BC0 and SP0 samples) in terms of processing methods. Positive loadings of dimension 2 corresponded to PEF treated samples associated with juicy, brown, livery and brothy attributes, while negative loadings further separated non-PEF treated samples.

4.2.2 Initial Tenderness of Frozen Beef Muscles

The initial tenderness of cooked frozen meat samples was evaluated on the first bite by panelists prior to carrying out TDS. Tenderness intensity was measured using a scale from 0 (least tender) to 100 (most tender). There was no significant changes in initial tenderness of frozen beef muscles between 0 and 7 days samples for each treatment. According to Faridnia et al. (2015) frozen storage of more than 3 months did not result in a significant change in tenderness, because of the physical disruption on muscle cells from intracellular ice crystal.

Tenderness in both ST and BF beef samples however was significantly increased immediately and 7 days after PEF processing. Similarly, Faridnia et al. (2015) also reported a significant decrease on the shear force in PEF treatment of frozen-thawed beef samples compared to frozen-thawed control samples. The freezing-thawing process undergone by beef muscles can result in additional physical damage to muscle cells, making a lower tenderness from fresh beef muscles.

Table 14 Initial tenderness of frozen beef samples with different treatments, storage period, and cuts

Storage days	Control	PEF processing	F value		
			Storage	Processing	Storage*Treatment
		Semitendinosus			
0 day	24.73 ± 1.04^{by}	42.01 ± 1.69^{ay}	355.144*	17.945*	745.229*
7 days	35.68 ± 0.33^{bx}	59.30 ± 1.64^{ax}			
		Biceps femoris			
0 day	40.86 ± 1.68^{by}	57.90 ± 7.73^{ay}	7.638*	24.817*	1.083
7 days	51.63 ± 3.23^{bx}	62.78 ± 4.81^{ax}			

[a,b] Means within a row of aging and processing for beef sample with different letters are different ($P<0.05$).
[x,y] Means within a column of aging and processing for beef sample with different letters are different ($P<0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

4.2.3 Changes in Colour of PEF Processed Frozen Beef Muscles

Table 15 summarizes the colour profiles of both control and PEF treated frozen ST and BF beef muscles. There was no significant change ($P>0.05$) in colour of ST samples caused by either storage or PEF processing, except for the decrease in L value in PEF treated samples stored for 7 days. As for BF samples, L, b*, and C values of control samples significantly increased ($P<0.05$), and redness decreased ($P<0.05$) after 7 days of storage. Similarly, Brewer and Wu (1993) reported that the red colour of frozen ground beef in vacuum packaging decreased during storage. Furthermore, BF samples became darker ($P<0.05$), while redness and Chroma increased ($P<0.05$) significantly immediately after PEF treatment compared to control sample. Frozen samples showed fewer changes after storage and PEF processing suggesting that the colour of frozen beef muscles was more stable. In addition, changes in beef colour might have been restrained by the vacuum environment in which the meat was stored. Oxidation is important in influencing beef colour stability.

Table 15 Mean values of colour profile for frozen raw beef samples with different cuts, treatment and aging days

	Aging days	Treatment processing		F value		
		Control	PEF	Storage	Processing	Storage*Processing
			Semitendinosus			
Lightness[1] (L)	0	58.34±0.56^{ax}	55.53±1.80^{ax}	0.382	18.450*	0.299
	7	59.22±0.19^{ax}	55.59±1.78^{bx}			
Redness[2] (a*)	0	9.08±0.21	9.47±0.64	0.231	0.586	0.694
	7	9.40±0.29	9.39±0.43			
Yellowness[3](b*)	0	17.55±0.23	17.42±0.29	0.162	1.135	0.350
	7	17.83±0.81	17.36±0.40			
Chroma[4](C)	0	19.76±0.29	19.83±0.51	0.419	0.509	1.001
	7	20.16±0.59	19.74±0.15			
			Biceps femoris			
Lightness[1] (L)	0	46.10±1.06^{by}	50.59±0.50^{ax}	38.825*	20.245*	8.472*
	7	51.64±0.97^{ax}	52.61±1.46^{ax}			
Redness[2] (a*)	0	9.45±0.18^{ax}	7.67±0.49^{bx}	19.723*	35.213*	17.443*
	7	7.93±0.30^{ay}	7.62±0.09^{ax}			
Yellowness[3](b*)	0	15.32±0.39^{ay}	15.69±0.11^{ax}	29.535*	0.021	2.320
	7	17.17±0.22^{ax}	16.73±0.80^{ax}			
Chroma[4](C)	0	18.01±0.26^{ay}	17.47±0.16^{bx}	15.521*	5.289	0.000
	7	18.92±0.23^{ax}	18.39±0.71^{ax}			

^{a,b} Means within a row of L, a, b and Chroma for beef sample with different letters are different (P<0.05).
xy Means within a column of L, a, b and Chroma for beef sample with different letters are different (P<0.05).
[1]Measure of darkness to lightness (a greater value indicates a lighter colour).
[2]Greater value indicates redder colour.
[3]Greater value indicates more yellow colour.
[4]Chroma or saturation index is measure of the total colour/vividness of colour (greater value indicates greater total colour/more vivid colour).
*significant differences in student t values of processing and storage within 8 different samples at 5% level.

4.2.4 Effect of PEF on the Lipid Oxidation of Frozen Beef Muscles

TBARS values above about 0.5 are considered critical since they indicate a level of lipid oxidation products which produce a rancid odour and taste which can be detected by consumers (Wood et al., 2008). Values of TBARS in our study with beef muscles before and after PEF treatment at 0 and 7 days storage was well below 0.5. Table 16 showed that the level of lipid oxidation in both ST and BF beef muscles significantly increased ($P<0.05$) after 7 days storage. This result is in agreement with a previous study by Faridnia et al. (2015) who reported increasing TBARS values of frozen-thawed beef samples over 18 days of storage. Similarly Bekhit et al. (2014) reported that the lipid oxidation of beef loins and topsides were significantly increased during a storage period of 21 days.

Lipid oxidation also significantly increased ($P<0.05$) after PEF processing compared to non-PEF treated sample. Similarly, Faridnia et al. (2015) reported that the PEF application can significantly increase the TBARS values of frozen-thawed beef samples. For both BF and ST beef muscles, the PEF treated samples had the highest level of lipid oxidation (approximately 0.3 mg MDA/kg meat) after 7 days storage. Hansen et al. (2004) reported that the lipid oxidation in meat muscles was accelerated by the frozen-thawing processing. Benjakul and Bauer (2001) further explained that the freezing process of meat muscle tissues would produce small ice crystals that can break cell membranes and release pro-oxidants (such as haem iron). Hence after thawing, this damage could result in accelerated TBARS accumulation. Furthermore, the influence of PEF processing on lipid oxidation in frozen beef samples was more pronounced for frozen ST muscle compared fresh ST beef samples.

Table 16 Evolution of lipid oxidation marker (TBARS) in PEF treated and control frozen ST and BF beef samples during 0 and 7 days storage period

Samples				F value		
				Processing	Storage	Processing *Storage
Semitendinosus						
SC0	SC7	SP0	SP7			
0.157±0.001[b]	0.291±0.004[a]	0.192±0.003[b]	0.292±0.004[a]	63.004*	2771.220*	60.321*
Bicep femoris						
BC0	BC7	BP0	BP7			
0.210±0.007[c]	0.254±0.012[b]	0.236±0.006[bc]	0.313±0.012[a]	38.436*	79.379*	5.813

[a,b,c] Mean of fatty acids with different treatments within the same row differs significantly using Fisher's least significant difference ($p < 0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

4.2.5 Effects of PEF on the Fatty Acids of Frozen Beef Muscles

The fatty acids composition of frozen beef samples was different from fresh beef samples, the SFAs occupied about 45% of total fatty acids. Most unsaturated fatty acids were MUFAs which were approximately 45% in each sample. There were only about 10% of fatty acids were PUFAs. The fatty acid composition of beef with and without PEF treatments is shown in Table 17. In frozen BF beef samples, five fatty acids of C16:1 (n-7), C17:0, C18:0, C18:1 (n-9) and C18:2 (n-6), significantly decreased after PEF processing compared to 0 day control BF sample. This is supported by the lipid oxidation of frozen beef result in section 4.2.4, which showed higher level of lipid oxidation after PEF processing. This result is similar to fresh beef sample subjected to PEF processing.

However in frozen ST beef samples, several fatty acids C16:0, C17:0, C18:0, C18:2 (n-6) and C20:4 (n-6) increased with PEF treatment compared to 0 day control ST sample. So far there are limited studies to support the effect of PEF processing on fatty acid composition. Only, Zeng et al. (2010) who applied different high strength PEF processing (20 kV cm-1, 30 kV cm-1, 40 kV cm-1, and 50 kV cm-1) on peanut oil reported that the composition and content of unsaturated fatty acid increased with PEF processing. They claimed that PEF processing could restrain the oxidation and acidification speed of fatty acid. This might explain why the PEF treated frozen ST beef samples retained higher content of fatty acids compared to the 0 day control ST sample.

Table 17 The fatty acid composition of frozen beef with and without PEF treatments (mg/100 g dry meat) during 0 or 7 days of storage

Fatty acids	Samples				F value		
					Processing	Storage	Processing*Storage
			semitendinosus				
	SC0	SC7	SP0	SP7			
C14:0	98.98±3.31^{b}	91.57±7.92^{b}	96.58±1.27^{b}	115.07±1.99^{a}	11.257*	3.102	16.956*
C16:0	1116.63±46.66^{b}	1091.27±81.71^{b}	1247.46±59.58^{ab}	1384.15±25.38^{a}	27.524*	1.900	4.026
C16:1 (n-7)	120.91±5.21^{b}	145.66±10^{a}	112.18±1.45^{b}	117.23±2.66^{b}	20.273*	13.037*	5.701
C17:0	45.99±0.69^{c}	37.53±0.56^{d}	49.88±0.38^{b}	55.14±0.99^{a}	490.151*	10.815*	199.721*
C17:1	39.97±2.44^{a}	40.76±1.6^{a}	43.02±0.13^{a}	40.70±1.08^{a}	1.851	0.487	1.996
C18:0	584.31±23.95^{c}	444.10±31.76^{d}	663.14±37.49^{b}	773.41±12.48^{a}	106.033*	0.571	39.937*
C18:1 (n-9)	1501.42±85.23	1516.56±109.52	1474.09±65.04	1679.78±22.04	1.541	4.069	3.029
C18:2 (n-6)	190.18±2.52^{b}	210.68±11.86^{a}	217.55±5.93^{a}	225.56±1.97^{a}	19.213*	8.741*	1.676
C18:3 (n-3)	46.01±0.97^{a}	39.92±1.91^{b}	49.53±0.96^{a}	49.35±1.77^{a}	39.077*	9.156*	8.142*
C20:2 (n-6)	9.49±0.47^{ab}	7.70±1.3^{b}	10.61±0.47^{a}	9.59±0.4^{ab}	7.947*	6.924	0.521
C20:3 (n-6)	21.68±0.07^{a}	19.72±0.27^{b}	18.65±1.33^{b}	18.44±0.33^{b}	19.288*	4.888	3.178
C20:4 (n-6)	41.59±0.92^{c}	53.46±0.61^{a}	43.29±1.02^{bc}	44.28±0.79^{b}	39.225*	116.065*	83.194*
C20:5 (n-3)	26.36±0.35^{b}	29.24±1.26^{a}	25.95±0.31^{b}	25.05±1.11^{b}	13.956*	2.588	9.418*
C22:6 (n-3)	36.16±1.14^{ab}	33.41±4.09^{b}	45.22±0.44^{ab}	48.50±9.13^{a}	11.506*	0.006	0.719
SFA[1]	1845.91±74.6^{c}	1664.46±121.93^{c}	2057.05±20.46^{b}	2327.76±38.86^{a}	68.404*	0.713	18.289*
MUFA[2]	1662.3±92.87^{a}	1702.97±121.11^{a}	1629.29±63.72^{a}	1837.71±25.77^{a}	0.739	4.430	2.009
PUFA[3]	371.47±0.38^{b}	394.13±18.69^{ab}	410.80±4.26^{a}	420.78±9.73^{a}	18.840*	4.611	0.696
total n-3[4]	108.53±1.76^{bc}	102.57±7.26^{c}	120.70±0.22^{ab}	122.90±6.25^{a}	22.303*	0.298	1.410
total n-6[5]	262.94±2.13^{b}	291.56±11.44^{a}	290.10±4.05^{a}	297.87±3.48^{a}	13.680*	16.178*	5.311
n-6:n-3	2.42±0.06^{b}	2.85±0.09^{a}	2.40±0.03^{b}	2.43±0.1^{b}	18.058*	18.531*	14.970*
PUFA: SFA	0.20±0.01^{b}	0.24±0.01^{a}	0.20±0.01^{b}	0.18±0.01^{c}	61.897*	5.112	54.822*
MUFA: SFA	0.90±0.02^{b}	1.02±0.01^{a}	0.79±0.04^{c}	0.79±0.01^{c}	136.384*	16.877*	18.458*

	bicep femoris						
	BC0	BC7	BP0	BP7			
C14:0	102.65±5.12	106.05±10.53	119.12±15.65	123.21±9.07	5.602	0.278	0.002
C16:0	1160.08±4.62	1190.46±95.93	1343.56±217.92	1305.33±8.3	9.277*	0.006	0.488
C16:1 (n-7)	152.39±3.03^{b}	186.22±14.08^{a}	129.88±34.71^{b}	128.98±3.62^{b}	24.783*	4.226	4.698
C17:0	51.31±1.12^{a}	43.29±2.47^{b}	43.44±6.61^{b}	47.13±0.18^{ab}	2.202	2.532	18.574*
C17:1	46.97±0.64	47.39±3.91	46.91±6.38	43.59±1.16	0.852	0.483	0.801
C18:0	635.47±0.72^{a}	527.83±47.32^{b}	556.5±107.23^{ab}	527.03±5.47^{b}	2.733	8.074*	2.625
C18:1 (n-9)	1852.19±19.8^{a}	1861.98±176.34^{a}	1504.75±449.35^{b}	1452.33±22.38^{b}	25.744*	0.082	0.174
C18:2 (n-6)	294.73±2.66^{a}	243.98±5.76^{b}	245.37±64.86^{b}	250.13±20.63^{b}	4.673	5.291	7.713*
C18:3 (n-3)	54.95±0.64	48.87±1.84	54.04±7.69	52.55±0.01	0.765	5.747	2.115
C20:2 (n-6)	11.75±1.1^{ab}	9.61±0.08^{c}	10.03±0.27^{bc}	12.43±0.43^{a}	1.265	0.069	21.260*
C20:3 (n-6)	20.81±1.48	24.17±0.34	21.39±0.17	27.68±0.91	1.352	7.521	0.694
C20:4 (n-6)	56.5±1.52^{b}	66.88±0.21^{a}	44.09±6.59^{c}	66.91±0.43^{a}	23.053*	165.882*	23.281*
C20:5 (n-3)	31.88±0.2^{b}	35.72±0.07^{a}	25.97±0.85^{c}	32.94±0.31^{ab}	25.105*	38.804*	3.248
C22:6 (n-3)	16.92±0.56	16.01±0.09	17.15±1.28	15.96±3.02	0.007	0.991	0.018
SFA[1]	1949.51±10.15	1867.63±156.24	2062.62±347.39	2002.7±4.53	2.538	0.828	0.020
MUFA[2]	2051.55±23.47^{a}	2095.58±194.32^{a}	1681.54±490.43^{b}	1624.9±19.92^{b}	25.024*	0.006	0.359
PUFA[3]	487.54±2.43^{a}	445.24±8.2^{ab}	418.04±63.74^{b}	458.61±24^{ab}	4.772	0.005	10.399
total n-3[4]	103.75±0.99	100.6±1.81	97.17±5.57	101.44±3.32	0.851	0.032	1.426
total n-6[5]	383.78±1.44^{a}	344.64±6.39^{ab}	320.88±58.17^{b}	357.16±20.68^{ab}	6.060	0.019	13.581*
n-6:n-3	3.7±0.03^{a}	3.43±0.01^{b}	3.3±0.29^{b}	3.52±0.08^{ab}	5.203	0.185	14.002*
PUFA: SFA	0.25±0.01^{a}	0.24±0.02^{a}	0.2±0.01^{b}	0.23±0.02^{a}	20.278*	1.443	8.541*
MUFA: SFA	1.05±0.01^{a}	1.12±0.02^{b}	0.82±0.01^{c}	0.81±0.01^{c}	2316.450*	33.199*	41.258*

[1]SFA = C14:0 + C16:0 + C17:0 +C18:0;
[2]MUFA = C16:1 (n-7) + C18:1 (n-9)
[3]PUFA = C18:2 (n-6) + C18:3 (n-3) + C20:2 (n-6) + C20:3 (n-6) + C20:4 (n-6) + C20:5 (n-3) + C22:6 (n-3)
[4]Total n-3 = C18:3 (n-3) + C20:5 (n-3) + C22:6 (n-3)
[5]Total n-6 = C18:2 (n-6) + C20:2 (n-6) + C20:3 (n-6) + C20:4 (n-6)
[a,b,c] Mean of fatty acids with different treatments within the same row differs significantly using Fisher's least significant difference ($p < 0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

4.2.6 Flavour Volatile Composition of PEF Treated Frozen Beef Muscles

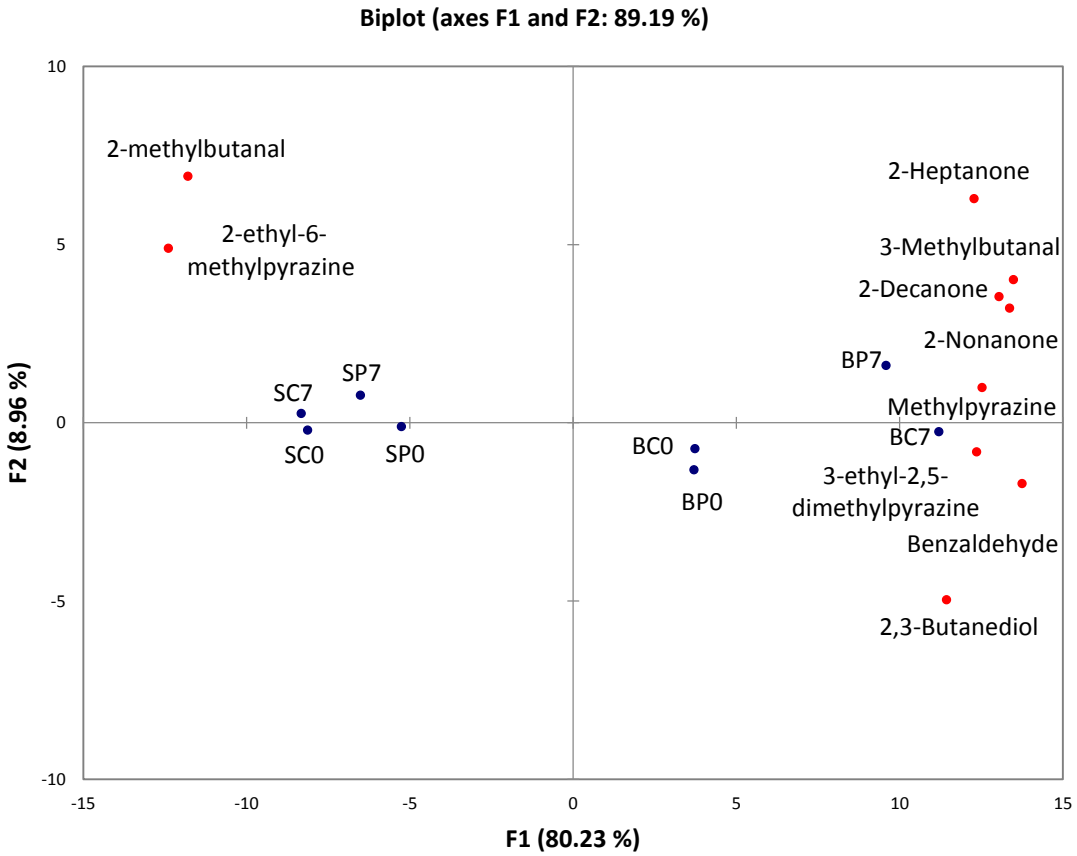

Figure 17 Bi-plot of volatile compounds in cooked frozen ST and BF beef muscles with or without PEF processing during 0 or 7 days storage

In order to illustrate differences between BF and ST beef samples with or without PEF processing during 0 and 7 days storage on the basis of individual volatile compounds, PCA was carried out (Figure 17). The PCA described 80.23% and 8.96% of the total variation of factor 1 (F1) and factor 2 (F2), respectively. Dimension 1 separated the samples in terms of different cuts, where negative loadings of the PCA corresponded to ST beef samples, and positive loadings corresponded to BF samples that were associated with most volatile compounds. There was no significant difference between BF beef samples except for BP7 which had significantly higher content of 2-methylbutanal. Lipid oxidation of fresh beef meat result in section 4.2.4 showed that frozen BF beef muscles had higher oxidation level than frozen ST beef muscles that may account for the higher volatile composition in BF samples. Faridnia et al. (2015) stated that the cooked meat flavour was influenced by the changes in volatile profiles, which were related to the degradation products derived from lipid and protein oxidation.

Table 18 Volatile compounds in cooked frozen BF and ST beef muscles with or without PEF processing during 0 or 7 days storage

Volatile compounds	Samples				F value		
					Processing	Storage	Processing*Storage
			Semitendinosus				
	SC0	SC7	SP0	SP7			
3-methylbutanal	0.07±0.01[b]	0.10±0.02[b]	0.10±0.01[b]	0.14±0.00[a]	17.807*	18.912*	1.332
2-methylbutanal	1.00±0.05[b]	0.98±0.07[b]	1.01±0.04[b]	1.30±0.01[a]	153.827*	180.827*	159.956*
2,3-butanediol	0.87±0.02[ab]	0.84±0.01[b]	0.91±0.03[ab]	0.94±0.05[a]	7.933*	0.039	1.294
methylpyrazine	0.84±0.04[b]	0.89±0.1[b]	1.28±0.19[a]	1.31±0.00[a]	31.057*	0.251	0.005
2-heptanone	0.32±0.01	0.36±0.03	0.37±0.03	0.38±0.00	4.990	2.454	0.832
benzaldehyde	1.00±0.03[b]	0.86±0.01[b]	1.46±0.21[a]	1.63±0.11[a]	52.974*	0.041	3.516
2-ethyl-6-methylpyrazine	2.29±0.03	3.33±0.04	2.44±2.82	3.84±0.06	3.367	0.080	2.848
3-ethyl-2,5-dimethylpyrazine	1.65±0.05[c]	1.75±0.08[c]	4.11±0.07[a]	3.02±0.04[b]	1756.950*	127.133*	177.915*
2-nonanone	0.40±0.05[b]	0.36±0.08[b]	0.79±0.05[a]	0.49±0.08[b]	29.002*	12.924*	6.696
2-decanone	0.44±0.2[c]	0.64±0.01[b]	0.14±0.00[c]	0.76±0.1[a]	1.299	26.204*	6.809
			Bicep femoris				
	BC0	BC7	BP0	BP7			
3-methylbutanal	0.33±0.03	0.59±0.4	0.3±0.01	0.69±0	0.059	5.263	0.201
2-methylbutanal	0.25±0[b]	0.25±0.02[b]	0.29±0.01[b]	0.61±0.03[a]	228.478*	155.751*	155.751*
2,3-butanediol	1.12±0.86	1.83±1.02	1.41±0.07	1.1±0.02	0.228	0.174	1.187
methylpyrazine	1.85±0.35	1.96±0.44	2.4±0.47	2.75±0.16	6.457	0.793	0.202
2-heptanone	0.49±0.3	0.67±0.26	0.36±0.05	0.73±0.11	0.077	3.552	0.416
benzaldehyde	2.96±1	3.5±0.15	2.91±0.59	3.12±0.08	0.257	0.821	0.158
2-ethyl-6-methylpyrazine	0.39±0.18	0.48±0.1	0.54±0.05	0.68±0.03	5.635	2.355	0.146
3-ethyl-2,5-dimethylpyrazine	4.34±3.65	6.23±2.79	3.83±0.16	4.57±0.43	0.442	0.648	0.126
2-nonanone	0.92±0.01	2.04±1.63	1.27±0.67	2.27±0.04	0.221	2.897	0.010
2-decanone	1.53±0.96	2.01±0.67	1.1±0.1	2.28±0.06	0.040	3.963	0.705

[a,b,c] Mean of fatty acids with different treatments within the same row differs significantly using Fisher's least significant difference ($p < 0.05$).
*significant differences in main effect of processing, storage, and interaction of processing and storage effects with 8 different samples at 5% level.

Chapter 5 Conclusion

This research was primarily carried out to determine the effects of pulsed electric field (PEF) processing on the sensory attributes, initial tenderness, colour, lipid oxidation, fatty acids, and flavour of beef meat. Our studies have mainly centred on the effects of PEF on the physicochemical characteristics and flavour of fresh and frozen-thawed beef muscles. PEF processing had similar effects on fresh and frozen beef meat. However, due to the damage on cell membrane during frozen-thawing processing, frozen beef samples had higher oxidation level. Therefore, the oxidation caused by PEF processing and storage in frozen samples were not as obvious as fresh samples.

Fresh beef meat

TDS was carried out to determine the temporal changes of dominant sensory attributes in cooked beef samples. Both storage and PEF treatment affected the temporal flavour of brothy and oxidized flavour attributes. The Initial tenderness increased with storage and PEF processing. In general colour profiles decreased in ST beef samples but increased in BF beef muscles with PEF processing and storage. The L, a*, and b* values of BF muscles after 0 day storage significantly increased after PEF processing. L and b* values were decreased by storage in PEF treated BF muscles. This corresponded well to significantly higher TBARS value of BF muscles compared to ST muscles in terms of storage and PEF processing. Most fatty acids significantly decreased after PEF processing in BF muscles. However, in ST muscles, there were no significant differences between PEF treated and non-treated samples during 0 day storage. In addition, PEF treatment and storage also influenced the composition of volatile compounds in fresh beef meat. However, most significant differences were between BF and ST muscles.

Frozen beef meat

TDS results of frozen beef meat samples showed that the dominances of brothy and oxidized sensory attributes were influenced by both PEF processing and storage. Initial tenderness of frozen beef samples increased with PEF processing but with no significant differences between 0 and 7 days of storage. In frozen beef samples the L value of ST

after 7 days of storage significantly increased with PEF treatment. The L, b*, and C values of BF muscles significantly increased after 7 days of storage. Moreover, the composition of fatty acids in PEF processed frozen beef was different from fresh beef, with the PUFAs in all the frozen beef samples being much lower for all treatments. Similar to fresh beef meat, most fatty acids decreased in BF muscles. However fatty acids increased in ST muscles with PEF treatment. Finally, BF and ST beef muscles varied in volatile composition in frozen beef meat, ST beef muscles contained more 2-methylbutanal and 2-ethyl-6-methylpyrazine than BF muscles.

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Appendices

1. Pulsed Electric Field Processing Operating Parameters Checklist

Pulsed Electric Field Processing of beef meat

Date/Time:			
Sample description			
Electrode distance (cm)			
Weight of chamber (g)			
Weight of water (g)			
Total weight (g)			
Conductivity before PEF (mS)			
Temperature before PEF (ºC)			
Conductivity after PEF (mS)			
Temperature after PEF (mS)			
Input voltage (%)			
Pulse width (µS)			
Frequency (Hz)			
Time (s)			
Field strength (kV/cm)			
Pulse voltage (kV)			
Pulse current (A)			
Pulse power (kW)			
Pulse energy (J)			
Total energy (kJ)			
Pulse number			
Pulse resistance (ohm)			
Flashover before PEF			
Flashover after PEF			
Calculated energy (kJ/kg)			
Comment			

2. Letter of Approval from AUT Ethics Committee (AUTEC)

AUTEC
SECRETARIAT

12 November 2013

Nazimah Hamid
Faculty of Health and Environmental Sciences

Dear Nazimah
Re Ethics Application: **13/317 Descriptive sensory analysis and consumer testing of cooked lamb and beef meat.**
Thank you for providing evidence as requested, which satisfies the points raised by the AUT University Ethics Committee (AUTEC).
Your ethics application has been approved for three years until 12 November 2016.
As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online throughhttp://www.aut.ac.nz/researchethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 12 November 2016;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/researchethics. This report is to be submitted either when the approval expires on 12 November 2016 or on completion of the project.

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.
AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this. If your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply there.
To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.
All the very best with your research,

Kate O'Connor
Executive Secretary
Auckland University of Technology Ethics Committee
Cc: Qianli Ma mql081228@hotmail.com

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Student		**Date**	8/10/2015
Primary Supervisor	Nazimah Hamid Nazimah Hamid	**Date**	9/10/2015
Secondary Supervisor	Michelle Yoo Michelle Yoo	**Date**	9/10/2015
Additional Supervisor		**Date**	

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