Effects of Pulsed Electric Field (PEF) processing on the physical, chemical and microbiological properties of Red-Fleshed Sweet Cherries (*Prunus avium* var. Stella)

By

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Abstract

The effects of mild or moderate intensity pulsed electric field (PEF) processing on the chemical, flavor and microbiological properties of Stella sweet cherries were studied. The specific aims of this study were to determine (1) the effect of different PEF treatments on the physico-chemical properties of cherry samples, (2) the effects of different PEF treatments and storage conditions on the volatile profile of cherry samples, (3) the effects of different PEF treatments and storage conditions on the extraction or release of bioactive compounds including the anthocyanins and polyphenols on PEF-treated samples, and finally (4) the growth of probiotic bacteria specifically the lactic acid bacteria on PEF-treated cherry samples,

The cherry samples were treated at a constant pulse frequency of 100 Hz and a constant pulse width of 20 μs with different electric field strengths between 0.3 and 2.5 kV/cm used. Cherry fruits used in this study that were subjected to PEF processing were analysed (1) immediately after PEF (S2) and (2) 24 hours of incubation at 4 °C (S3). PEF treated samples were significantly different to control sample in terms of juice yield, pH, titratable acidity (TA), total soluble solids (TSS) and moisture content (MC). There was a significant increase in juice yield in all PEF-treated samples compared to control. The pH values of samples incubated for 24 hours after PEF (S3) significantly increased compared to control and samples immediately after PEF treatment (S2). On the other hand, the values of TA and TSS significantly decreased for most S3 samples compared to the control and S2 samples. It was observed that as the intensity of PEF treatments increased, the TSS value also increased for S3 samples.

In terms of flavour volatiles, S3 samples generated higher concentrations of volatiles than S2 samples. The moderate intensity applied to the samples induced higher amounts of volatile compounds characteristic of cherry flavour. In addition, with the low energy intensities applied, no undesirable compounds were detected for all samples. In terms of anthocyanins, only cyanidin glucoside was significantly affected by PEF treatments. S3 samples had significantly higher cyanidin glucoside content compared to most S2 samples. A contrasting trend to anthocyanins was observed for polyphenols. Only four compounds, namely rutin, 4-hydroxybenzoic acid, isorhamnetin rutinoside, and myricetin, were significantly affected by storage and PEF treatments. S2 samples generated higher content of rutin, 4-hydroxybenzoic acid and isorhamnetin rutinoside

compared to S3 samples. On the other hand, myricetin was the only compound that was significantly higher in S3 samples than S2 samples.

Overall, the growth of LAB in cherries was affected by the different PEF treatments. Stimulatory (more nutrients were released after PEF application) effects by PEF were observed on samples immediately after PEF application (S2) which means the LAB count was positively increased while, inhibitory effects was observed for samples incubated for 24 hours after PEF (S3) which resulted in a decrease in LAB counts.

Overall, it can be concluded that the application of mild or moderate PEF intensities influenced chemical, microbiological and flavour characteristics of cherries. The findings from this study offer both methodological and practical contributions. The use and capability of PEF processing in terms of extracting valuable components of cherries that included flavour volatiles and bioactive compounds have been demonstrated. Hence this study would provide the food processors with a technological edge when using a non-thermal processing method, like PEF in meeting the current consumer demand for minimally-processed and fresh-like food products.

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Abbreviations

APHA American Public Health Association

ANOVA analysis of variance

AOAC Association of Official Analytical Chemists

APCI Atmospheric pressure chemical ionization

APPI Atmospheric pressure photoionization

AUT Auckland University of Technology

CFU colony forming unit

CMMF Compendium of Methods for Microbiology of Foods

C control

DAD diode array detector

DVB divinylbenzene

ESI Electrospray ionization

E-Nose electronic nose

EC *Escherichia* coli

EU European Union

F-C Folin-Ciocalteu

FD Folin-Denis

FAO Food and Agriculture Organization

FAS Foreign Agricultural Service

GC-FID gas chromatography – flame ionisation detector

GC-O gas chromatography olfactometry

GC-MS gas chromatography – mass spectrometry

GLP good laboratory practice

HS - SPME headspace solid phase microextraction

HIPEF high intensity pulsed electric field

HPLC high performance liquid chromatography

HPP high pressure processing

IQL individually quick-frozen

IPA isopropanol

IPA isopropyl alcohol

LAB lactic acid bacteria

LSD Least significant difference

LC-MS liquid chromatography - mass spectrometry

LOD limit of detection

LOQ limit of quantification

LOX Lipoxygenase

LLE Liquid-liquid extraction

LDL low-density lipoprotein

MS mass spectrometry

MUG methylumbelliferyl β-D- galactopyranoside

MO microorganisms

MIPEF mild or moderate intensity pulsed electric field

MT million tons

MA modified atmosphere

MAP modified atmosphere packaging

MC moisture content

MW molecular weight

MDPI Multi Interdisciplinary Publishing Incorporated

MMI Multimode ionization

MPS multi-purpose sampler

MRM Multiple Reaction Monitoring

NIST National Institute of Standards and Technology

OMF oscillating magnetic fields

PME Pectin methyl esterase

POD Peroxidase

PDMS polydimethylsiloxane

PPO Polyphenoxidase

PEF pulsed electric field

PFN pulse-forming network

RFEF radio frequency electric fields

ROS reduce reactive species

RI retention indices

SPME solid phase microextraction

SD standard deviation

S2 cherry samples immediately after PEF

S3 cherry samples 24 hours after PEF

S1 control or untreated cherry samples

TA titratable acidity

TOF time-of-flight

TAMB Total aerobic mesophilic bacteria

TAA total antioxidant activity

TIC total ion scan

TMY Total Molds and yeast

TPC Total plate counts

TP total polyphenolic

TSS total soluble solids

UAE ultrasound-assisted extractions

UV ultraviolet

USDA United States Department of Agriculture

WHO World Health Organization

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, 'Effects of Pulsed Electric Field (PEF) processing on the chemical, microbiological and flavour properties of Red-Fleshed Sweet Cherries (*Prunus avium* var. Stella)' contains neither material previously published or written by another person (except where explicitly defined in the acknowledgements) nor materials 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.

Name: Kristine Ann Gualberto Sotelo

Signed: 

Date: May 22, 2015

Co-authored Work

The flavour analysis part of this thesis has been submitted, accepted, and published in Molecules open access journals which belongs to the special issue called “Aromas and Volatiles of Fruits” by the Molecular Diversity Preservation International (MDPI) publisher. This was a jointly authored research paper, however, the candidate or the researcher itself was the principal author. Thus, this paper is the candidate’s own work.

The authors’ contributions are outlined below:

- Study, conception and design - Hamid, N., Oey, I. and Leong, S.Y.
- Acquisition of data – Sotelo, K.A.G.
- Analysis and interpretation of data - Sotelo, K.A.G. and Ma, Q.L.
- Drafting of manuscript - Sotelo, K.A.G. and Hamid, N.
- Critical revision - Hamid, N., Oey, I., Gutierrez-Maddox, and Sotelo, K.A.G.

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Chapter 1. Introduction

1.1 Thesis Statement/Motivation

Sweet cherry (*Prunus avium L.)* fruit is one of the most popular temperate fruits (Di Cagno et al., 2011; Bastos et al., 2015). Globally, only 7% of sweet cherries are grown in the southern hemisphere, of which New Zealand, Australia, and Chile are the world's biggest exporters (Paterson, 2003). Due to the relatively short growing season, a large part of fresh cherry is processed as brined, canned, frozen or dried products. Alternatively, cherries can be processed into jams, juices, jellies, puree, concentrates or syrups (Chaovanalikit & Wrolstad, 2004; Mark & Olga, 2004; Sinha et al., 2012; and Di Cagno et al., 2011). Frozen cherries on the other hand are commonly used as intermediate raw materials for a variety of food products including jams, preserves, and smoothies (Patras et al., 2009; Sinha et al., 2012).

According to Skinner, Hunter and others (2013) and Bastos and others (2015), sweet cherry is an essential fruit with high commercial importance worldwide that has been largely studied by the scientific community. Consumers generally like cherries because of its taste, balanced sweetness, colour (which is associated with anthocyanins), nutritional values, and bioactive (antioxidant) properties (Crisosto et al., 2003; Ferretti, Usenik, Fabcic, & Stampar, 2008; Bacchetti et al., 2010; Serra et al., 2010; Usenik et al., 2010; Liu et al., 2011; Pacifico et al., 2014; Serradilla et al., 2011; Ballistreri et al., 2013). However, processed cherry fruits require strict treatment conditions to protect its quality, especially the flavour and bioactive compounds, which are important attributes in determining consumers' acceptability.

Cherries are also rich with essential vitamins, minerals, fibers, and most especially phytochemicals collectively known as 'bioactives' such as phenolics and anthocyanins. Cherries contain liposoluble (fat-soluble) vitamins such as A, E, and K, and hydrosoluble (water-soluble) vitamins such as C and B, minerals such as calcium, magnesium, phosphorus, and potassium, and some carotenoids particularly beta-carotene (Yıgıt, Baydas, & Guleryuz, 2009; Schmitz-Eiberger & Blanke, 2012). However, the most important compounds that are gaining much attention and interest by the scientific community and consumers nowadays are the phytochemicals in

cherries. These include the anthocyanins and polyphenols that are very powerful in their observed protective effect as an antioxidant (Schreiner and Huyskens-Keil, 2006). Anthocyanins and polyphenols have been reported and recognized to be effective in strengthening the body's immune system, prevention of several diseases like cancer, cardiovascular diseases, and other oxidative stress related diseases (Beattie, Crozier, & Duthie, 2005; Serra, Duarte, Bronze, & Duarte, 2011; Kamei et al., 1998; Changwei et al., 2008; Allen & Silver, 2010; Iezzoni, 2008; Sinha et al., 2012). Because of these health benefits, studying means of enhancing them would be of significant importance.

Pulsed electric field (PEF) processing is a non-thermal food processing technology based on the application of short pulses of high voltage applied on a food product, whether semi-solid or liquid form placed between two electrodes (Asavasanti et al., 2011). The application of PEF on food induces cell membrane permeabilization through a phenomenon called "electroporation" (Asavasanti et al., 2011). This can induce pore formation within cellular components due to the localised structural changes and the breakdown of the cellular membrane that in turn, allows the extraction of plant compounds (Toepfl et al., 2005). This process is carried out at either low or moderate temperature and is a promising non-thermal extraction technique (Zeuthen & Bogh-Sorensen, 2003). The effect of low or moderate intensity PEF has been mainly studied on the extraction of bioactive compounds from fruits and vegetables. Toepfl (2006) reported that the extractability of fruit and vegetable juices or intracellular compounds could be enhanced after PEF-treatment. Higher juice yield (5% more) with higher total anthocyanin content (3 times higher) was obtained after PEF treatment at 3 kV/cm applied to grape samples (Tedjo et al., 2002). Higher concentration of bioactive compounds such as polyphenols in tomatoes was also reported after PEF treatment (Vallverdú-Queralt et al., 2012). PEF treatment at 0.5 kV/cm also increased the total polyphenolic (TP) content in fresh pressed grape juice by 13% in comparison to reference sample (Balasa et al., 2006).

To date, the effect of PEF treatment at low or moderate intensity on the flavour profile of fruit and vegetables has not been studied. Vallverdú-Queralt and others (2013). indicated that PEF treatment at moderate intensity increased the concentration of hexanal and (E)-2-hexenal flavour compounds in tomatoes processed into juice. The effect of PEF treatment on the volatile profiles has been mainly studied at high intensity PEF treatment required for preservation (28 to 40 kV/cm electric field strengths).

Studies showed that PEF processing changed the flavour volatiles of watermelon juice during storage (Aguiló-Aguayo et al., 2010), strawberry juices (Cserhalmi et al., 2006), citrus juices (Aguiló-Aguayo et al., 2009), apple juice (Aguilar-Rosas et al., 2007), longan juice (Zhang et al., 2010), and orange juices Yeom et al., 2000; Ayhan et al., 2001; Min et al., 2003; Jia et al., 1999).

Furthermore, the positive effect of different PEF treatments in stimulating growth of probiotic bacteria has not been studied. The use of mild or moderate PEF intensity can induce reversible pore formation that could extract bioactive compounds such as anthocyanins and polyphenolics. These compounds can affect the growth of probiotic bacteria by either exhibiting stimulatory (Sun-Waterhouse et al., 2012; Hap, 2010; Hervert-Hernández and others, 2009; Koren et al., 2009) or inhibitory effects (Hap and Gutierrez, 2012).

1.1.1 Objectives

The general objective of this study was to determine the effects of low or moderate pulsed electric field (PEF) processing on the chemical, flavour, and microbiological quality properties of red-fleshed sweet cherries.

The specific objectives of this study were to (1) determine the basic physical properties such as the size range and average fruit weight of cherry samples, and to determine the effect of different PEF treatments on the physico-chemical properties of cherry samples such as juice yield, conductivity, pH, colour, titratable acidity, total soluble solids, and moisture, (2) perform method development for the optimum extraction of anthocyanins and polyphenols in cherry samples using Liquid Chromatography Mass Spectrometry (LCMS), and to determine the effects of different PEF treatments and storage conditions on the extraction or release of these bioactive compounds.(3) determine the effects of different PEF treatments and storage conditions on the volatile profile of cherry samples, and finally (4) determine the growth of probiotic bacteria specifically the lactic acid bacteria on PEF-treated cherry sample,

1.1.2 Significance

Interest in the role of antioxidants in human health has prompted research in the fields of horticulture and food science to assess fruit and vegetable antioxidants. Food

processors are exploring new processing methods to gain a technological edge in the marketplace to meet the current consumer demand for minimally-processed and fresh-like food products without synthetic chemical preservatives. Hence in this study, the capability of low or moderate PEF treatments on the physicochemical properties of cherries, as well as the extraction and enhancement of flavours and bioactive components of cherries were evaluated.

1.2 Thesis structure

This chapter is arranged in five chapters. The first chapter provides a background to the study carried out. Thereafter, the context of the study is presented in which the research problem, central research question, and research objectives are outlined. Moreover, the significance of the study is also presented. Chapter 2 presents a detailed literature review for this research detailing physical, chemical, and microbiological studies on pulsed electric field processing of fruit and vegetable products. Chapter 3 outlines the methodology of the study, and explains the research approach used. Chapter 4 presents the main contribution of the thesis in which findings are presented and discussed. This chapter explains the effect of PEF processing on the physical, chemical, and microbiological properties of cherries. Chapter 5 is the final chapter of this thesis that summarizes the main results or the key findings of this research. Moreover, the implications of the study and limitations are acknowledged. Finally some recommendations and considerations for further future related researches are addressed.

Chapter 2. Literature Review

2.1 Cherry Fruit

2.1.1 Sweet and Sour Cherries

A cherry fruit is a fleshy stone fruit or drupe, which is obtained from plants of the genus *Prunus* (Dirlewanger et al., 2007). There are two major species of cherries that are commercially grown, namely the sweet or wild cherry (*Prunus avium*) and the sour or tart cherry (*Prunus cerasus*) (Iezzoni, 2008).

2.1.1.1 History and Origin

Prunus avium includes sweet cherry trees cultivated mainly for human consumption and for the use of their woods, also known as mazzards. In parts of Asia, sweet cherries (Figure 1) are indigenous, especially in northern Iran, Ukraine, and countries south of the Caucasus mountains (Dirlewanger et al., 2007). The descendants of the modern cultivated sweet cherries are reckoned to have originated around the Caspian and Black Seas, where they have spread slowly (Mark & Olga, 2004; McCune, Kubota, Stendell-Hollis, & Thomson, 2010; Dirlewanger et al., 2007). Presently, sweet cherries are commercially cultivated in more than 40 countries around the world in temperate Mediterranean and subtropical regions (Dirlewanger et al., 2007). Its natural range encompasses the temperate regions of Europe, from the north of Spain to the southeastern part of Russia (Hedrick et al., 1915; Dirlewanger et al., 2007). Regions with warm and dry summers are preferred. However, adequate rainfall and irrigation during the growing season are also required to produce fruits with appropriate size for marketing purposes (Dirlewanger et al., 2007).

Figure 1 Satellite image of the Caucasus Mountains (origin of sweet cherries or Prunus avium) (O'Connell, 2008)

The fruit of *Prunus cerasus* includes sour cherry trees. This species is mainly used for processed products including pie jam or liquor (Dirlewanger et al., 2007)). They originated around the Caspian Sea close to Istanbul (Dirlewanger et al., 2007; Iezzoni, 2008). Though less widely cultivated compared to sweet cherries, large quantities of sour cherries are produced in many European countries and in the United States of America (USA) (Dirlewanger et al., 2007).

2.1.1.2 Sweet Cherry Varieties

There are more than 30 varieties of sweet cherry with two main colour categories, namely dark (red and black) and yellow (Sinha, Sidhu, Barta, Wu, & Cano, 2012). Some of the popular sweet cherry varieties include Lapins, Stella, Bing, Starkrimson, Ambrunes, Van, Sweetheart, Lambert, Summit, Salmo, Chelan, Skeena, and etc. These varieties of sweet cherry are commonly grown in temperate zones but can also withstand cold winter weather (Di Cagno et al., 2011).

Sour cherry has lesser variability and its colours are either light or dark red (Sinha et al., 2012). Montmorency, Meteor, Morello, and Northstar are examples of sour cherry cultivars. Unlike sweet cherry, sour cherry can be grown in various regions because it can adapt better to climatic conditions without major damages (Sinha et al., 2012).

2.1.1.3 Growing season

The growing season for cherries is relatively short wherein summer is the peak season. In the northern hemisphere countries, cherries are available from late December to July. Cherries are usually at their peak in late December in Australia and New Zealand; June in Southern Europe and North America; mid-July in United Kingdom; and July to mid-August in Canada.

2.1.2 Stella Cultivar

Stella is the first self-fertile sweet cherry cultivar named by the Canada Department of Agriculture Station in Summerland, BC in 1968 (Lapins, 1971). The parent of this self-fruitful pollen originated through a program designed to produce self-fruitful sweet cherries at the John Innes Institute, Colney Lane, Norwich, England (Lapins, 1971). Stella cherry is described as a large, heart-shaped fruit, red to dark red in colour, and sweet flavoured cherry (Lapins, 1971). The flesh is medium, coarse, and moderately firm; the juice is coloured; the flavour is fair to good; and the fruit contains high levels of both soluble solids and titratable acids.

2.1.2.1 Structure

All cherries morphologically share common characteristics, which differentiate them as cherries. These characteristics comprise the seed surrounded by the pericarp and stalk that provide nutrients through plant's vascular components as the fruit grows, matures, and ripens on the tree. All cultivars have the same features displayed in Figure 2. A cherry fruit is composed of pericarp and a seed. The pericarp consists of three components, namely, endocarp, mesocarp, and exocarp. Endocarp is the layer that lies inside the fruit. It can either be thick and hard or slightly fleshy or is the part that covers the seed forming part of the stone (Vursavuş, Kelebek et al., 2006). The mesocarp is the

fleshy layer of the fruit that lies between the exocarp and the endocarp, and therefore is the most edible part of the fruit. The exocarp is commonly known as the skin or the outer layer of the fruit. This part usually forms and stores pigments and nutrients and commonly dictates the appearance of the fruit. Lastly, the seed is the part of a fruit that houses the embryo that has the capability of germinating to make new plants grow.

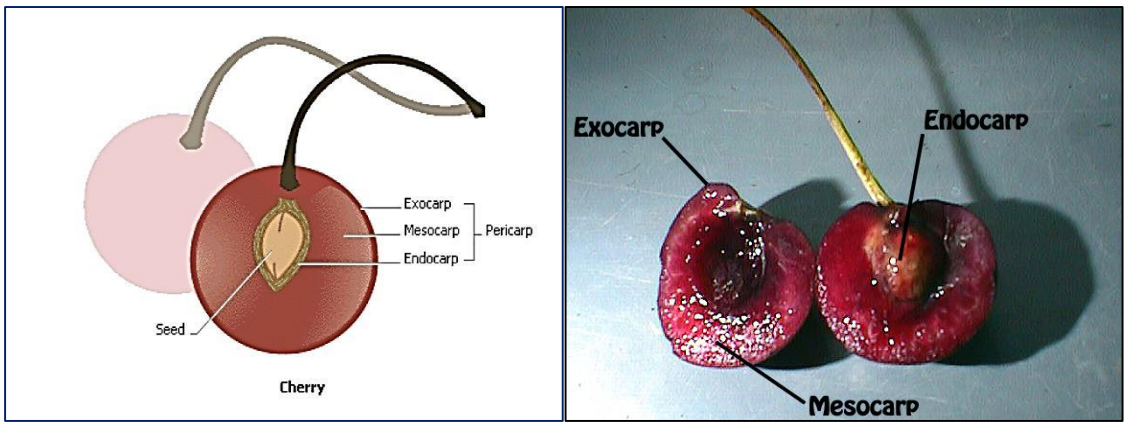


Figure 2 Basic cherry structure (Department of Plant and Soil, 2001)

2.1.2.2 Production and Consumption

2.1.2.2.1 Popularity of stone fruits

The consumption of fruits is becoming more popular all over the world. Fruits are an essential part of the human diet that can contribute important nutrients to the human body. Consumption of fuits can prevent major diseases such as cardiovascular diseases and certain cancers, through sufficient daily consumption (World Health Organization (WHO), 2015). Since the discovery of the role of vitamins and minerals in the human diet, and the awareness of fruits' health benefits, there have been substantial changes in daily fruit consumption (Kennedy and Stepien, 2008). Hence there has been an increasing demand for fruits, with many programs seeking to improve the production of fruits and expansion of productions zones into the milder winter zones to allow year-round availability of stone fruit (Bryne, 2005).

Stone fruits are becoming more popular to western consumers and include peaches, nectarines, plums, apricots, and cherries. They are all members of the *Prunus* genus and are therefore closely related. They commonly are referred to as "stone fruits" because the seed is very large and hard (College of Agricultural Sciences, 2015).

2.1.2.2.2 Global production and exports of cherry fruits

The world's largest sweet cherry producing countries according to the Food and Agriculture Organization of the United Nations (FAO) are Turkey, United States of America, Iran, Italy, Spain, and Russia (Table 1). Cherry (sweet and sour) fruit annual world production is about 2.8 million tons. The world's total production of sweet cherry is approximately 1.6-1.8 million tons (MT) in 2011, and 50% are produced in Western Europe (Sinha et al., 2012, Allen & Silver, 2010). Turkey is still the leading and highest sweet cherry producer since 2000 with a 9.6% increase in 2012 to that of the previous year at a total of 42, 198 MT. In terms of exports, the top cherry exporting countries are listed in Table 2 with United States of America in the lead from 2000 to 2011. In 2000, these top cherry exporting countries exported a total of 136, 269 MT of cherries. The same cherry exporters reached a total of 295, 183 MT of cherry exports in 2010. After a year (2011), an increase of 14 % in cherry exports was recorded at a total of 41, 433 MT. According to Food Agricultural Services (FAS) of United States Department of Agriculture (USDA), the world cherry production in 2014/15 is forecasted to increase by 2% to 2.4 million tons, as gains in China and Chile offset reductions in the (European Union) EU and the United States. Consumption is up, driven largely by China, while processing is lower due to reduced supply in the EU and United States. Meanwhile, exports are forecasted at a record 304,000 tons. With these, it is clear that the global cherry industry is extremely productive, and thus research in improving cherry quality is worthwhile and beneficial to the industry through agriculture, food science, and food processing.

Table 1 World cherry production from 2001 to 2012 in metric tons (MT), (FAOSTAT, 2013)

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Turkey	250 000	210 000	265 000	245 000	280 000	310 254	398 141	338 361	417 694	417 905
U.S.A.	209 010	164 564	160 844	256 824	227 522	266 349	281 862	225 073	401 792	284 130
Iran	218 584	220 000	222 000	174 576	224 892	225 000	200 000	198 768	225 000	255 500
Italy	111 000	126 000	102 000	95 169	101 295	110 910	106 189	134 407	116 179	115 476
Spain	85 600	115 182	107 975	83 467	95 726	91 672	75 738	72 466	96 400	80 300
Russian Fed*	88 000	85 000	90 000	100 000	93 000	50 000	100 000	73 000	76 000	66 700
Romania	91 200	66 300	98 500	50 988	117 859	104 791	65 163	67 664	67 874	70 290
Ukraine	54 900	72 500	73 800	85 300	100 200	48 900	68 200	74 700	53 000	73 000
France	55 579	68 779	50 826	61 748	69 024	68 122	47 557	40 356	53 575	45 905
Syria	50 795	39 728	54 800	35 400	53 441	62 966	75 034	48 300	56 900	58 100
Greece	40 015	47 090	44 618	46 714	44 201	43 700	60 700	57 200	61 128	38 200
Uzbekistan	20 500	21 400	19 500	14 500	22 000	53 605	55 000	61 000	67 000	75 000
Chile	27 950	29 000	29 500	32 000	32 000	41 000	45 000	56 000	56 000	59 000
Poland	44 645	40 756	44 122	48 442	37 508	38 364	20 186	40 818	50 505	35 462
Lebanon	42 300	33 600	36 900	30 700	29 500	23 400	30 000	31 000	34 662	38 700
Germany	38 418	27 768	33 386	39 076	27 911	31 637	34 452	25 166	39 463	30 831
Austria	32 069	21 818	29 384	27 250	25 909	27 243	33 890	26 790	30 276	39 960
Serbia**	21 635	17 257	27 158	32 295	21 509	23 302	28 546	29 551	29 228	22 201
China	12 000	12 500	14 000	20 000	25 000	19 000	22 000	25 000	27 000	28 500
Bulgaria	28 487	15 654	17 243	21 369	18 235	20 504	18 427	16 067	17 456	24 951
Japan	19 600	21 200	19 300	16 400	19 100	20 800	16 600	17 000	16 600	19 700
Portugal	11 981	19 990	14 135	16 149	15 612	15 561	9 289	10 528	11 227	8 700
Czech Rep***	12 194	10 289	9 474	18 291	9 094	2 244	2 059	2 379	2 404	2 107
Argentina	7 602	6 800	6 912	6 700	7 267	6 750	6 800	4 813	5 061	6 000

Russian Fed*= Russian Federation, Serbia** = Serbia Montenegro untill 2005, Czech Rep***=Czech Republic

World cherry production	2011	World cherry production	2012
Turkey	438 550	Turkey	480 748
U.S.A.	303 363	U.S.A.	384 646
Iran	241 117	Iran	200 000
Italy	112 775	Italy	104 766
Spain	101 729	Spain	98 400
Russian Fed*	76 000	Russian Fed*	72 000
Romania	81 842	Romania	70 542
Ukraine	72 800	Ukraine	72 600
France	48 082	France	30 440
Syria	62 195	Syria	82 341
Greece	44 200	Greece	60 300
Uzbekistan	82 000	Uzbekistan	84 000
Chile	61 008	Chile	90 000
Poland	37 984	Poland	41 063
Austria	92 520	Austria	38 680
Germany	37 035	Germany	23 005
Lebanon	21 000	Lebanon	22 500
Serbia**	28 557	Serbia**	22 213
China	32 000	China	35 500
Bulgaria	30 063	Bulgaria	19 512
Japan	20 400	Japan	17 800
Portugal	13 144	Portugal	10 500
Czech Rep***	2 662	Czech Rep***	1 539
Argentina	6 215	Argentina	6 500

Table 2 World cherry exports from 2000 to 2011 in metric tons (MT) (FAOSTAT, 2013)

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
U.S.A.	36 502	38 879	33 359	45 391	40 629	48 394	42 804	54 935	50 905	69 754	64 305	78 298
Turkey	11 940	24 553	19 042	32 688	39 732	34 793	53 867	57 019	28 549	50 785	65 294	46 477
Chile	6 927	8 763	12 787	12 816	11 308	17 916	22 462	26 885	44 782	23 474	44 311	64 612
Austria	5 153	9 685	10 229	15 108	22 555	19 160	22 494	18 509	10 947	18 553	20 526	17 342
Spain	13 623	9 147	11 465	10 956	11 747	13 676	22 117	8 401	13 462	25 498	22 951	29 268
Syria	9 108	6 372	12 723	3 108	4 700	7 110	9 512	3 639	1 371	11 618	13 769	9 036
Italy	11 295	5 213	5 721	7 234	2 497	11 671	6 436	8 216	9 230	4 850	5129	11 189
Poland	6 462	0	5 695	14 266	11 408	8 598	12 035	2 045	11 139	10 995	2 489	5 049
France	7 677	6 467	8 986	7 687	8 261	8 482	8 517	3 844	3 026	6 814	4 763	7 065
Greece	4 988	3 491	3 550	1 201	2 649	4 439	3 728	6 439	9 998	6 737	7 291	9 448
Netherlands	2 998	3 042	3 302	4 977	6 060	4 565	3 859	5 383	5 054	5 129	5 670	4 790
Germany	3 656	2 882	1 827	2 721	5 201	3 530	4 610	7 418	2 890	5 139	4 442	7 625
Belgium	2 436	1 675	2 445	4 517	4 122	3 629	4 885	4 095	4 827	2 371	3 064	4 699
Kyrgyztan	179	46	462	162	686	377	2065	2 964	6 765	12 881	5 8 058	5 264
Lebanon	1 572	2 120	2 291	2 777	2 386	4 255	4 355	4 029	3 640	2 150	3 420	2 611
Serbia*	3 624	0	3 137	223	1 567	385	165	2 918	4 171	9 563	7 084	10 771
Canada	249	1 047	1 908	2 050	3 042	2 758	3 665	4 763	2 465	5 559	5 206	7 087
Iran	1 661	1 522	1 252	1 054	566	635	5 180	6 459	5 022	5 022	3 292	8 375
Czech Rep**	6 098	4 673	5 095	4 969	1 253	704	2 019	459	877	1 353	1 751	1 809
Moldova	301	782	472	918	2 480	4 071	3 440	1 704	1 744	4 232	2 368	5 801

2.1.2.3 Market-driven

Sweet cherries are being produced based on the requirements outlined through commercial selection or what is called ‘market-driven’. The market requirements, which influence variety selection include availability, fruit size (to compete with others), fruit colour, fruit flavour (for consumer likeness), and fruit firmness (for good transport) (Lang, 2011).

2.1.3 Physicochemical properties of sweet cherries

The composition of cherries depends mainly on the physical and physiological factors that can influence postharvest quality. These factors are discussed below.

2.1.3.1 Physical factors

Several physical factors influence cherry composition. They can be grouped as either soil or climate factor. These are important to consider when selecting a suitable site for

cherry cropping. Soil factors include type of soil, depth, drainage, pH, and also status of nutrients (Longstroth and Perry, 1996). For sweet cherries cultivation, deep-layered soil with pH range of 5.5 to 7.5 rich in nutrients, and root system with aeration (Simon, 2004) are important requirements. Climate factors include temperature, wind, light quality, rainfall, and photoperiod. Sweet cherries are mostly grown within the temperate zone. Since it is a composite genus, it needs warm and light conditions. However, it can also withstand cold winter weather. Sweet cherries require an annual precipitation of 550 to 600 millimeters per annum (Simon, 2004). All of these factors vary extensively according to the region in which they are grown.

2.1.3.2 Physiological factors

Physiological factors that can influence the composition of cherry fruits include maturation and ripening, which will then define the quality recognized by consumers. These two key physiological factors also affect the final size and weight of the fruit (Sinha et al., 2012). When a fruit is at its maturation age, its palatability is improved as its size and weight reach a maximum and its growth rate decreases (Sinha et al., 2012).

Ripening of a fruit refers to the physiological and biochemical changes that it undergoes to attain desirable color, flavour, aroma, sweetness, texture, and thus consumption quality (Sinha et al., 2012). This process usually does not occur until a fruit reaches its full maturity with either fruit ripening on the plant or after harvest (Sinha et al., 2012). For sweet cherries, the ripening and increase in size and weight occur during the last few weeks prior to harvest (Barrelt & Gonzalez, 1994).

2.1.3.2.1 Physiological changes of a fruit during maturity

The colour, seed, carbohydrate content, acids content, aroma and flavour, and firmness of cherry can change with maturity in addition to fruit size, shape, and weight. The colour of a fruit changes when it grows to full maturity. There will be a decline in the chlorophyll in the chroroplast of the skin cells, and thus results in the fading of colour (Sinha et al., 2012).

In addition, an attractive colour develops on the fruit's skin mainly due to the accumulation of anthocyanins, carotenoids, or flavones in vacuoles of epidermal cells

(Fernandez-Lopez et al., 1992; Ikoma et al., 2001). Sucrose, fructose, and glucose are the major sugars found in cherries. For the carbohydrate profile, a significant amount of sucrose is transported during fruit maturation and then partially changed to fructose and glucose (Holland et al., 1999). As the sweetness of the fruit increases with maturity, the acid content decreases. The major acids found are malic, citric, and tartaric acids. In cherries, malic acid is the predominant acid.

The aroma and flavour compounds in fruits starts to develop and accumulate when they reach full maturity, and then decline as the fruit enter the senescence phase (fruit passes maximum ripeness) (Sacher, 1973). The development and accumulation would however be determined not only by their genetics but also by other factors such as the environment, agrichemicals, cultural practices, and nutrition that can impact on fruit development (Mattheis and Fellman 1999). Firmness decreases as fruit becomes mature. This results when the cell walls become less interconnected due to pectin degradation and intercellular space expansion (Brummel et al., 2004).

2.1.3.4 Physical properties of sweet cherries

2.1.3.4.1 Shape, size, colour

The shape of wild sweet cherry is quite variable, and can be spherical, lengthwise, or heart-shaped (Sinha et al., 2012). Botanically, "bird cherry" or *Prunus avium* species is classified as "heart-cherry" or heart shaped sweet cherry varieties (Terpo 1974; Brown et al., 1996). The size could be small, medium, large, and very large with weights about 3 to 5, 5 to 7, 7 to 9, and 9 to 12 grams respectively (Sinha et al., 2012). In terms of colour, dark and yellow sweet cherries are the two main types.

2.1.3.5 Chemical properties of sweet cherries

2.1.3.5.1 Carbohydrates, sugars, and organic acids

According to Rodler (2005), two-third of total carbohydrates in sweet cherry comprise fructose (4.2 g/100 g), which is significantly higher than glucose (2.2 g/100 g). It also contains sorbitol (0.76 g/100 g), which is believed to have a laxative effect (Rodler, 2005). All fruits contain significant amount of organic acids that produce sour sensation

or acidity in fruits. According to the study of Usenik and others (2008), the acid content of sweet cherry is between 0.2 and 0.7 %, in which malic acid is the predominant acid present in cherries. The acid to sugar ratio of cherry is 0.07 (Sinha et al., 2012).

2.1.4 Flavour Qualities

Flavour is one of the important attributes in determining fruit quality and consumers' acceptability. It refers to the impressions perceived via the chemical senses from the product in the mouth (Caul, 1957). These impressions include olfactory perceptions called 'aromatics', which are caused by volatile substances released from a product in the mouth via the posterior nares, taste perceptions (salty, sweet, sour, bitter and umami) caused by soluble substances in the mouth, and lastly, the chemical feeling factors such as astringency, cooling, bite, spice heat, and metallic flavour, which stimulate nerve ends in the soft membranes of the buccal and nasal cavities (Caul, 1957). In fruits, flavour involves interaction of diverse metabolites such as sugars, organic acids, and aroma compounds (Baldwin, 2002) that are responsible for the flavour in cherries.

2.1.4.1 Cherry flavour

Flavour compounds are formed during growth, maturation, ripening, and senescence of fruits. These compounds are nevertheless influenced by factors such as genetic, preharvest, harvest, and postharvest (Mattheis and Fellman, 1999). Fruit flavours in fruits involve both volatile and non-volatile components. Both volatile and non-volatile compounds will determine the flavour characteristic of fruits, which play an important role in consumer flavour perception.

2.1.4.1.1. Non-volatile compounds

Sugars and organic acids are the primary constituents contributing to taste of cherries. Fructose and glucose are the most abundant sugars in cherries, and malic acid is the predominant acid. Sucrose is also present but in lower quantity. Non-volatile compounds like the sugar alcohols (sorbitol and mannitol), ascorbic acid, citric,

fumaric, and succinic acid are considered minor compounds present in cherries (Girard & Kopp, 1998; Roddler, 2005).

Fruits undergo carbohydrate metabolism in which the starch is converted to sugars during maturation (Sinha et al., 2012). During the ripening process, starch undergoes degradation which will result in accumulation of glucose and fructose in the fruit flesh. In the case of cherry fruits, sugar accumulation is only possible when they are attached to the plant (Revell, 2009; Sinha et al., 2012). Hence, it is very important to harvest cherries that are in full maturity to ensure that flavours are fully developed.

2.1.4.1.2 Volatile compounds

The volatile compounds in cherries refer to the odour and aroma of fruit itself. Flavour volatiles in cherries are very important because these distinguish them or make them unique from other fruits (Revell, 2009). The flavour profile of cherries is relatively complex. Sweet cherries contain a great number of volatile compounds, wherein the major constituents are aldehydes, alcohols, acids, esters, alkanes, aromatic hydrocarbons, ketones, and terpenes.

Several researchers have studied the flavour volatiles of cherries. According to Schmid and Grosch (1986), volatiles such as benzaldehyde, (E)-2-hexenal, and hexanal were reported to be the major flavour compounds found in cherries. Mattheis (1992) reported that a total of 28 aroma compounds were detected in "Bing" sweet cherry fruit using headspace sampling, wherein 2-propanol and ethanol had relatively high concentrations while benzaldehyde and hexanal had high aroma values and were highly correlated with fresh cherry aroma. In the study of Girard and Kopp (1998), 50 volatile compounds were identified in 12 sweet cherries using a dynamic headspace method coupled with GC-MS. They reported that (E)-2-hexenol, benzaldehyde, hexanal and (E)-2-hexenal were the predominant flavour volatiles in sweet cherries. Using the same technique Bernalte et al. (2007) identified 62 volatile compounds in sweet cherry. Of these, hexanal and (E)-2-hexenal were the major compounds found. Zhang and co-workers (2007) studied the aroma components of "Hongdeng" sweet cherry using HS-SPME, followed by GC-MS, which led to the identification of 37 compounds. Six compounds, including hexanal, (E)-2-hexenal, benzaldehyde, (E)-2-hexen-1-ol, ethyl acetate, and hexanoic acid ethyl ester were found to be characteristic aroma components of sweet cherry fruit.

2.1.4.1.2.1 Flavour analysis

Identification of flavour volatiles involves the use of analytical instruments such as gas chromatography flame ionization detector (GC-FID), gas chromatography mass spectrometry (GC-MS), gas chromatography olfactometry (GC-O), and also the electronic nose (E-Nose). Moreover, different techniques of sample preparation have also been studied. Common sample preparations include direct injection, headspace methods (direct and dynamic), solvent extraction, distillation methods, and solid-phase microextraction (SPME).

The SPME method is gaining much interest because of its advantages of being simple, low-cost, solventless (which prevents matrix effects and interferences present in liquid sample), environmentally friendly, relatively fast, and reasonably sensitive when it comes to volatile analysis (Reineccius, 2007; Roberts, Pollien et al., 2000; Jeleń, Majcher et al., 2012; Steffen and Pawliszyn, 1996; Sides, Robards et al., 2000; (Jeleń, Majcher et al., 2012). SPME devices can be either used manually (direct injection) or automated (using an auto sampler) (Jeleń, Majcher et al., 2012).

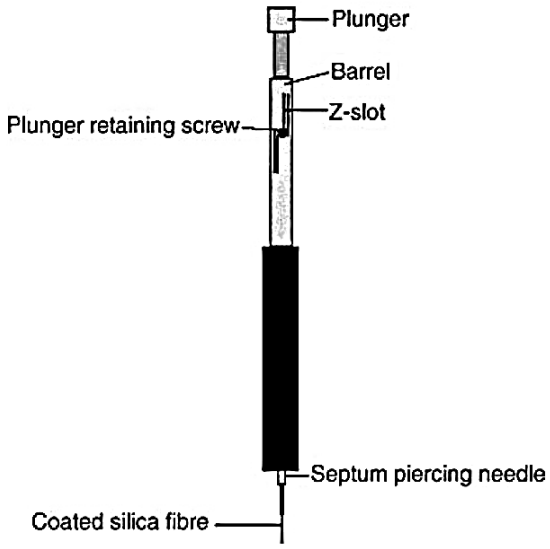


Figure 3 Parts of a SPME device (King, Readman et.al 2003)

2.1.4.1.2.1.1 Solid Phase Microextraction (SPME)

2.1.4.1.2.1. 1.1 SPME device

A SPME device is comprised of a syringe-shaped device (Figure 3) that holds a polymer-coated fiber (designed to adsorb different polar and non-polar groups of volatiles) in the needle, which is 1 cm in length attached to a stainless steel plunger and installed in a holder (King, Readman et al., 2003; Wampler, 2001).

2.1.4.1.2.1.1.2 Mode of operation

SPME works by exposing the fiber into a sample matrix whether immersing it in the liquid sample or to the headspace above it until equilibrium is achieved between the analyte in the sample (Figure 4). The volatile compounds are concentrated by the fiber while adsorption is taking place. Once equilibrium is reached, the fiber is taken out from the sample matrix and then introduced or injected into a gas chromatography injection port, where the adsorbed analytes are thermally desorbed (King, Readman et al., 2003; Supelco, 1998).

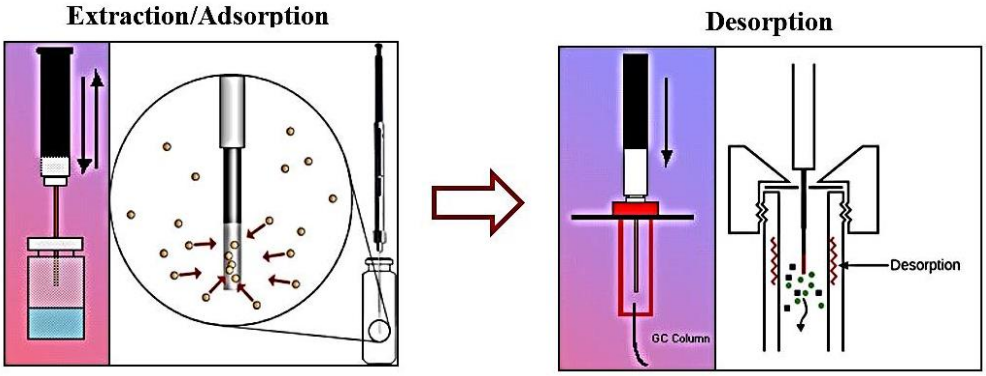


Figure 4 Principles of SPME (extraction and desorption procedures)

2.1.4.1.2.1.2 Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS is a powerful analytical technique combining the features of gas chromatography and mass spectrometry to identify and quantify complex mixtures of chemicals present in a given sample (Figure 5). The GC separates the mixture with respect to time and the mass spectrometer provides information about the structural properties of compounds, which lead to their identification (Kitson, Larsen et al., 1996; Kitson, Sparkman et al., 2011). Mass spectrometry or MS utilizes high-energy electrons to break a certain molecule into fragments. The separation and analysis of the fragments gives information about the structure and molecular weight of compounds. The synergistic combination of GC-MS provides a complete mass spectrum of an analyte.

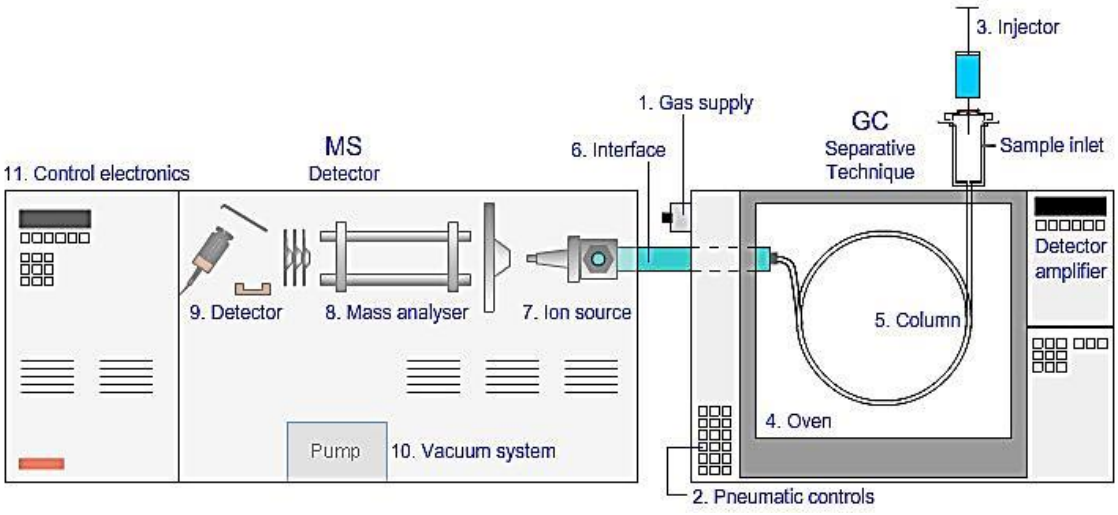


Figure 5 Schematic diagram of combined GC-MS (CHROMacademy, 2010)

2.1.4.1.2.1.3 Applications of combined HS-SPME and GCMS in extraction, identification and quantification of volatile in fruits

According to Jeleń, Majcher and others (2012), fruits and vegetables are the second biggest group food next to wine that applies SPME in flavour or volatile analysis. Table 3 summarizes some studies on flavour analysis of various fruits using SPME.

Table 3: HS-SPME coupled with GC-MS analysis of flavour profile of various fruits.

Matrix	Analytes	Fiber/GC column	Desorption time/temperature	Reference
Fresh fruits				
passion fruit , cashew, tamarind, acerola, and guava	alcohols, esters, carbonyl compounds, and terpernes	polydimethylsiloxane (PDMS) / Varian VF-5MS (5% diphenyl-95% polymethylsiloxane) capillary column	3 min./ 250 ºC	Carasek and Pawliszyn, 2006
peaches, nectarines,	terpenoids, esters, aldehydes, lactones, terpenoids, ketones, and alcohols	PDMS/DVB/ HP-5 MS (5% phenyl-polymethylsiloxane) capillary column	2 min./220 ºC	Wang, Yang et al., 2009
Durians	esters, alkanes, and alcohol	DVB/CAR/PDMS / Supelcowax-10 capillary column	5 min./ 250 ºC	Chin, Nazimah et al., 2007
Grapes	alcohols, aldehydes, terpenes, and benzenic compounds	PDMS/DVB / BP-21 capillary column	5 min./ 250 ºC	Sánchez-Palomo, Díaz-Maroto et al., 2005
grape berries	aldehydes, alcohols, carbonyl compounds, esters, and terpenoids	PDMS/DVB / HP-5MS capillary column	3 min./220 ºC	Yang, Wang et al., 2009
Fruit juices				
Mandarin and hybrid juices	terpenes, alcohols, aldehydes, hydrocarbons	PDMS, carbowax/divinylbenzene (CW/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)/ fused-silica capillary columns Rtx-1 and Rtx-Wax	5 min./ 260 ºC	Barboni, Luro et al., 2009
Orange, mosambi, dalandan juice	terpenes, alcohols, carbonyls, esters, hydrocarbons, limonenes	DVB/PDMS / BPX-5 (5% phenyl 95% methyl polysil-phenylene-siloxane) column	3 min/ 270 ºC	Dharmawan, Kasapis et al., 2007
Raspberry	alcohols, aldehydes, terpenes, esters, ketones, acids	DBV/CAR/PDMS / HP-Innowax fused-silica capillary column	5 min./ 250 ºC	Aprea, Biasioli et al., 2009

2.1.5 Nutritional Qualities

Cherry fruit has been long been known as a 'superfruit' (Allen & Silver, 2010). The United States Department of Agriculture (USDA) commission has linked cherries to a number of health benefits with important health implications (Young, 2013). This include the capability of cherries to promote better sleep, decrease inflammatory markers in blood, protection against Alzheimer's, and reduce the risk of cardiovascular diseases, diabetes, cancer, and other kind of diseases. The components of cherries that promote these health benefits will be discussed in the following sections.

2.1.5.1 Vitamins, dietary fiber, and minerals in cherries

Cherry fruit contains essential vitamins, minerals, and fibers (Table 4). According to Sinha and others (2012), and Ferretti, Bacchetti and others (2010), cherries contain both liposoluble (fat-soluble) vitamins such as A, E, and K and hydrosoluble (water-soluble) vitamins such as C and B, minerals such as calcium, magnesium, phosphorus, and potassium, and some carotenoids particularly beta-carotene (Yıgıt, Baydas, & Guleryuz, 2009; Schmitz-Eiberger & Blanke, 2012). Vitamin C (ascorbic acid) and E (tocopherol) play significant roles as antioxidants in the body, hindering the formation of peroxides and the release of damaging free radicals. Potassium reduces the risk of stroke and hypertension (Allen & Silver, 2010; McCune et al., 2010; Sinha et al., 2012).

In addition, cherries are also rich in dietary fiber (Tomás-Barberán, Ruiz et al., 2013). Dietary fibers are equally important because they absorb fat, reduce blood total and low-density lipoprotein (LDL) cholesterol levels, improve the control of blood glucose, prevent constipation, and hinder the development of various diseases such as colon cancer, stomach, and intestinal disorders (Erkikila et al., 1999; Lunn and Buttriss, 2007; and Sinha et al., 2012). Moreover, cherries have a low glycemic index, which is good for diabetic patients (Brand-Miller and Foster-Powell, 1999; Allen & Silver, 2010).

Table 4 Sweet cherry nutritional values per 100 g of cherry fruit (Sinha et al., 2012)

Proximates	USDA 2010	Souci et al. 2008	Minerals	USDA 2010	Souci et al. 2008	Vitamins	USDA 2010	Souci et al. 2008
Water (g)	82.25	82.8	Calcium (mg)	13	17	Vitamin C (mg)	7	15
Energy (kcal/kJ)	63/263	62/265	Iron (mg)	0.36	0.35	Thiamin (μg)	27	39
Protein (g)	1.06	0.90	Magnesium (mg)	11	13	Riboflavin (μg)	33	42
Total lipid (fat) (g)	0.20	0.31	Phosphorus (mg)	21	24	Niacin (mg)	0.154	–
Ash (g)	0.48	0.49	Potassium (mg)	222	235	Pantothenic acid (mg)	0.199	0.190
Carbohydrate (g)	16.01	13.3	Sodium (mg)	–	2.7	Vitamin B_6 (mg)	0.049	0.045
Fiber, total dietary (g)	2.1	1.31	Zinc (mg)	0.07	0.085	Folic acid (μg)	–	52
Sugars, total (g)	12.82	–	Copper (mg)	0.06	0.100	Carotene, beta (μg)	38	35
Sucrose (g)	0.15	0.193	Manganese (mg)	0.07	0.086	Vitamin A (IU)	64	–
Glucose (g)	6.59	7.134	Fluoride (μg)	2	18	Vitamin E (mg)	0.07	0.13
Fructose (g)	5.37	6.32	Selenium (μg)	0	1.2	Vitamin K (μg)	2.1	1.5

2.1.5.2 Bioactive compounds

Cherries also contain phytochemicals collectively known as ‘bioactive compounds’. Bioactive compounds that produce physiological effects can prevent health problems (Aluko, 2011). These compounds are found in plants especially fruits and vegetables (Skinner, Hunter et al., 2013). According to Emilio (2007) and Weston (2010), the bioactives present in fruits show antimicrobial activity, anticancer activity, anti-inflammatory activity, immuno-stimulatory activity, antioxidant activity and other health beneficial activities.

2.1.5.2.1 Phenolics

Structurally, phenolics are a diverse class of phytochemicals. They are identified by the presence of at least one aromatic ring, which bears one phenol or more (polyphenols), together with their functional derivative such as esters and glycosides (Maffei, 2003; Dai and Mumper, 2010). Phenolics are widely distributed in the plant kingdom with more than 8,000 different phenolic structures (Dai and Mumper, 2010), and are said to be natural antioxidants. Antioxidants are compounds that can impede the oxygen’s damaging effects in cell tissues. In fruits, the main natural antioxidants are phenolics, which are grouped into tocopherols, flavonoids, cinnamic acid derivatives, phenolic acids, lignans, tannins, and coumarins (El Gharras, 2009 and Shahidi and Naczk, 1995).

2.1.5.2.2 Flavonoids

Among the phenolics, the flavonoids and phenolic acids are abundant in cherries (Dai and Mumper, 2010). Flavonoids are the most significant bioactive compounds in many

fruits, and within this group is a larger group of natural antioxidants (Figure 6) responsible for the colours of the cherries (Sinha et al., 2012; Haminiuk et al., 2012).

The flavonoid structure consists of C6-C3-C6 carbon framework or two aromatic rings connected by a three carbon aliphatic chain (Shahidi and Naczk, 1995). They are polyphenolic compounds that are water-soluble and exhibit powerful antioxidant activities (Skinner, Hunter et al., 2013). Several clinical studies have reported that flavonoids have positive physiological effects on human body (Sinha et al., 2012). Flavonoids also enhance the nutritional qualities of vitamins C and E. They hinder the development of cardiovascular diseases and are proven to be anti-carcinogenic (Allen & Silver, 2010; Sinha et al., 2012). Under the flavonoids group are the following subgroups namely, flavones, flavonols, flavans, flavanones, isoflavones, anthocyanidins, and anthocyanins as seen in Figure 6.

2.1.5.2.2.1 Anthocyanins

2.1.5.2.2.1.1 Pigments

Anthocyanins are the main phenolic compounds in cherries (Sinha et al., 2012). They contribute to the red, blue, orange, and purple pigments in plants, which is an important indicator of maturity and fresh quality of cherries (Gao and Mazza, 1995; Kallay 2008; Steger-Mate et al., 2010; Dai and Mumper 2010). Anthocyanins concentrations vary from one cultivar to another. According to Diaz-Mula and others (2008), cultivars that are light-coloured ones such as ‘Brooks’, ‘Prime giant’, ‘Somerset’, and ‘Sweetheart’ have the lowest concentrations of anthocyanins (40 mg/100 g cherry); while dark-coloured ‘Cristalina’ and ‘Sonata” cultivars have the highest content of anthocyanin (about 225 mg/100 g of cherry fruit).

Colour is an indication of anthocyanin content in cherries. Among the anthocyanins, the cyanidin-3-rutinoside and cyanidin-3-glucoside are predominant and account for between 79 and 96% of total anthocyanins. Peonidin- and pelargonidin- 3- glucoside, and 3-rutinoside are present in lower amounts (Macheix et al., 1990; Goncalves et al., 2004; Mozetic et al., 2006; Usenik et al., 2008). Cyanidins give cherries its magenta and crimson coloration, while the orange, pink and red colours are provided by the pelargonidins (Sinha et al., 2012).

2.1.5.2.2.2 Polyphenols

Polyphenols contribute largely to the quality of fruit by modifying sensory attributes that include colour, taste, flavour and aroma (Tomas-Barberan and Espin, 2001). Phenolic compounds other than anthocyanins that are colourless are called polyphenols. Among the phenolic bioactive compounds, quercetin from the class of flavonols (flavonoids) has been reported to have a potent total antioxidant activity (TAA) and to be the dominant flavonols in cherries (18-59 mg/L) (Boots et al., 2008; Sinha et al., 2012). Quercetin can reduce reactive oxygen species (ROS) like hydrogen peroxide and superoxide anion, which are linked to chronic illnesses like cardiovascular and cancer (Wilms et al., 2005). Significant amounts of isorhamnetin rutinoside have been reported by Kirakosyan and others (2009), and Usenik and others (2008).

2.1.5.2.3 Phenolic Acids

Sweet cherries are rich in phenolic acids as well, in which the hydroxycinnamic acids including caffeic and p-coumaric acids are predominant (Dai and Mumper, 2010; Skinner, Hunter et al., 2013). The hydroxycinnamates have been reported by McCune and others (2011) as having health-promoting ability as an antioxidant. These compounds have chemopreventive properties and the ability to inhibit the oxidation of LDL as demonstrated by in vitro studies (McCune et al., 2011).

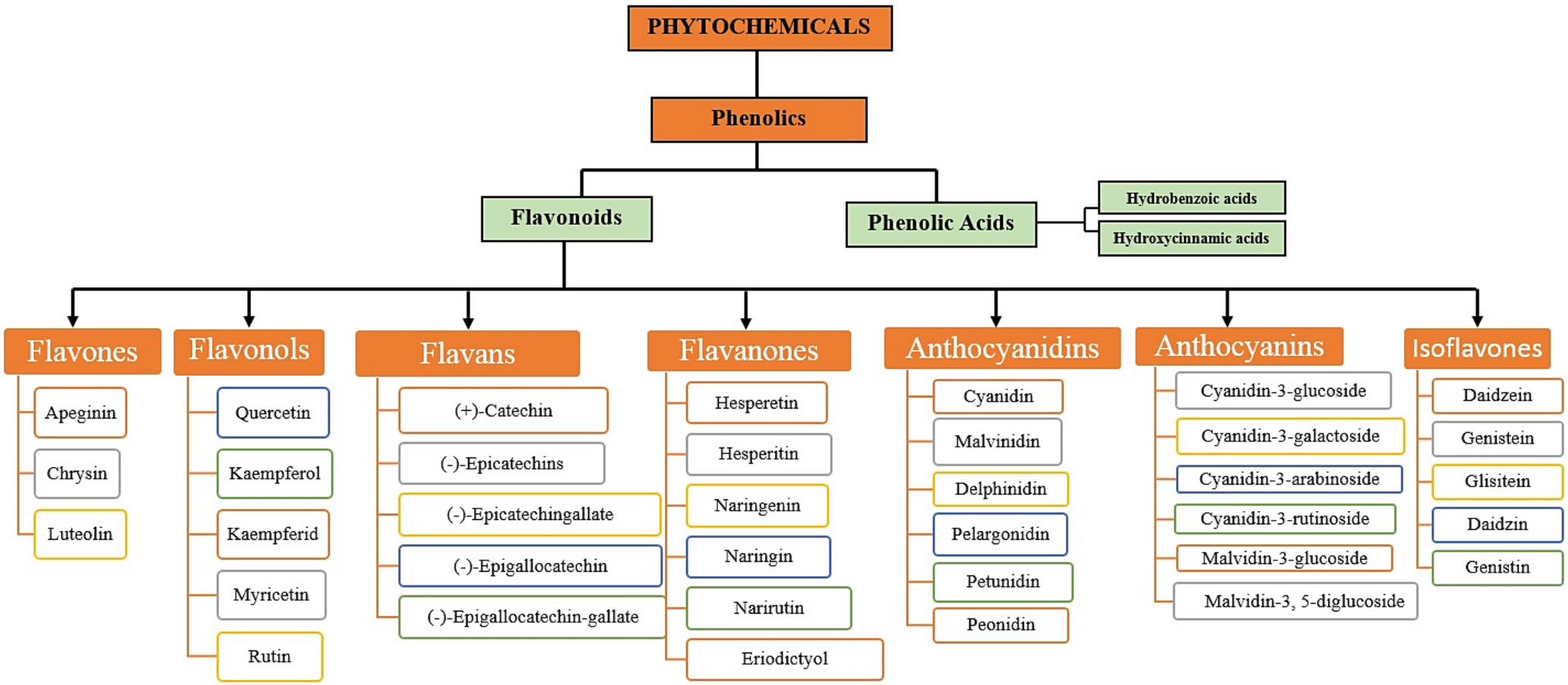


Figure 6 Bioactive components in cherry fruits (McCune et al., 2010; Liu, 2004; Von Elbe and Schwartz, 1996; Hollman and Katan, 1999; Tokusoglu and Hall, 2001; El Gharras, 2009; Naczk and Shahidi, 2006)

2.1.5.3 Analytical methods used in the extraction and identification of bioactive compounds

The extraction, identification, and quantification of bioactive compounds have been the focus of many researches, including studies related to health and medical research (Dai and Mumper, 2010).

2.1.5.3.1 Extraction and purification

The extraction of bioactive compounds from any plant material for identification and quantification involves selective separation of target compounds using different extraction methods (Sana and Fischer, 2007). The most commonly used is the solvent extraction method, which makes use of differential solubility and solvent immiscibility (Sana and Fischer, 2007). Prior to extraction of bioactive compounds, samples usually undergo milling, grinding, and homogenization before they are air or freeze dried (Dai and Mumper, 2010). Freeze-drying generally retains greater levels of phenolics content (Abascal et al., 2005). However, drying processes like freeze-drying can cause detrimental effects on the target compounds in plants by inactivating or diminishing some activity of phenolic compounds in plants resulting to their decrease in relative concentrations (Abascal et al., 2005).

2.1.5.3.1.1 Liquid-liquid extraction (LLE)

This technique involves the transfer of compounds from one liquid phase to another liquid phase by the addition of an immiscible solvent (which solubilizes the target compound) to the original solution (Sana and Fischer, 2007). Usually, organic solvents like chloroform (non-polar) are paired with polar and aqueous solutions like water to form a two-phase system.

Extraction yields are dependent on an array of factors. These include the solvent types (with varying polarities), extraction time and temperature, ratio of sample to solvent, and sample chemical composition and physical properties (Dai and Mumper, 2010). The solubility of phenolics is influenced by the chemical characteristics of samples and solvent polarity (Dai and Mumper, 2010). Hence there is actually no general extraction procedure appropriate for all plant phenolics extraction. The use of solvents like

ethanol, methanol, acetone, ethyl acetate, and their combinations with different water proportion have been explored to aid in the extraction of phenolics (Dai and Mumper, 2010).

According to Xu and Chang (2007), it is very important to select the right solvents for extraction, as it will affect the amount and rate of phenolics being extracted. Among the solvents, methanol and acetone have been reported to be more effective in extracting polyphenols with lower molecular weight and flavanols with higher molecular weight respectively (Metivier et al., 1980; Prior et al., 2001; Guyot et al., 2001). Another alternative solvent is ethanol that has been reported by Shi and others (2005) to be a good solvent that is also safe for human consumption.

For the extraction of anthocyanins in plants, the conventional solvent used is either methanol or ethanol, which is acidified. This technique dissolves the anthocyanins resulting in more efficient extraction. However this system with added acid could result in excess acid, which can hydrolyze labile, acyl, and sugar residues during purification procedures, leading to less extraction of target compounds (Dai et al., 2010). To prevent this from happening and to achieve higher concentrations of extracts, utilisation of weak organic acids like formic, citric, acetic, phosphoric, and tartaric acids, and low concentrations of strong acids, such as hydrochloric acid (less than 1%) and trifluoroacetic acid (0.5-3 %) have been proposed (Jackman et.al 1987; Revilla et al., 1998; Nicoue et al., 2007).

Other important parameters that are critical for effective extraction of compounds include temperature and time. When the temperature is high, the solubility of the analyte will increase, making the extraction rate faster. However, the use of high temperatures (70 ^{o}C and above) could oxidise and hydrolyse many of the phenolic compounds (Jackman et al., 1987; Havlikova et al., 1985). Thus, researchers generally use temperatures ranging from 20 to 50 ^{o}C to prevent rapid degradation of anthocyanins (Jackman et al., 1987; Havlikova et al., 1985). In the past, extraction steps such as maceration and soxhlet extraction were used. However, these methods involve not only long extraction time but also the use of possible environment pollutants (Dai and Mumper, 2010). In order to reduce extraction time, researchers have developed several extraction methods that include microwave, ultrasound-assisted extractions (UAE), and

other methods using compressed fluids. Of all these methods, the UAE has been reported to be a very useful and low-cost technology (Vinatoru, 2001).
The solvent-to-solid ratio and particle size of sample can also affect extraction. According to Cacace and Mazza (2003), Pinelo and others (2005), and Nepote and others (2005), the higher the solvent-to-solid ratio, the higher the phenol extracts yield. Decreasing the particle size can also increase the yield of phenolic compounds. In this study, solvent extraction method will be used. Solvents used may vary in their polarity, and their interactions with sample. Hence preliminary runs were conducted to determine solvent or a combination of solvents capable of extracting target compounds in high concentrations.

2.1.5.3.2 Identification, analysis and quantification of bioactive compounds

The identification and quantification of phenolic compounds are usually determined by spectrophotometric and chromatographic methods.

2.1.5.3.2.1 Spectrophotometric method

The choice of an appropriate analytical approach for studying bioactive compounds (phenolics) depends on the study aims, and also nature of the sample and analyte (Robards, 2003). According to Dai and Mumper (2010), the assays used for the analysis of phenolics are categorized as either measuring the total phenolics or quantifying a particular group of phenolic compounds.

Various methods have been used in the determination of total phenolics. Among them are ultraviolet (UV) absorbance, colorimetry using iron salts, permanganate titration, Folin-Ciocalteu method (F-C), and Folin-Denis (FD) method. Of all these methods, F-C is the most preferred because of its general chemistry nature (Singleton et al., 1999). Moreover, the assay of F-C is very straightforward, reproducible, and has been widely used for quantification of phenolic compounds in plant materials. According to Singleton and others (1999), F-C relies on the transfer of electrons from phenolic compounds to phosphomolybdic acid complexes, in an alkaline medium to form blue complexes, which are determined spectroscopically at approximately 500 nm.

If the purpose of the study is to determine a particular group, for example, the anthocyanin, the simplest assay involves measuring the absorption at wavelength 490 nm to 550 nm using a spectrophotomer (Fuleki and Francis, 1968a). However, overestimation of total anthocyanin content can happen, as their degradation products due to browning reactions are also co-determined (Naczk and Shahidi, 2004). The pH differential method can be applied so that anthocyanins are differentiated from their degradation products (Fuleki and Francis, 1968b). Using this method, the absorption of the extracts is measured at pH 1 (anthocyanins as coloured oxonium salts) and pH 4.5 (as colourless hemiketals). Then, the calculation of concentration is normally done based on the anthocyanin's molecular weight (MW), and molar extinction coefficient (ε).

2.1.5.3.2.2 Chromatographic method

2.1.5.3.2.2.1 Gas chromatography (GC)

The separation and quantification of phytochemicals (flavonoids and phenolic acids) involve the use of mainly GC.

2.1.5.3.2.2.2 Liquid chromatography

Due to the complexity of phenolics in plant samples, it is hard to obtain accurate and comparable results using spectrophotometric assays. Furthermore, these methods are inclined to interferences, which often result in over or under estimates. Thus, instrumentation techniques have emerged, which uses high-performance chromatography for the identification, profiling, and quantification of phenolic compounds. These instruments include gas chromatography (GC) and high performance liquid chromatography (HPLC) or its improved version, the liquid chromatography mass spectrometry (LCMS).

Presently, HPLC or alternatively LCMS is the most popular and reliable method of analysing phenolics in food samples. This technique can also analyse all components of interest simultaneously with their derivatives (Sakakibara et al., 2003 & Downey and Rochfort, 2008).

According to Petrovic and Barcelo (2013), the LC-MS techniques have advanced dramatically over the last 20 years due to their sensitivity, short run time, less use of organic solvents, specificity, and reliability, mostly because of the development of hyphenated chromatography–mass spectrometry techniques. It has been a powerful analytical tool for the identification of phytochemicals (Sulaiman & Gopalakrishnan, 2014). LC-MS systems take advantage of the technique that combines the physical separation (resolution) capabilities of liquid chromatography with the mass (qualitative) capabilities of mass spectrometry (Shimadzu Corporation, 2015).

2.1.5.3.2.2.1 The LC-MS components

The LC-MS system consists of a HPLC device for introducing the samples, an interface for connecting the device, an ion source for ionising the samples, an electrostatic lens for introducing the generated ions, a mass analyser unit for separation of ions on their mass-to-charge (m/z) ratio, and a detector for separated ions detection (Shimadzu Corporation, 2013). A schematic diagram of the modern LC-MS unit is presented in Figure 7.

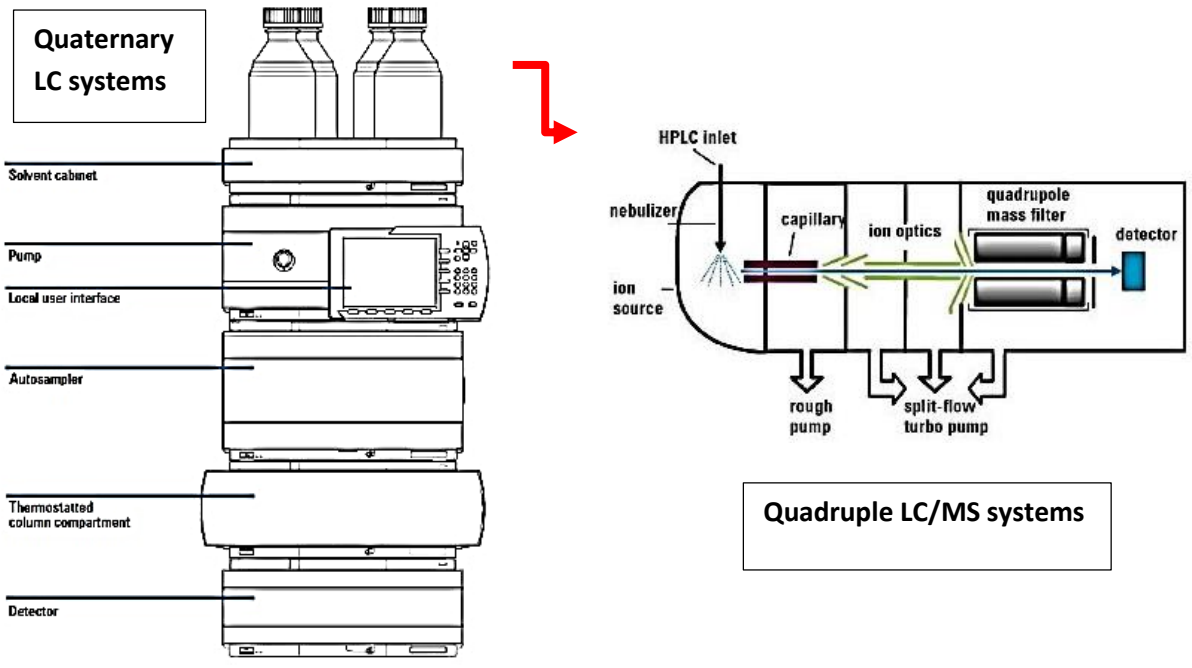


Figure 7 Block diagrams of modern LC-MS systems (Agilent Technologies, 2010; Agilent Technologies, 2014)

Mass spectrometry (MS) is based on the analysis of ions moving through a vacuum, which will give the mass spectra (Agilent Technologies, 2014). The obtained mass

spectra from the scanned measurements provides valuable information about the structure, molecular mass, identity, purity, and quantity of the eluted compounds, which complements the qualitative data based on retention times (Shimadzu Corporation, 2013, Agilent Technologies, 2014). The MS also adds specificity for both quantitative and qualitative analyses.

There are many types of ion sources available depending on the required method for the analysis. The ion source could be electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric chemical photoionization (APPI), and multimode ionization (MMI). The ESI is particularly useful for the analysis of large biomolecules but can also analyse smaller molecules, The APCI cannot withstand high temperature vaporization and is applicable for a broad range of molecular polarities, but suited for less polar compounds with molecular weights less than 1,500 Da. The APPI is valuable in the analysis of non-polar compounds and useful for compounds that are normally analysed by APCI. Finally, the MMI source is an ion source that can work in three different modes (APCI, ESI or simultaneous APCI/ESI) and is also very useful in determining and screening unknown compounds (Agilent Technologies, 2014). Of all these sources, the ESI source is the most commonly used.

Besides a good choice of ion source, the uses of reversed-phase (RP) columns are recommended at constant column temperature for good separation and reproducibility of different classes of phenolics (Stalikas, 2007; Oh et al., 2008). Mobile phases require to be acidified to prevent peak tailing. Mobile phases such as methanol and acetonitrile (acidified with formic, phosphoric, or acetic acids) are the most generally used organic modifiers. In this study, the LC-MS using MMI source will be used to identify the bioactive compounds in cherries.

2.1.5.3.2.2.2.1 Applications of LC-MS in the analysis of bioactive compounds in fruits

Table 5 shows some analysis of bioactive compounds in fruits using LC-MS methods.

Table 5 Applications of liquid chromatography coupled with mass spectrometry in the analysis of bioactive compounds (phenolics) in fruits

Matrix	Analytes	Mobile phase	Column	Ion source	Limits of Detection (LOD)	Limits of Quantification (LOQ)	Reference
Grapes(dried pomace)	Epicatechin gallate, Catechin hydrate, caffeic acid, gallic acid, ferulic acid, Isorhamnetin, Kaempferol, Myricetin, p-coumaric acid, Quercetin, p-hydrobenzoic acid, Protocatechuic acid, Vanillic acid, 3,4 Dihydroxyphenylacetic acid, Syringic acid, Cinnamic acid, Quinic acid, Catechin, Epicatechin, Phloretin, Caftaric acid, Caffeoylshikimic acid, Bis-HHDP-hexose, Quercetin 3-O-glucoside, Naringenin 7-O-glucoside, Cyanidin 3-O-glucoside1, Delphinidin 3-O-glucoside1, Malvidin 3-O-glucoside1, Pelargonidin 3-O-glucoside1, Petunidin 3-O-glucoside1, Peonidin 3-O-glucoside1, Delphinidin 3,5 diglucoside, Cyanidin 3 (acetylglucoside), Delphinidin3-O-β-glucopyranoside, Malvidin 3-gentiobiside, Delphinidin 3-O-p-coumaryl glucoside, Petunidin 3-O-p-coumaryl glucoside, Malvidin 3-O-p-coumaryl glucoside, Peonidin-malonylglucoside	Solvent A: 0.1% H3PO4 in MilliQ water; Solvent B: 0.1% H3PO4 in acetonitrile	RP Sun Fire™ C18 column	ESI	not determined	not determined	Ramirez-Lopez and DeWitt, 2014
Açaí-do-Amazonas, acerola, cashew apple, camu-camu, pineapple and taperebá	(−)-Epicatechin, Chlorogenic acid, Caffeic acid, Vanillic acid, Syringic acid, Ethyl gallate, Sinapinic acid, p-Coumaric acid, Ferulic acid, Propyl gallate, Myricetin, Quercetin, Luteolin, Kaempferol, Isorhamnetin, Apigenin	Solvent A: water; Solvent B: methanol	Shim-pack XR-ODS III 2.2 μm, 2.0 mm i.d., 150 mm column	ESI	28.85–333.3 pg/mL	96.15–1111 pg/mL	Bataglion, 2015

Apple and pear	5-HMF, Procyanidin B1, Catechin, p-Coumaroyl glucose b, Chlorogenic acid, p-Coumaroylquinic acidb, Procyanidin B2, Caffeic acid, Epicatechin, p-Coumaric acid, Quercetin-3-galactoside, Quercetin-3-glucoside, Quercetin-3-xyloside, Quercetin-3-rhamnoside, Phloretin-2′-xyloglucosidec, Phloridzin, Quercetin	Solvent A: 2% (v/v) acetic acid in water ; Solvent B: 0.5% acetic acid in water and acetonitrile (50:50, v/v)	Aqua 5 mm C18 column	ESI	not determined	not determined	Schieber, 2001
Strawberries	p-Coumaroyl-glucoside, Q-rutinoside, Methyl–EA–pentose, Q-glucoside, Q-glucuronide, Sanguiin-H6, K-coumaroyl-glucoside, Methyl–EA–pentose, K-glucuronide, K-glucuronide	Solvent A: H2O (1% formic acid); Solvent B: acetonitrile (ACN)	Symmetry C-18 column	ESI	not determined	not determined	Seeram et al., 2006
Black currant	3-caffeoylquinic acid, p-coumaric acid derivate, caffeoylglucose coumaric acid glucoside, 3-p-coumaroylquinic acid, p-coumaroylglucose ferulic acid glucoside, feruoylglucose sinapic acid, Myricetin glucoside, myricetin rutinoside, myricetin malonylglucoside, aureusidin glucoside, quercetin glucoside, quercetin rutinoside, quercetin malonylglucoside, kaempferol glucoside, kaempferol rutinoside, isorhamnetin rutinoside	Solvent A: 1% v/v formic acid ; Solvent B: 10% v/v acetonitrile in methanol	5 µm Vydac 218TP54 RP-18 column	ESI	not determined	not determined	Anttonen and Karjalainen, 2006
Star fruit	(−)epicatechin and gallic acid	Solvent A: 0.1% formic acid in water; Solvent B: methanol	Shim-Pack VP-ODS column	ESI	not determined	not determined	Shui and Leong, 2006

2.1.5 Sweet cherry fruit products

The majority of sweet cherries are eaten fresh and the remaining 40% are processed into different products in case of sweet cherries (Mark & Olga, 2004; McCune et al., 2010). Cherry fruit is processed mainly because of its short growing season (Di Cagno et al., 2011). Cherry products like quick-frozen, dried, purees and concentrates can be processed further and/or used as intermediate raw materials for other food products. Quick-frozen cherries can be used in baking, confectionery, dairy, and canning (Sinha et al., 2012). Purees and concentrates can be incorporated in jams, powdered juices, and sauces while, dried cherries can be used as ingredients for fruit snacks, fruit teas, muesli bars and mixes, and etc. (Sinha et al., 2012).

2.2 Different technologies used in the processing of cherry fruits

Advanced thermal processing technologies such as ohmic heating, microwave heating, and other related computer control technologies have been developed to produce quality foods with the use of rapid temperature profiling (Hosahalli et al., 2004). However, these treatments cannot fully eliminate the adverse effect on fruit nutrients. Thus, various non-thermal processing technologies, which are known to produce high quality products, have been regarded as alternatives to thermal processing. These include the use of high pressure processing (HPP) and pulsed electric field (PEF) processing

2.2.1. Thermal Processing versus Non-thermal Processing

2.2.1. 1 Thermal Processing

Thermal processing is the most common method of preserving foods and food products. However, despite the beneficial effect, thermal treatment results in undesirable effects (Gutierrez-Lopez & Barbosa-Canovas, 2003). During this process, the product undergoes a lot of changes that alter final product quality in terms of nutritional characteristics, colour, flavour, texture, and the entire product's general appearance (Jia, Howard Zhang, & Min, 1999; H. Q. Zhang et al., 2010). Thermal processing has been optimized in order to produce better quality food products. But despite these advanced techniques, nutrient degradation cannot be fully eliminated (Zhang et al., 2010).

Dehydration and pasteurization processing can destroy the bioactive pigments (phenolics like anthocyanin) present in cherries. Kim and Padilla-Zakour (2004) reported that the anthocyanin content and colour of cherry fruit jam were affected by heat processing. Anthocyanin content was significantly reduced and resulted in a colour change (from red to brown). Moreover, several studies have reported that thermal processing of cherries resulted in a decrease in antioxidant content including vitamin C (Rickman et al., 2007), phenolic compounds (Chaovanalikit and Wrolstad, 2004), anthocyanins, and other flavonoids (Jones, 2007).

2.2.1.2. Non-thermal Processing

Non-thermal processing is often called “cold-pasteurization” (H. Q. Zhang et al., 2010). This does not mean that no heat is applied on food. This process uses heat but at much lower levels than thermal processing, or the action of these non-thermal technologies do not increase food temperature (Barbosa-Canovas, Pothakamury, Palou, & Swanson, 1998; Gutierrez-Lopez & Barbosa-Canovas, 2003). Non-thermal processing also uses short processing times compared to thermal processing (H. Q. Zhang et al., 2010). Therefore the degradation of food’s quality (nutritional and sensorial) is expected to be minimal.

Non-thermal processing has been proven efficient not only in terms of inactivation of enzymes and microorganisms but also in the production and development of food products with novel and outstanding characteristics (H. Q. Zhang et al., 2010). Most of these technologies are environmentally friendly because of minimal waste, low cost, less energy utilization, and preservation of food quality attributes (Barbosa-Canovas et al., 1998; H. Q. Zhang et al., 2010).

2.3 Non-thermal Processing

2.3.1 Types

Non-thermally, foods can be processed using high hydrostatic pressure technology or high pressure processing (HPP), high intensity light or ultraviolet (UV), irradiation, ultrasound, oscillating magnetic fields (OMFs), radio frequency electric fields (RFEF),

and pulsed electric field processing (PEF). PEF will be discussed further as this method has been employed in this research.

2.3.1.1 Pulsed Electric Field (PEF)

Pulsed electric field (PEF) is a physical technology applied on food especially liquid foods, which uses power electronics that allow operations at low to moderate temperature (Zeuthen & Bogh-Sorensen, 2003). The electric field consists of short pulses that range from a few microseconds to milliseconds pulse duration (Barbosa-Canovas et al., 1998). Enzyme and microbial inactivation are the main beneficial effects of this method on food. Others include retention of flavour, physicochemical, nutritional properties, and extraction of bioactive compounds.

2.4 Pulsed Electric Field Processing

2.4.1 Principle

Pulsed electric field (PEF) processing involves the application of short electric pulses that is usually 1 to 20 μs at ambient or low temperatures for less than a second with field strength of 20 to 80 kV/cm to food samples positioned between two electrodes in either batch or continuous process chamber (Hosahalli et al., 2004; Zeuthen & Bogh-Sorensen, 2003). The operation of PEF can be classified as batch (single-pass) or continuous systems.

2.4.2 Engineering Aspects

2.4.2.1 Systems

As mentioned earlier, a typical pulsed electric field (PEF) processing system (Figure 8) consists of four parts, namely a control and monitoring system, pulse generator, treatment chamber, and sample handling with delivery and cooling system (Zhang et al., 2010). These parts also include a power supply (high-voltage source), capacitor bank, a switch, and treatment chamber (Barbosa-Canovas et al., 1998; Rahman, 2007; Zeuthen & Bogh-Sorensen, 2003; H. Q. Zhang et al., 2010).

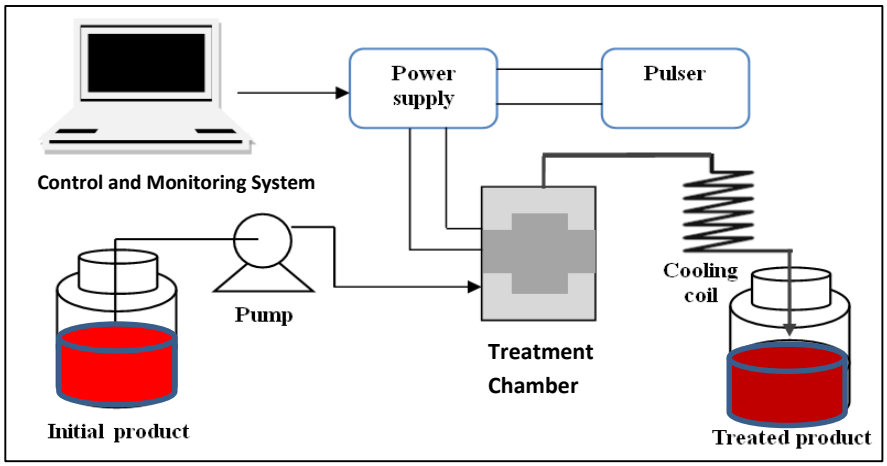


Figure 8 Schematic diagram of a PEF operation (Mohamed & Eissa, 2012)

2.4.3.2 Electrical Components

A power supply consisting of a pulse generator supplies high-voltage pulses to the system. The capacitor bank stores and collects these high-voltage pulses. The switch discharges the stored high-voltage pulses through the pulse-forming network (PFN) circuit instantly across the food samples held in a treatment chamber. PFN is an electrical circuit, which generates pulse electric fields, discharges electrical energy fast, and charge voltages, switches, capacitors, inductors, resistors, and treatment chamber (Zhang et al., 2010).

2.4.3.3 Process Flow

During processing, the food product is pumped into the treatment chamber to hold the sample. This treatment chamber consists of two electrodes. When high voltage electrical pulses are applied to these electrodes, high intensity electrical pulses are conducted to the food product (Zhang et al., 2010).

2.4.4 Applications of PEF in Food Processing

To date many researches have been carried out on the use of PEF on liquid foods, especially fruit juices and vegetable beverages. Application of PEF in semi-solids and solid foods is however slowly increasing. The effect of PEF on foods in terms of

microbial and enzyme inactivation, retention of physical and chemical characteristics, flavour, nutritional properties, shelf-life, extraction of bioactive compounds, and growth of probiotic bacteria are evaluated and discussed below.

2.4.4.1 Microbial Inactivation

Microorganisms (*Lactobacillus brevis and Saccharomyces cerevisiae*) in apple juice can be inactivated by PEF processing with 6.3 and 4.2 log reductions using a frequency of 1200 pulse per second and an intensity of 35 kV/cm based on the study of Aguilar-Rosas, Ballinas- Casarrubias, Nevarez- Moorillon, Martin-Belloso, & Ortega-Rivas (2007). Inactivation of *Penicillum expansum* and *Botritis cinerea* in apricot nectars, sour cherry juice, and peach nectars in terms of germination tube elongation and spore germination were also investigated by Evrendilek, Tok, Soylu, & Soylu (2008). Results showed that an increase in electric field strengths (0, 13, 17, 20, 23, 27, 30, and 34 kV/cm), completely inhibited tube elongation and spore germination (Evrendilek et al., 2008). A summary of researches that use PEF processing to inactivate microorganisms and other food-borne pathogens is shown in Table 6.

The level of inactivation varies depending on how intense the electric field strength is, as well as the frequency and pulse duration. Thus, these are important factors to consider when carrying out research. In the present study, the aim of PEF processing of cherries is not to inactivate microorganisms, but rather, to determine if probiotic microorganisms could grow after samples are PEF treated.

Table 6 Microbial inactivation of different foods using PEF processing

Samples	Microorganisms	Treatment Conditions	Log Reductions / Effects	Germination tube elongation and Spore germination	References
Apple Juice	*Lactobacillus brevis* *Saccharomyces cerevisiae*	Batch, 35 kV/cm, 1200 pps, 4µs bipolar pulse	6.3 4.2	not determined	Aguilar-Rosas et al., 2007
Apricot nectars Cherry juice (sour) Peach nectars	*Penicillum expansum* *Botritis cinerea*	Continuous,13,17,20,23,27,30, and 34 kV/cm, 500 pps, 3 µs pulse	not determined	Completely inhibited on both microorganisms (99.0 & 100.0%)	Evrendilek et al., 2008
Orange juice	Total plate counts (TPC) Yeast and Molds count	Batch, 30 kV/cm, 240 and 480 µs pulse duration	TPC and yeast and molds were reduced	not determined	Jia. et al., 1999
	Leuconostoc mesenteroides	Continuous, 30 and 50 kV/cm electric field stengths, 12 µs pulse duration	4.75-log10 and 6.2-log10 inactivation	not determined	McDonald et al., 2000
	Escherichia coli O157:H7 (EHEC)	Batch, 13.1, 19.7, and 23.7 kV/cm; 75 µs pulse duration	1.0, 2.4, and 3.4-log10 reduction		Gurtler et al., 2010
Carrot juice (formulated)	Total aerobic mesophilic bacteria (TAMB) *Escherichia* coli (EC) Total Molds and yeast (TMY)	Continuous, 13,17,20,23,27 kV/cm, 20µs pulse	0.71, 1.58, 2.27, 3.34, 4.30 for TAMB 1.09, 1.91, 2.84, 3.52, 4.46 for TE 1.23, 1.58,2.07, 2.66, 3.57 for EC 3.19,3.46, 4.23, 4.43 for TMC	not determined	Akin and Evrindelek, 2009
Grape juice	Yeast Bacteria (*K.apiculata*, *S.cereviasiae*, lactic and acetic bacteria)	Continuous, 35.0 kV/cm, 1µs pulse	2.24 3.94	not determined	Marselles-Fontanet et al., 2009
Cherry juice (sour)	*Escherichia coli O157:H7, Staphylococcus aureus, Listeria monocytogenes, Erwinia carotowora, Pseudomonas syringae subs. syringae, Botrytis cinerea and*	0 (control), 17, 20, 23, 27, and 30 kV cm)1, 3µs pulse duration, 500 pps repetition rate, and 131 µs treatment time, 22.5, 27.0, 36.5, 48.0 and 67.5 J s)1 energy input	7–8 log cfu mL)1 inactivation	not determined	Atluntas et al., 2010

2.4.4.2 Enzyme Inactivation

Researches on enzyme inactivation by PEF are still on-going. Some researchers claim that enzymes are highly inactivated by the PEF method (Sinha et al., 2012). A summary of studies on the inactivation of enzymes of some liquid foods using PEF treatment is shown in Table 7.

Table 7 Enzyme inactivation in juices using PEF processing

Samples	Enzymes	Treatment Conditions	% Inactivation	Reference
Strawberry juice	Lipoxygenase (LOX) β-glucosidase (β-GLUC)	Continuous, 35 kV/cm, 1700 pps, 4μs wave pulses	66.7	Aguilo-Aguayo et al., 2009
Strawberry juice	Polyphenoxidase (PPO)	Continuous, 35 kV/cm, 1000 - 2000 pps, 1-7μs pulses	97.5	Aguilo-Aguayo et al., 2010
Tomato juice	Lipoxygenase (LOX)	Continuous, 35 kV/cm, 1500 pps, 4μs wave pulses	70.2	Aguilo-Aguayo et al., 2010
Orange juice	Pectin methyl esterase (PME)	Continuous, 35 kV/cm, 59 μs pulses	88.0	Min et al., 2000
	PME	Batch, 25 kV/cm, 250 μs,	90	Yeom et al., 2002
Apple juice	Peroxidase (POD) polyphenoloxidase (PPO)	Continuous, 30 kV/cm, 3 - 8 μs pulses	100.0 100.0	Shilling et al., 2008

2.4.4.3 Effect of PEF processing on the physicochemical, flavour and nutritional properties of food

PEF treatment is thought to retain the physico-chemical characteristics such as pH, soluble solids, viscosity, color, electrical conductivity, as well as flavour and other nutritional properties of fruit juices. A summary of the studies on the effects of PEF on the physico-chemical characteristics (Table 8), flavour (Table 9) and nutritional qualities (Table 10) of liquid foods is presented below. It can be observed that most physico-chemical characteristics and flavours of juices are retained except for vitamin C and phenols.

Table 8 Effects of PEF treatment on the physico-chemical properties of some liquid foods

Samples	Physico-chemical properties	Treatment Conditions	Effects	Reference
Tomato juice	Color (red)	Continuous, 40 kV/cm, 2000 pps, 57 µs pulse duration	Lower non-enzymatic browning Higher redness	Min and Zhang, 2003
Tomato juice	pH Soluble solids Color (L* and h*) Electrical conductivity	Continuous, 35 kV/cm, 1500µs, 4 µs pulses	No effect No effect Slight difference No effect	Vallverdu-Queralt et al., 2013
Strawberry juice	Color (L*)	Continuous, 35 kV/cm, 1700 µs, 4 µs pulses	Higher colour retention	Aguilo-Aguayo et al., 2009
Longan juice	Viscosity Color pH Titratable acidity Total soluble solids	Batch, 32 kV/cm, 90 µs pulse duration, 3µs bipolar pulses	Higher values No significant difference between untreated samples	Zhang et al., 2009
Cherry juice (sour)	pH, ºBrix, titratable, acidity, conductivity, colour (L*, a* and b*)	0 (control), 17, 20, 23, 27, and 30 kV cm)1, 3µs pulse duration, 500 pps repetition rate, and 131 µs treatment time, 22.5, 27.0, 36.5, 48.0 and 67.5 J s)1 energy input	No significant change	Atluntas et al., 2010
Grapefruit, lemon, orange, tangerine	pH, total soluble solids, colour	Continuous, 28 kV/cm with 50 pulses	no significant differences found between untreated and treated samples	Cserhalmi et al., 2006
Orange juice	Colour	Continuous, 30 kV/cm with 100 µs pulses	Colour similar to untrated samples / no significant difference between untreated	Cortes et al., 2008
Apple mash	pH, total soluble solids, total acidity	Batch, 1, 3, 5 kV/cm, 30 pulses, 400 µs pulse duration	Did not significantly differ from the controls	Schilling et al., 2007

Table 9 Effects of PEF treatment on the volatile compounds of some liquid foods

Samples	Important flavour volatiles	Treatment Conditions	Effects	Reference
Tomato juice	trans-2-hexenal, 2-isobutylthiazole, cis-3-hexanol	Continuous, 40 kV/cm, 2000 pps, 57 µs pulse duration	Higher flavour retention	Min and Zhang, 2003
Tomato juice	flavour compounds:hexanal, 2-hexenal, 2,2-decadienal, 3-hexenol, hexanol, 1-penten-3-one,6-methyl-5hepten-2-one geranyl, acetone, and 2-isobutylthizole	Continuous, 35 kV/cm, 1500µs, 4 µs pulses	Flavour compounds maintained right after processing	Vallverdu-Queralt et al., 2013
Grapefruit, lemon, orange, tangerine	ethyl butyrate, linalool, decanal and valencene , neral, geranial, and nootkatone	Continuous, 28 kV/cm with 50 pulses	Did not decrease significantly/no flavour loss	Cserhalmi et al., 2006
Watermelon juice	hexanal, (E)-2-nonenal, nonanal, 6-methyl-5-hepten-2-one and geranylacetone	Batch, 35 kV/cm for 1727 µs using bipolar pulses of 4-µs at 188 Hz	20% increase in concentration of volatiles.	Aguilo-Aguayo et al., 2010
	(Z)-6-nonenal, 1-nonanol and (Z)-3-nonen-1-ol		Retained/unchanged	

Table 10 Effects of PEF treatment on the bioactive compounds of some liquid foods

Samples	Bioactive compounds and other nutrients	Treatment Conditions	Effects	Reference
Watermelon juice	Lycopene Vitamin C Antioxidant capacity Vitamin C	Continuous, 35 kV/cm, 50μs pulse duration, 7 μs bipolar pulses 2050 μs pulse duration	113 % retention attained 72% retention attained 100% retention attained Content was reduced	Oms-Oliu et al., 2009
Longan juice	Vitamin C Total phenols	Batch, 32 kV/cm, 90μs pulse duration, 3μs bipolar pulses	4% reduction 18 % reduction	Zhang et al., 2009
Cherry juice (sour)	Vitamin C Total anthocyanins	0 (control), 17, 20, 23, 27, and 30 kV cm)1, 3μs pulse duration, 500 pps repetition rate, and 131 μs treatment time, 22.5, 27.0, 36.5, 48.0 and 67.5 J s)1 energy input	Increased but not significant	Atluntas et al., 2010
Orange juice-milk	Ascorbic acid	Continuous, 15, 25, 35 and 40 kV/cm, 40 to 700 μs	No significant reduction; 90% retention	Zulueta et al., 2010
Strawberry juice	Phenolic acids, flavonoids, vitamin C and antioxidant capacity	35 kV/cm for 1,700 μs in bipolar 4-μs pulses at 100 Hz	ellagic acid was enhanced, maintained higher amounts of phenolic acids (ellagic and p-coumaric acid) and total anthocyanins	Odriozola-Serrano et al., 2008

2.4.4.4. Shelf-life Extension

A number of researches have been conducted to determine the effectiveness of PEF in producing shelf stable foods. Most of the studies focused on the impact of PEF on the shelf-life of orange juice using high intensity electric fields. These studies are summarized in Table 11 wherein, the stabilities of these juices became stable after PEF processing.

Table 11 Effects of PEF treatment on the stability of liquid foods

Sample	Treatment Conditions	Shelf-Life	Reference
Orange juice	Continuous, 37 kV/cm 59μs pulse duration	112 days *same with thermally-treated samples	Yeom et al., 2000
Orange juice	Continuous, 40 kV/cm 150μs pulse duration	168 days *still stable *has higher microbial count (but within safe limits) than thermally-treated	Cronin et al., 2009
Orange juice-milk based	Continuous, 30 kV/cm 50μs pulse duration	28 days *still stable and same with thermally-treated	Geveke et al., 2009

2.4.4.5 Extraction of bioactive compounds and changes in nutritional properties using moderate or mild PEF processing

The application of high intensity pulsed electric field (HIPEF) is more for pasteurization purposes. Application of mild or moderate intensity pulsed electric field (MIPEF) has been mainly used for the purpose of extracting valuable compounds, and drying purposes to a lesser extent. PEF can induce stress reactions in plant systems, which enhance and stimulate the bioproduction of certain compounds (Toepfl et al., 2006). The mechanism behind this is the so-called 'electroporation' or'electropermeabilization' effect. It involves application of external electric fields on biological cells including plants and animals, which results in disruption of cell membranes and induces pore formation. The pore formation could be either reversible or irreversible depending on the intensity of PEF applied. According to Angersbach and others (2000), when pores induced are small compared to the membrane area and when generated using a low-intensity PEF, the electric breakdown is reversible. The disruption of cell membrane using MIPEF may results in reversible pore formation, which permits the extraction of intracellular compounds and additional biosynthesis of secondary metabolites will be triggered as response to the stress induced by PEF (Angersbach et al., 2000).

Several researches were conducted on the applicability of mild or moderate intensity PEF in the extraction of intracellular metabolites in fruits. With regards to the extraction of bioactive compounds (anthocyanins and polyphenolics) using mild intensity PEF, Balasa and others (2006) reported an increase in total phenolics in pressed grapes after the application of 0.5 to 2.4 kV/cm electric field strength. Interestingly, they observed that an increasing amount of phenolics was obtained as they increased the electric field intensity up to 2.4 kV/cm. Tedjo and others (2002) studied the effect of mild PEF treatment on the release of anthocyanins in grapes. Application of 0.3 kV/cm electric field intensity on grapes resulted in a three-fold increase in the amount of anthocyanins compared to untreated samples. On the other hand, in the study of Schilling and others (2007), there were no significant differences between PEF-treated apple mash samples and untreated samples after the application of 1, 3, and 5 kV/cm electric field intensities. Moreover, the application of 1 and 3 kV/cm electric field intensity did not result in an increase in polyphenolics, and a slightly lower content (not significant) of polyphenolics was observed after the application of 5 kV/cm. A summary of the effects of mild or moderate PEF treatments on anthocyanin and polyphenol content in fruits is

presented in Table 12. Based on this, MIPEF treatments enhanced the phytochemicals in fruits using MIPEF in majority of the studies conducted.

Table 12 Extraction of anthocyanins and polyphenols in fruits using moderate pulsed electric field intensity

Samples	Anthocyanins and polyphenolics	Treatment Conditions	Effects	Reference
Grapes	Polyphenolics (compounds not specified)	0.5 kV/cm, 50 pulses, 0.1 kJ/kg 2.4 kV/cm, 50 pulses 2.3 kJ/kg	Increase in total polyphenolic (TP) content (28%) in fresh pressed grape juice compared to the untreated grape juice	Balasa et al., 2006
Grapes	Anthocyanins: (compounds not specified)	3 kV/cm, 50 pulses	Total anthocyanin content was 3 times higher than the untreated grapes	Tedjo et al., 2002
Apple	Polyphenolics: chlorogenic acids, catechins, procyanidins, quercetin glycoside	1, 3 5 kV/cm, 30 pulses	No significant difference in the polyphenolic content of PEF-treated samples using 1 and 3 kV/cm, and slightly lower content obtained after application of 5 kV/cm	Schilling et al., 2007
Tomato	Polyphenols (compounds not specified)	0.4 to 2.0 kV/cm, 5 to 30 pulses	Total polyphenols content increased by 44% after the application of 1.2 kV/cm at 30 pulses	Vallverdu-Queralt et al.,2012

2.4.4.6 Growth of probiotic bacteria after PEF treatments

Probiotic bacteria have long been known to be beneficial to human. There are many factors that affect the growth of probiotic bacteria in foods. Studies have reported the effects of phytochemicals (anthocyanins and polyphenols) on the growth of probiotic bacteria. Fruit extracts have been reported to exert stimulatory and inhibitory effects on the probiotic species. Sun-Waterhouse and others (2012) reported that the addition of apple polyphenols extracts in drinking yoghurt boosted the growth of *Lactobacillus* compared to the control. Sutherland and others (2009) reported that the growth of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* was enhanced by the acqueous polyphenols extracts of apple, banana, and orange in their study. Another study by Hervert-Hernández and others (2009) reported the stimulating effect of phenolics on *L.acidophilus* in grapes.

In the study by Hap (2010), high concentrations (20 to 30%) of polyphenolics extract from strawberry, blueberry, green kiwi fruit, and feijoa stimulated probiotic (*Bifidobacterium* and *Acidophilus*) growth. However, in their study, an inhibitory effect of polyphenols extracts was observed at low concentration of extracts (0.01 to 0.94 g/L). However high concentrations of phenolics and organic acids may interact and result in an inhibitory effect on probiotic bacteria (Hap and Gutierrez, 2012). The stimulatory effect of these bioactive compounds could be due to additional energy provided by these compounds to the probiotic bacteria that can enhance their growth (Koren et al., 2009; Parker et al., 2008). On the other hand, the inhibitory effects could be due to the interactions of the complex bioactive compounds composition. Hence in our study, the effects of mild or moderate PEF processing of cherries on the growth of probiotic bacteria will be investigated. To our knowledge, there has been no report yet on the effects of different PEF treatments on the growth of probiotic bacteria.

Chapter 3. Materials and Methods

The primary purpose of this study is to determine the effect of PEF on the physical, chemical, and microbiological properties of cherry fruits. This chapter outlines the research methods used. Most of the analyses conducted were based on the official methods of the Association of Official Analytical Chemists (AOAC), and methods described by other researchers. However, a new method was developed for the analyses of bioactive compounds (anthocyanins and polyphenols) using LC-MS. Finally, data analysis techniques used in this study are presented.

The collection and PEF processing of cherry samples were done at the Department of Food Science, University of Otago. All other analyses (physicochemical analysis, microbiological tests, volatile analysis, and antioxidants analyses (anthocyanins and polyphenols) were conducted at Auckland University of Technology (AUT).

3.1 Cherry Fruits

Red-fleshed sweet cherries (*Prunus avium variety* Stella) with stalks still attached were handpicked between January and early February 2013 at commercial maturity as determined by the grower from an orchard located at Alexandra (New Zealand). The cherries were immediately dispatched after harvest and transported overnight to the Department of Food Science, University of Otago (Dunedin, New Zealand) using a refrigerated (4 °C) truck. Upon arrival (within 24 h after harvest), the cherries were screened. Cherries that were mouldy or severely damaged flesh were excluded from the study. The stalks of the cherries were carefully removed, while the seeds were pitted (Cherry-It pitter, Progressive, Washington, DC, USA). The remaining edible portion was immediately frozen in liquid nitrogen, vacuum packed in aluminium foil bags (100 grams each) and stored at −20 °C until usage.

3.2 Pulsed Electric Field Treatments

The frozen cherries were randomly sampled before PEF treatments. Frozen cherry samples were taken out from the storage room (−20 °C) and prepared by cutting cherries into four cuts, weighing, and then placing them directly into the PEF treatment

chamber. No thawing out of the samples was done to prevent juice loss prior to PEF treatments. The chunks were submerged in distilled water (15 °C) at a ratio of 60:40, and then subjected to PEF processing. PEF treatments were carried out using a pilot scale PEF apparatus (Elcrack, HVP 5, DIL, German Institute of Food Technologists, Quakenbruck, Germany) with batch treatment configuration (chamber size of 100 mm length × 80 mm width × 50 mm height) (Figure 9). This chamber was comprised of two parallel stainless steel electrodes of 5 mm thickness with a separate distance of 80 mm.

For each PEF treatment, a total of 100 g of cherry chunks-water mixture was used. Each PEF treatment combination was carried out in triplicate using three independent batches of cherry chunks-water mixture. The temperature and conductivity of each sample were measured prior to and after PEF treatment, using an electrical conductivity meter (CyberScan CON 11; Eutech Instruments, Singapore). The weight of the samples (cherry chunks and water) was recorded. The PEF operating settings applied were: constant pulse width of 20 μs, different electric field strengths ranging from 0.3 to 2.5 kV/cm, constant pulse frequency of 100 Hz and different pulse numbers ranging from 385 to 10,000. Square wave bipolar pulse shape was monitored on-line with oscilloscope (Model UT2025C, Uni-Trend Group Ltd., Hong Kong, China) during treatment for all samples.

All treatments were conducted at an ambient temperature (~20 °C). In this study, the samples were grouped into three based on storage conditions after PEF treatment. The samples used were: (i) control (untreated) samples before PEF (S1); (ii) samples immediately after PEF (S2); and (iii) samples after PEF treatment and stored for 24 h at 4 °C (S3). Control sample (untreated samples) was similarly prepared to the PEF treated-samples by incubating cherry chunks using the same proportion water. Cherry chunks were separated from the liquid/juice after processing and storage. All cherry samples were frozen in liquid nitrogen, ground using an analytical grinding mill, and stored at −20°C prior to volatile analysis. Schematic representation of the experimental procedure carried out in this research is illustrated in Figure 10.

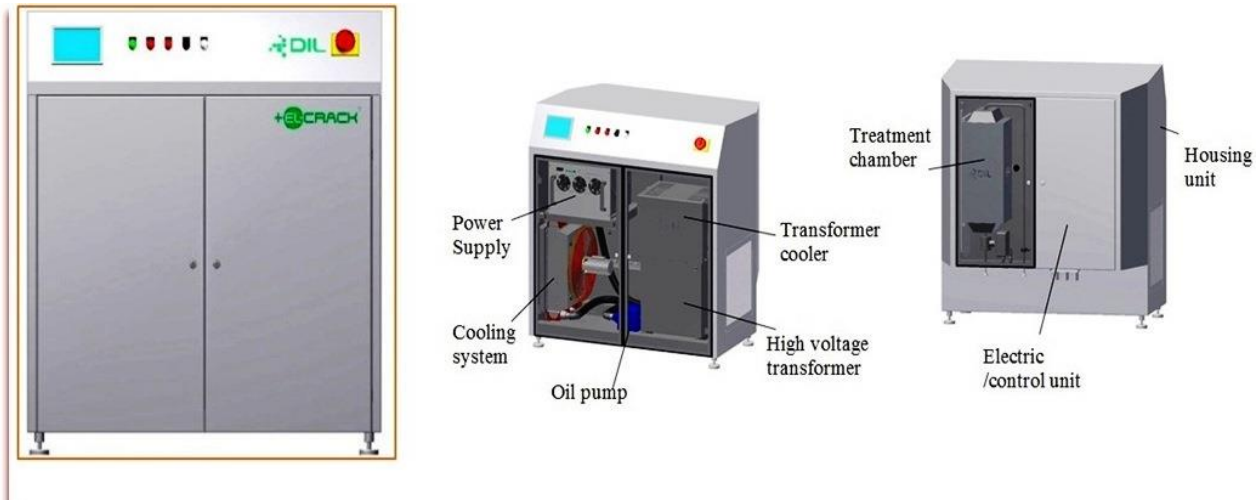


Figure 9 PEF laboratory unit

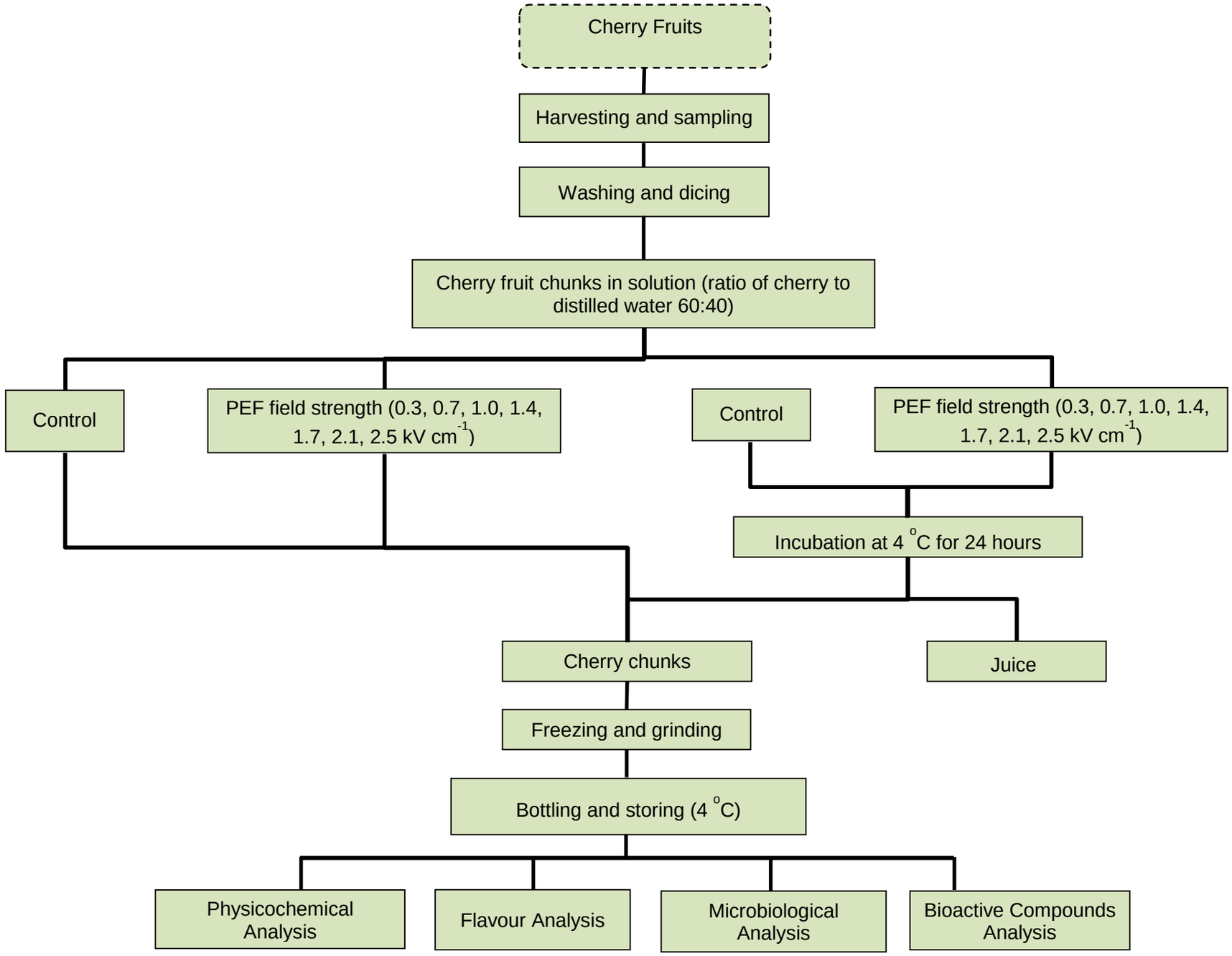


Figure 10 Schematic representation of experimental procedure

Table 13 summarizes the PEF processing settings applied to frozen cherry fruit chunks in solution and include changes in temperature, and conductivity of samples. The processing settings applied resulted in effective electric field strengths between 0.3 and 2.5 kV/cm, and pulsed electrical energy between 30 to 55 kJ/kg. These settings ensured that changes in temperature were kept at a minimum and that the final temperature did not exceed 25 °C. The calculation for the specific energy input (Wspec) generated during PEF treatment of cherry samples was adapted from the equation used in the study of Zhang, Barbosa-Cánovas, and Swanson (1995) (Equation (1) :

$$W_{spec}\ (kJ/kg) = VIN\tau/m \qquad (1)$$

where V is the pulse peak voltage in kilo volts (kV), I is the electric current in ampere (A), N is the number of pulses (dimensionless), τ is the pulse width of square pulses in microsecond (μs), and m is the total weight of the sample in kilogram (kg).

The standard deviations reported for the application of pulsed electric field (Table 13) refer to the instrumental error on repeatability or variability of the processing conditions delivered by the machine to the samples. Repeatability describes the closeness of output readings when the same input is applied repetitively over a short period of time, with the same measurement conditions, same instrument and observer, same location and same conditions of use maintained throughout (Morris, 2001). The input settings (energy input, pulse frequency, pulse width, pulse number) applied were the same but the indicative readings were different for all replicates.

Table 13 Summary of PEF processing conditions showing changes in temperature and conductivity (Mean ± SD) of cherry chunks in solution.

Sample Codes	Electric Field Strength (kV/cm)	Pulse Voltage (kV)	Pulse Number	Calculated Energy (kJ/kg)	Change in Temperature [A] ΔT (°C)	Change in Conductivity of Chunks in Solution
			Samples Immediately after PEF Treatment			
S2-P1	0.30 ± 0.06	2.83 ± 0.06	10000	39.92 ± 0.10	1.90 ± 0.82 [a]	1.51 ± 1.03 [ab]
S2-P2	0.70 ± 0.00	5.50 ± 0.10	6100	54.75 ± 6.16	4.10 ± 1.04 [a]	1.60 ± 0.51 [ab]
S2-P3	1.00 ± 0.06	8.33 ± 0.06	2350	48.31 ± 1.37	0.73 ± 0.42 [a]	0.46 ± 3.30 [ab]
S2-P4	1.40 ± 0.00	11.10 ± 0.10	900	34.63 ± 1.41	0.47 ± 0.87 [a]	0.68 ± 1.27 [b]
S2-P5	1.70 ± 0.06	13.97 ± 0.06	720	43.22 ± 1.06	0.60 ± 1.70 [a]	0.77 ± 0.74 [b]
S2-P6	2.10 ± 0.00	13.63 ± 5.92	520	41.86 ± 2.16	0.10 ± 0.52 [a]	0.12 ± 0.46 [b]
S2-P7	2.50 ± 0.06	19.60 ± 0.10	385	45.30 ± 1.71	0.33 ± 1.92 [a]	1.02 ± 1.23 [b]
			Samples 24 h after PEF Treatment			
S3-P0	-	-	-	-	Nd	Nd
S3-P1	0.30 ± 0.06	2.77 ± 0.06	10000	29.80 ± 0.09	1.30 ± 1.25 [a]	3.59 ± 0.53 [a]
S3-P2	0.70 ± 0.00	5.43 ± 0.06	6100	34.43 ± 3.51	0.87 ± 1.46 [a]	0.81 ± 0.43 [b]
S3-P3	1.00 ± 0.06	8.30 ± 0.26	2350	34.26 ± 4.87	0.40 ± 4.35 [a]	0.26 ± 1.55 [b]
S3-P4	1.40 ± 0.06	11.47 ± 0.21	900	30.82 ± 2.26	2.10 ± 2.81 [a]	1.63 ± 0.60 [b]
S3-P5	1.70 ± 0.06	14.00 ± 0.17	720	42.91 ± 2.13	0.17 ± 1.10 [a]	1.85 ± 0.49 [ab]
S3-P6	2.10 ± 0.00	16.73 ± 0.15	520	47.52 ± 2.57	1.23 ± 0.68 [a]	1.93 ± 0.24 [ab]
S3-P7	2.50 ± 0.06	19.70 ± 0.30	385	45.46 ± 4.70	1.47 ± 2.90 [a]	0.76 ± 0.24 [b]
Control						
S1	-	-	-	-	Nd	Nd

Mean ± S.D: Standard deviation based on three independent samples and treatments. S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. [A] Changes in temperature due to PEF treatment. The initial temperature of cherry chunks in solution sample prior subjected to PEF treatment averaged at 20.4 ± 0.00 °C. Means within the same column not bearing common superscripts differ ($p < 0.05$; one-way ANOVA with Fisher's LSD comparison test). Nd: not determined.

3.3 Physicochemical Analysis

All physicochemical tests were carried out on different cherry samples from three different treatments (control or untreated, immediately after PEF, and 24 hours after PEF), and each analysis was replicated three times. Physicochemical analyses include conductivity, pH determination, colour measurement, titratable acidity test, and total soluble solids. In addition physical properties such as size range, moisture content, and ratio of fruit to juice were also conducted. Prior to analysis, each sample was partially thawed according to the AOAC official method 974.12 (AOAC International, 2002), in which samples were thawed in an unopened container at room temperature.

3.3.1 Size range

Prior to cutting into chunks, whole cherry fruits were randomly picked for the size range test. The following fruit characteristics were determined: fruit diameter (mm), fruit volume (cm^3), total fruit weight (g), and flesh/seed ratio. The width and length of cherry samples were measured by using a Vernier caliper with a sensitivity of 0.01 mm. The volume of the fruit was estimated using the equation for a sphere:

$$V = \frac{4}{3}\pi r^3 \quad (2)$$

Fruit weights were measured using a digital electronic balance with a sensitivity of 0.001 g (Scaltec Company, Gottingen, Germany; model SPB31) with a maximum capacity of 210 g. The flesh to seed ratio was calculated using the formula adapted from the study of Demir and Kalyoncu (2003):

$$\text{Fruit weight} - \text{seed weight/seed weight} \quad (3)$$

3.3.2 Ratio of fruit to juice and juice yield

Ratios of fruit to juice for each sample were also quantified. This was done by separating the cherry chunks from the liquid or juice by sieving after PEF processing. The weights of the chunks and the juice were both recorded. The weights were determined using a top loading scale.

The calculation of the juice yield was based on the weight of the cherry and of the juice obtained (Schilling et al., 2009):

$$\% \text{ Yield} = (\text{wt. of juice} / \text{wt. of cherry}) \times 100 \quad (4)$$

3.3.3 Conductivity

The conductivity (Figure 11) of each samples was recorded prior to (control samples and samples for PEF treatments) and after PEF treatments (samples immediately after PEF and samples 24 hours after PEF) using an electrical conductivity meter (CyberScan CON 11; Eutech Instruments, Singapore, Singapore). The conductivity of the liquid matrix in which the samples were treated was also determined.

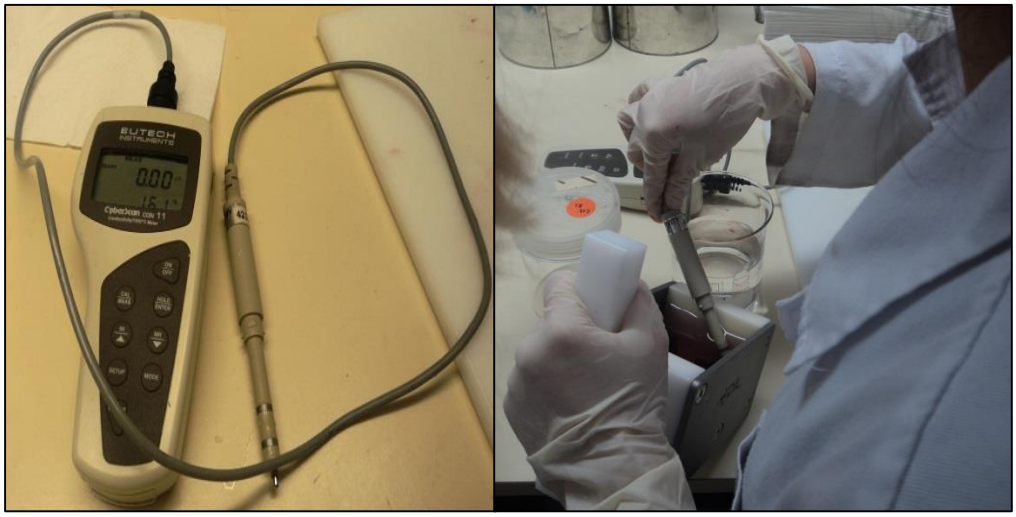


Figure 11 Conductivity testing of cherry samples

3.3.4 pH measurement

pH measurement was conducted using a bench top pH meter (AOAC 251.58, AOAC International 2002). Cherry samples were thawed out at room temperature prior to analysis. The electrode of the pH meter was calibrated and standardized at 25 ^{o}C in pH 7.0 and pH 4.0 standard buffer solutions, rinsed with distilled water, and wiped dry with a paper towel before analysis. The electrode was immersed in the sample and the pH

reading was recorded after allowing the pH meter to stabilize. Rinsing of electrode and calibration with distilled water was done after every sample for correct measurement.

3.3.5 Colour measurement

Colour was determined using a Hunter Lab (45/0, Colorflex EZ) color analyser (Figure 12). Approximately 6.0 grams of samples were used for each sample during analysis. As red cherries oxidized readily, colour was examined while some ice crystals remained in the product (AOAC Method 974.2, AOAC 2002). Readings were taken in triplicate for each sample and recorded based on the colour space system L*, a*, b* as defined by the Commission Internationale de l'Eclairage (CIE, 1986). Within this system, L* measures the lightness of the color of the sample, a* measures the red and green characteristics and b* measures the yellow and blue characteristics (McCaig, 2002). According to Hunterlab (2012), the two colour scales namely Hunter L, a, b and CIE L*, a*, b* are based on the Opponent- Color Theory where the receptors in the human eye perceive color as the following pairs of opposites below:

- L scale: Light vs. dark, low number (0-50) indicates dark and high number (51-100) indicates light.
- a scale: Red vs. green, a positive number indicates red and a negative number indicates green.
- b scale: Yellow vs. blue, a positive number indicates yellow and a negative number indicates blue.

The colour analyser was standardized in accordance with the method described by Hunterlab (2013) prior to analysis. Diagnostic tests were run to verify instrument performance, and white and black tile calibration was conducted to check for good repeatability and matching values.

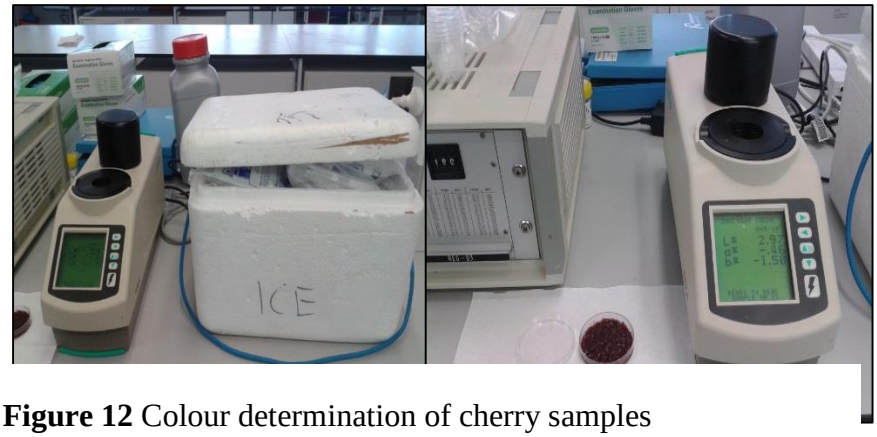


Figure 12 Colour determination of cherry samples

3.3.6 Titratable acidity (TA)

Percentage titratable acidity (Figure 13) of cherry samples was determined according to AOAC method 942.15 (AOAC International, 1995). Sample was weighed using an analytical balance and analysed for titratable acidity. Distilled water (100 ml) was then added. A potentiometric method (glass electrode method) using a pH meter was used to determine the endpoint since samples are coloured. The pH meter was calibrated using pH 4.0 and 7.0 buffer solutions before use. A magnetic stirrer was used to aid in stirring of the samples. Samples were titrated using standard 0.1 M NaOH solution until the pH reached an endpoint of 8.1. The results were expressed as % malic acid (fruit acid for cherries) using a 0.067 factor (equivalent weights). The formula for titratable acidity calculations is given below:

$$\% \text{ Titratable Acidity} = \left(\frac{\text{equivalent weights of acid x Normality of NaOH x Volume of titer}}{\text{weight of sample}}\right) \text{x } 100 \qquad (5)$$

Figure 13 Titratable acidity analysis of cherry samples

3.3.7 Total soluble solids (TSS)

Total soluble solids were determined using the Reichert automatically temperature compensated hand-held refractometer (Reichert, Inc, Walden Avenue, Depew, New York, USA) (Figure 14). The method was adapted according to the study of Yilmaz et al. (2009). The unit was washed with distilled water before and after measurements of each sample. The measuring range of the refractometer was between 0 and 30% solids (^{o}Brix scale). TSS was determined at room temperature (22^{o}C) by extracting and mixing one drop of thawed homogenized cherry from each sample onto a hand held refractometer.

Figure 14 Refractometor used in TSS analysis

3.3.8 Moisture analysis

Moisture content was analysed using the laboratory convection oven (Sanyo model MOV-112F, Sanyo Electric Co., Ltd., Biomedical Division, Japan) (Figure 15). The method used was according to AOAC 945.15 (AOAC, 2000). Sample (5.0 g) was weighed in dried crucibles using an analytical balance and dried in a vacuum oven at 105^{o}C for 16 hours. After drying, the samples were allowed to cool down for 30 minutes in desiccators with dried silica gel (Figure 16). Then the weight of samples was recorded. Percentage moisture was calculated using the formula:

$$\% \text{ Moisture} = \frac{\text{moisture loss in grams}}{\text{original weight of sample}} \times 100 \qquad (6)$$

Figure 15 Vacuum oven method used in cherry analysis

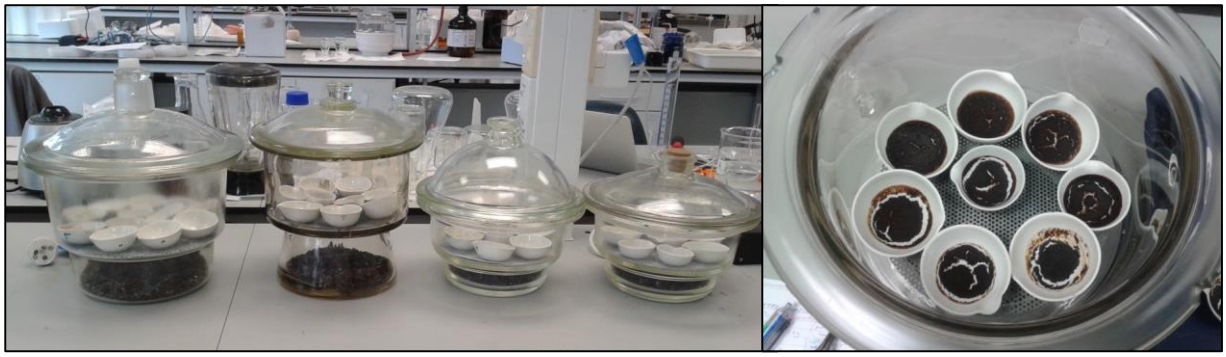


Figure 16 Moisture analysis of cherry samples

3.4. Bioactive Compound Analyses

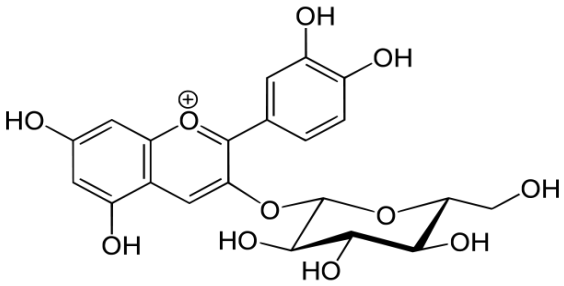
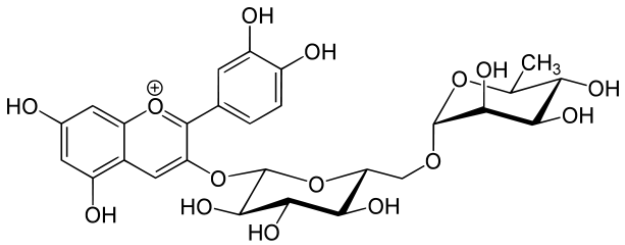
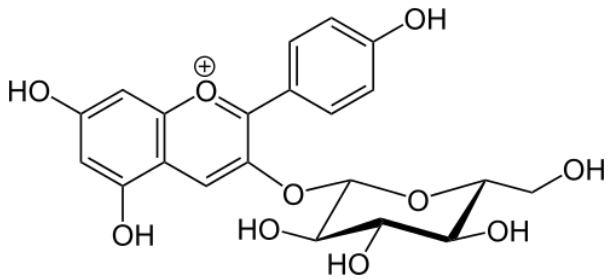
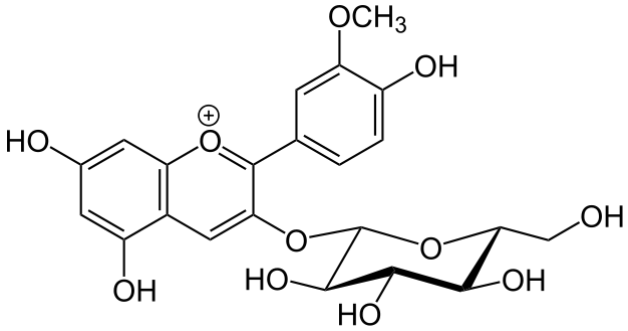
In this research, the effect of PEF on the extraction of bioactive compounds was investigated. This involved the identification and quantification of bioactive compounds present in cherries before and after PEF processing that included anthocyanins and polyphenols using the liquid chromatography mass spectrometry (LC-MS) instrument. The extraction and analysis of these compounds using LC-MS were optimized prior to analysis.

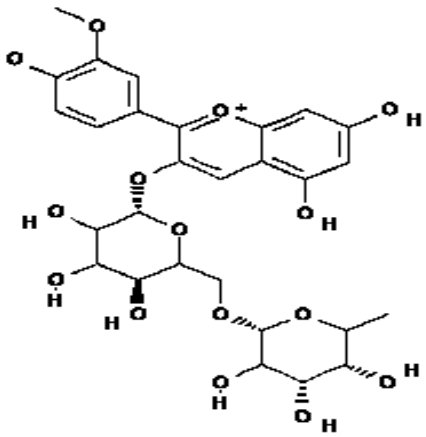
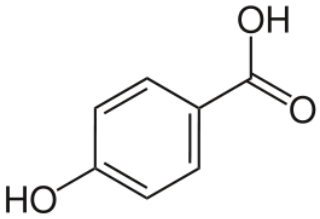
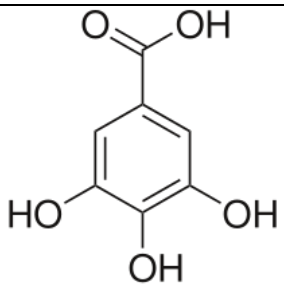
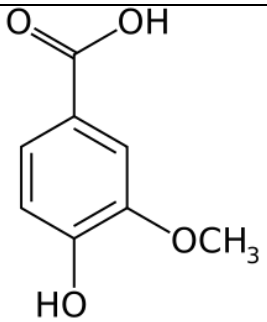
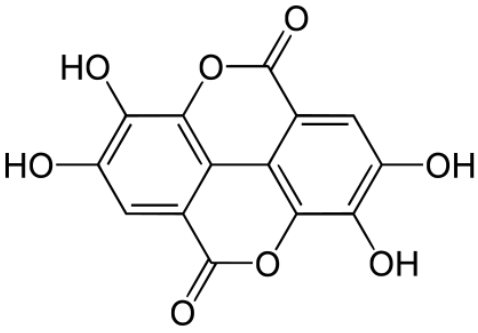
3.5.1 Liquid Chromatography – Mass Spectrometry (LC-MS) Method Development

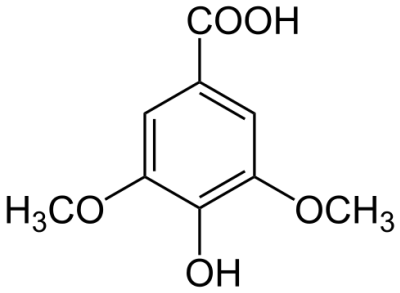
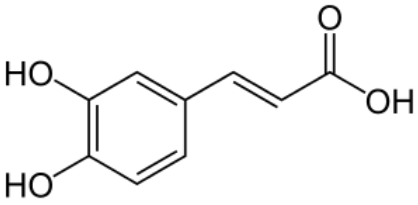
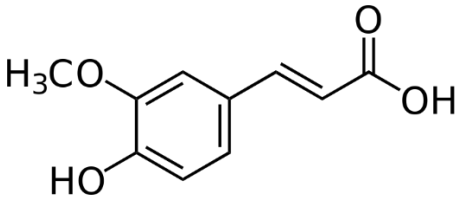
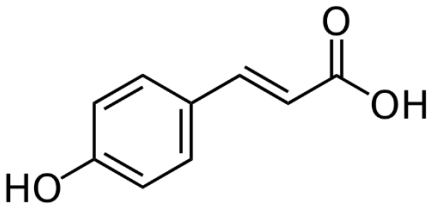
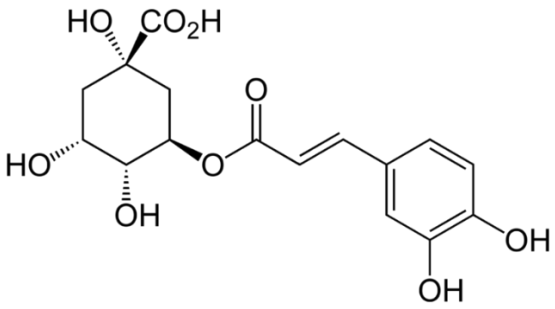
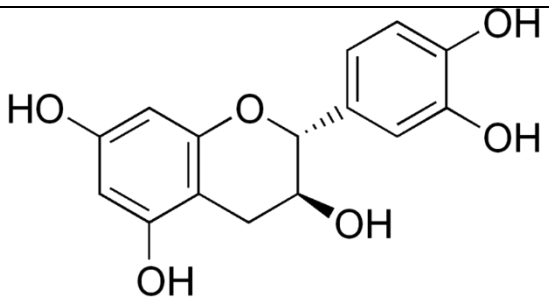
Identification and quantification of 23 chemical standards (5 anthocyanins and 18 polyphenolics) in cherry samples were carried out. These anthocyanins included cyanidin glucoside (1), cyanidin rutinoside (2), pelargonidin glucoside (3), peonidin glucoside (4), and peonidin rutinoside (5). Polyphenolics investigated included 4-hydroxybenzoic acid (6), gallic acid (7), vanillic acid (8), ellagic acid (9), syringic acid (10), caffeic acid (11), ferulic acid (12), ρ-coumaric acid (13), chlorogenic acid (14), (+) catechin (15), (-) epicatechin (16), quercetin (17), myrecitin (18), rutin (19), kaempferol (20), kaempferol rutinoside (21), isorhamnetin (22), and isorhamnetin rutinoside (23). Their chemical structures together with their names, chemical formula, and monoisotopic mass are shown in Table 14. Standard compounds 6, 15, 8, 10, 11, and 13 were purchased from Sigma (St. Louis, MO, USA), while the rest were purchased from Extrasynthese (Genay Cedex, France). All of these standards were suspended in 100% methanol, dried under a stream of nitrogen gas, and stored at -80 ^{o}C prior to analysis.

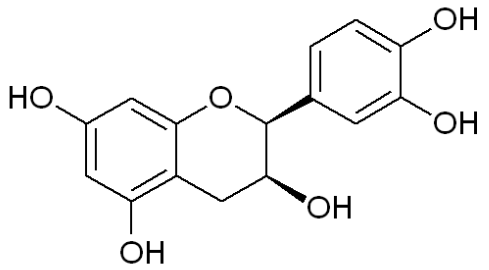
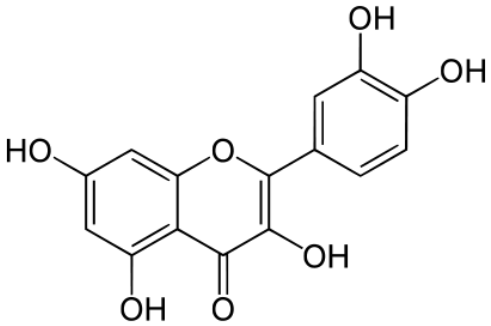
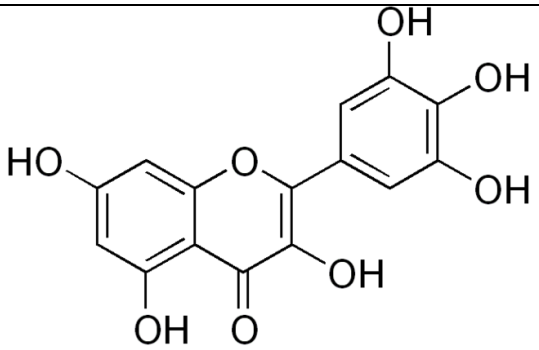
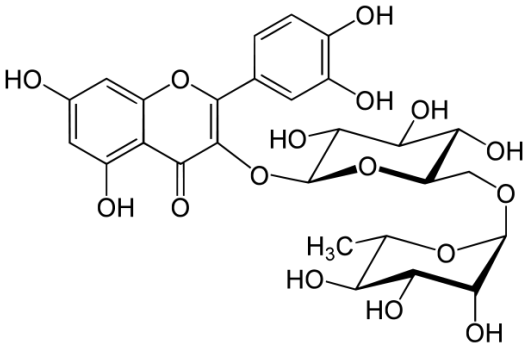
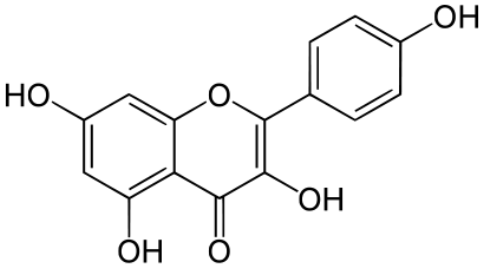
Initially, all standards were run using the same LCMS gradient program. However, the analysis of two classes of phenolics (anthocyanins and polyphenols) had to be carried out separately because the unique requirements for anthocyanin chromatography were not suitable for some polyphenols. Hence separate methods for analyses were carried out in this study and discussed in the following section. Chlorobenzoic acid and 4-methylumbelliferyl β-D- galactopyranoside (MUG) were chosen as internal standards for polyphenolics and anthocyanins respectively. These two standards were selected because of their similarity in properties and structures to the target compounds.

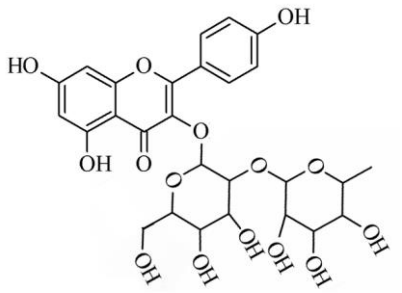
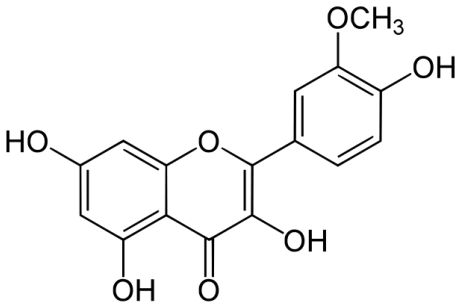
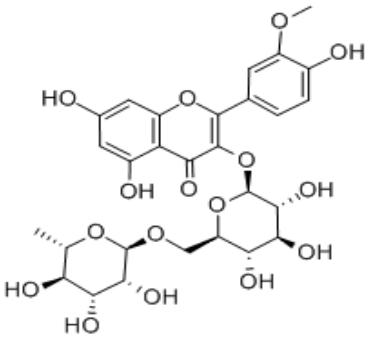
Table 14 Chemical structures of 23 phenolic compounds used in this study.

Anthocyanins	Chemical Structures
(1) Cyanidin glucoside Common name: Chrysanthemin Formula: $C_{21}H_{21}O_{11}$ Exact monoisotopic mass: 449.108390	
(2) Cyanidin rutinoside Common name: Antirrhinin Formula: $C_{27}H_{31}O_{15}$ Exact monoisotopic mass: 595.166300	
(3) Pelargonidin glucoside Common name: callistephin Formula: $C_{21}H_{21}O_{10}$ Exact monoisotopic mass: 433.113475	
(4) Peonidin glucoside Common name: Peonidin-3-glucoside; Peonidin 3-O-glucoside Formula: $C_{22}H_{23}O_{11}$ Exact monoisotopic mass: 463.124040	

(5) Peonidin rutinoside Common name: Peonidin 3-rutinoside Formula: $C_{28}H_{33}O_{15}$ Exact monoisotopic mass: 609.181950	
Hydroxybenzoic Acids	Chemical Structures
(6) 4-hydroxybenzoic acid Common name: p-Hydroxybenzoic acid, para-Hydroxybenzoic acid, PHBA 4-hydroxybenzoate Formula: $C_7H_6O_3$ Exact monoisotopic mass:138.031695	
(7) Gallic acid Common name: Gallate Formula: $C_7H_6O_5$ Exact monoisotopic mass: 170.021525	
(8) Vanillic acid Common name: Vanillate Formula: $C_8H_8O_4$ Exact monoisotopic mass: 168.042260	
(9) Ellagic acid Common name: Benzoaric Acid Formula: $C_{14}H_6O_8$ Exact monoisotopic mass: 302.006270	

(10) Syringic acid Common name: Syringate, cedar acid Formula: $C_9H_{10}O_5$ Exact monoisotopic mass: 198.052824	
Hydroxycinnamic Acids	Chemical Structures
(11) Caffeic acid Common name: Sodium caffeate Formula: $C_9H_8O_4$ Exact monoisotopic mass: 180.042260	
(12) Ferulic acid Common name: Ferulate, Coniferic acid Formula: $C_{10}H_{10}O_4$ Exact monoisotopic mass: 194.057910	
(13) ρ-Coumaric acid Common name: Naringeninic acid Formula: $C_9H_8O_3$ Exact monoisotopic mass: 164.047345	
(14) Chlorogenic acid Common name: Chlorogenate Formula: $C_{16}H_{18}O_9$ Exact monoisotopic mass: 354.095085	
Flavans or Flavanols	
(15) (+) Catechin Common name: Cianidanol, D-Catechin, Catechinic acid, Catechuic acid, Cianidol Formula: $C_{15}H_{14}O_6$ Exact monoisotopic mass: 290.079040	

(16) (+) Epicatechin Common name: Catechinic acid, catergen, cianidanol Formula: $C_{15}H_{14}O_6$ Exact monoisotopic mass: 290.079040	
Flavonols	Chemical Structures
(17) Quercetin Common name: Sophoretin, Meletin, Xanthaurine, Quercetol, Quertine, Flavin, meletin Formula: $C_{15}H_{10}O_7$ Exact monoisotopic mass: 302.042654	
(18) Myricetin Common name: Cannabiscetin, Myricetol Formula: $C_{15}H_{10}O_8$ Exact monoisotopic mass: 318.037570	
(19) Rutin Common name: rutoside, quercetin-3-O- rutinoside and sophorin Formula: $C_{27}H_{30}O_{16}$ Exact monoisotopic mass: 610.153390	
(20) Kaempferol Common name: Robigenin; Pelargidenolon; Rhamnolutein; Rhamnolutin; Populnetin; Trifolitin Formula: $C_{15}H_{10}O_6$ Exact monoisotopic mass: 286.047740	

(21) Kaempferol rutinoside Common name: Kaempferol 3-O-rhamnosyl-glucoside, Nicotiflorine Formula: $C_{27}H_{30}O_{15}$ Exact monoisotopic mass: 594.158475	
(22) Isorhamnetin Common name: Isorhamnetol, isorhamentin, isorhamnetine Trifolitin Formula: $C_{16}H_{12}O_7$ Exact monoisotopic mass: 316.058305	
(23) Isorhamnetin rutinoside Common name: Narcisin Formula: $C_{28}H_{32}O_{16}$ Exact monoisotopic mass: 624.169040	

3.5.1.2 Methods and method development

The instrument used in this study was an Agilent 1260 Infinity Quaternary LC System (Santa Clara, CA 95051 USA) that was made up of the following components: quartenary pump VL (model number: G1311C), 1260 infinity ALS sampler (model number: G1329B), 1200 series autosampler thermostat FC/ALS/Therm (model number: G1330B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), attached to a 6420 Triple Quadrupole LC/MS System with multimode ionization (MMI) interface source (model number: G1978B).

The LC-MS method was adapted from the method described by Chaovanalikit & Wrolstad (2004) with some modifications. The chromatographic separation was performed on a reversed-phase Cortecs C18 column (2.7 µm 2.1 x 100 mm; Waters, Ireland). The mobile phase was made up of 0.1 % formic acid in Milli Q (solvent A), 0.1% formic acid in acetonitrile (solvent B), and 1% formic acid in Milli Q (solvent C).

The LC-MS instrument used the Mass Hunter Workstation Software (Agilent Technologies Incorporated, 2012, CA 95051, United States), which provides powerful control of the data for method development, data acquisition, and qualitative and quantitative data analysis. Prior to analysis, the instrument was calibrated according to the Good Laboratory Practice (GLP) manual of the instrument. The calibrations included inspection, cleaning (especially the source), and maintenance of the instrument. The resolution, response, and sensitivity were also checked as part of the calibration. Tuning was also performed on the mass spectrometer to optimize the ion source and transmissions, calibrate the mass spectrometer, and to maximize the detection of one or more particular ions.

3.5.1.2.1 Multiple Reaction Monitoring (MRM) of target compounds

All standards exhibited higher sensitivity in the negative ion mode than the positive ion mode under the MMI conditions. Both qualitative and quantitative analyses were carried out first in the full scan mode using the MMI source to check the total ion scan (TIC) of the analytes. Then the mass spectrometer was run in the negative ion multiple reaction monitoring (MRM) mode to transmit different precursor and product ion pairs.

Pure compounds were injected in an isocratic flow of 40% acetonitrite and 0.1% formic acid. The MassHunter Optimizer software automated multiple injections of each compounds to determine optimal fragmentor voltages, collision energies, and parent to daughter ion transitions (Table 15). A single product ion scan of one of the targets, cyanidin glucoside for instance, is presented in Figure 17 showing the parent ion and the most abundant fragment that was selected for MRM.

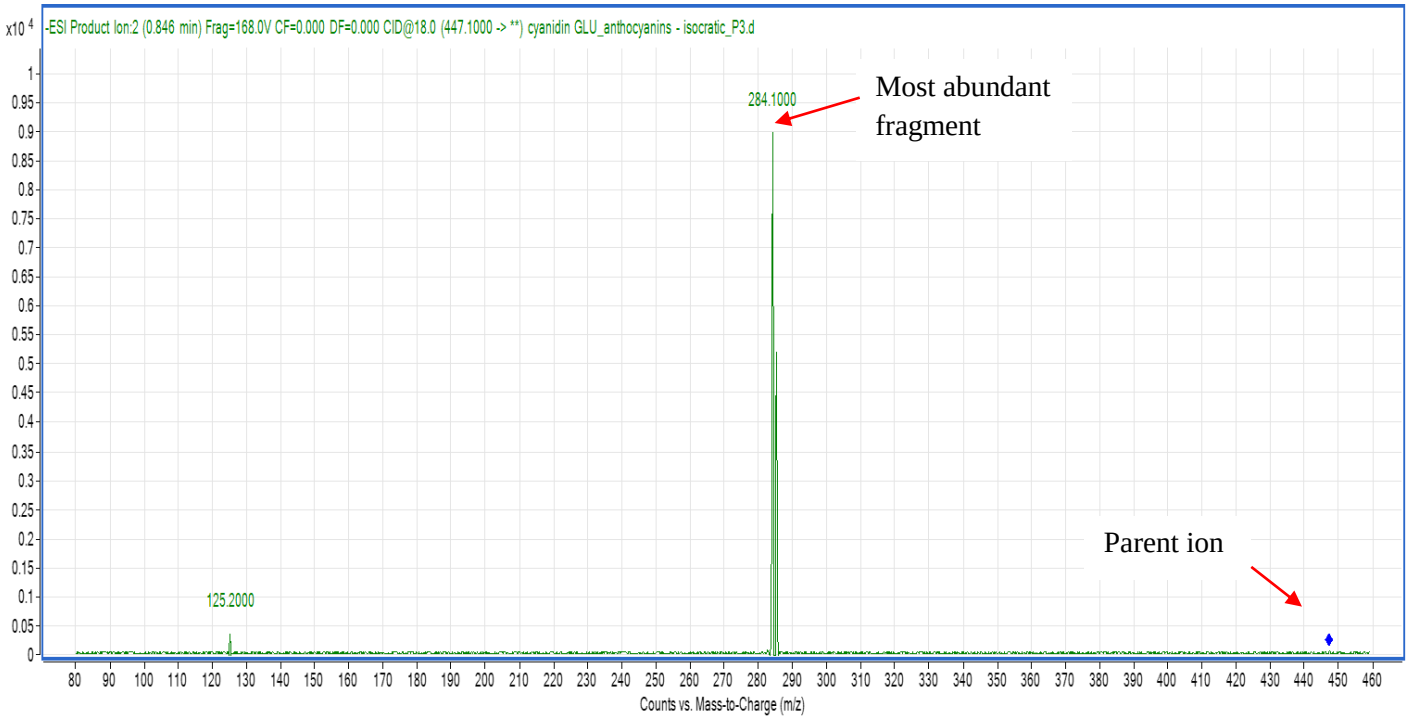


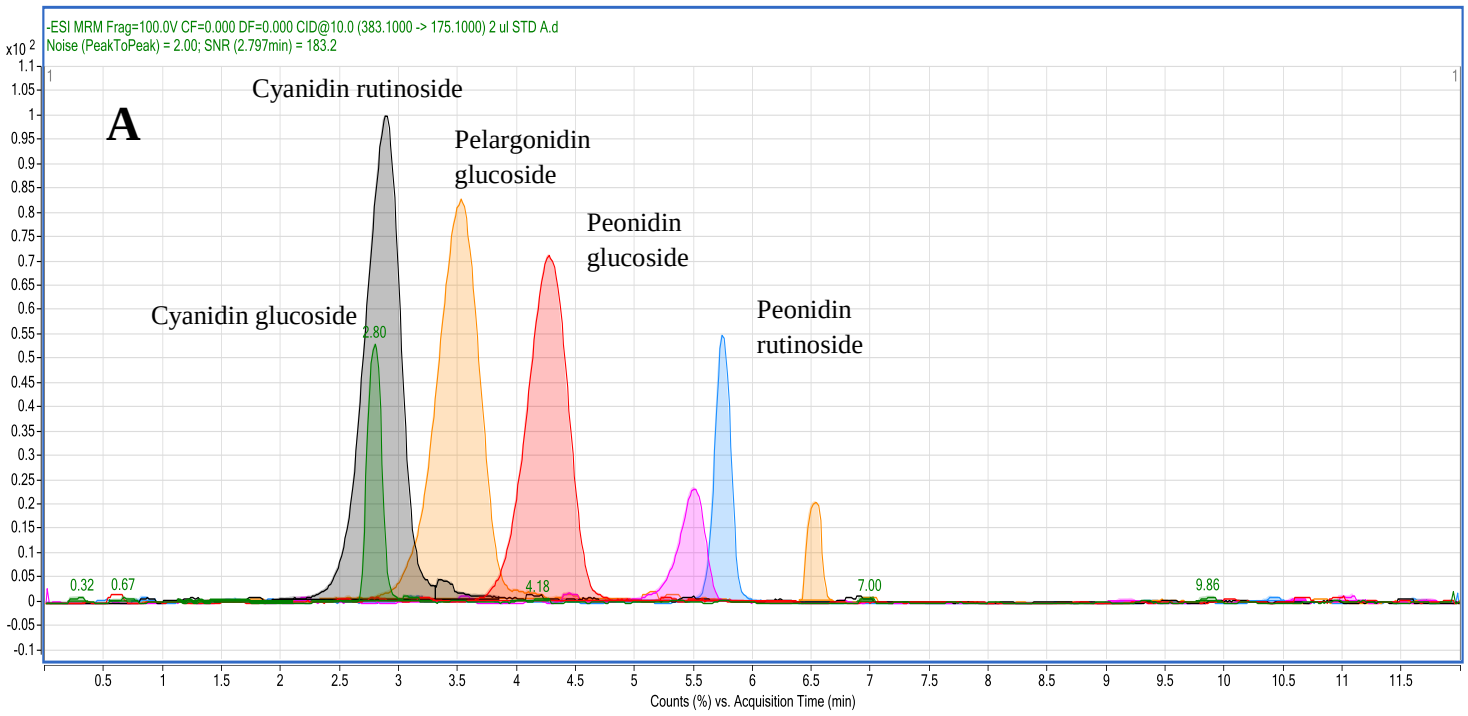
Figure 17 Product ion scan of cyanidin glucoside

Table 15 Optimised MRMs of 23 target compounds and 2 internal standards

Anthocyanins	ISTD	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
4-methylumbelliferyl-b-D-galactopyranaside (IS)	False	383.1	Unit	175.1	Unit	150	100	10	7	Negative
pelargonidin glucoside	False	431.09	Unit	269.1	Unit	150	163	14	7	Negative
cyanidin glucoside	False	447.09	Unit	284.1	Unit	150	168	22	7	Negative
peonidin glucoside	False	461.1	Unit	299.1	Unit	150	178	12	7	Negative
cyanidin rutinoside	False	593.15	Unit	284.1	Unit	150	226	34	7	Negative
peonidin rutinoside	False	607.16	Unit	299.2	Unit	150	211	12	7	Negative
Polyphenolics										
chlorobenzoic acid (IS)	False	154.99	Unit	111.1	Unit	150	86	9	7	Negative
4-hydroxybenzoic acid	False	137.02	Unit	93.1	Unit	150	92	13	7	Negative
p-coumaric acid	False	163.04	Unit	119.1	Unit	150	92	13	7	Negative
vanillic acid	False	167.03	Unit	152	Unit	150	98	9	7	Negative
gallic acid	False	169.01	Unit	125.1	Unit	150	98	9	7	Negative
caffeic acid	False	179.03	Unit	135	Unit	150	110	13	7	Negative
ferulic acid	False	193.05	Unit	134.1	Unit	150	92	13	7	Negative
syringic acid	False	197.04	Unit	123	Unit	150	98	21	7	Negative
Kaempferol	False	285	Unit	93.15	Unit	150	170	28	7	Negative
(+) catechin	False	289.1	Unit	245.1	Unit	150	140	10	7	Negative
(+) epicatechin	False	289.07	Unit	288.4	Unit	150	134	5	7	Negative
ellagic acid	False	301	Unit	228.7	Unit	150	164	28	7	Negative
Quercetin	False	301	Unit	151	Unit	150	146	16	7	Negative
Isorhamnetin	False	315	Unit	300	Unit	150	158	22	7	Negative
Myricetin	False	317	Unit	151	Unit	150	152	18	7	Negative
chlorogenic acid	False	353.09	Unit	190.7	Unit	150	104	13	7	Negative
kaempferol rutinoside	False	593.15	Unit	285.1	Unit	150	224	33	7	Negative
Rutin	False	609.14	Unit	300.1	Unit	150	218	44	7	Negative
isorhamnetin rutinoside	False	623.16	Unit	315	Unit	150	212	33	7	Negative

3.5.1.2.2 Chromatography

As mentioned earlier, separate methods were used for anthocyanins and polyphenolics because of their unique chromatography characteristics. The figures below are chromatograms derived from preliminary method development trials. As seen in Figures 18 and 19, some peaks were co-eluting. Hence the gradient had to be optimised to improve the elution of each compound before actual analysis of the samples.



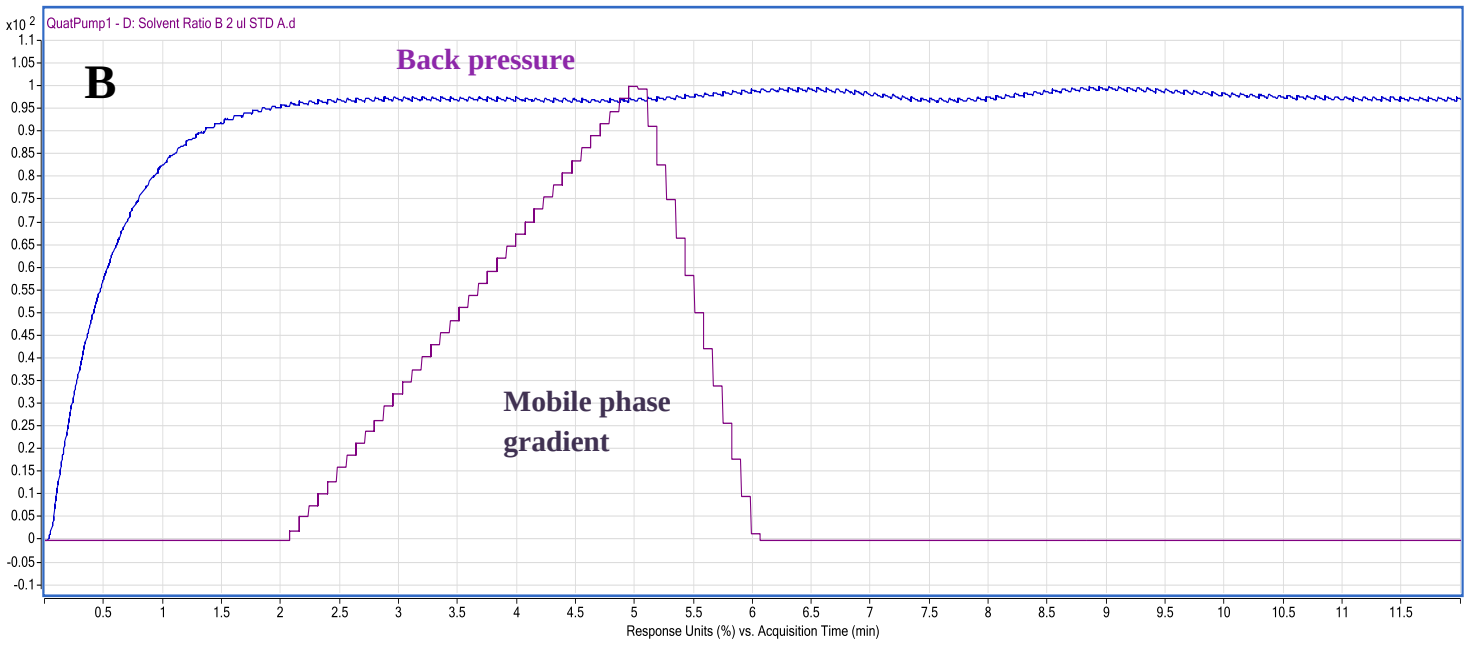
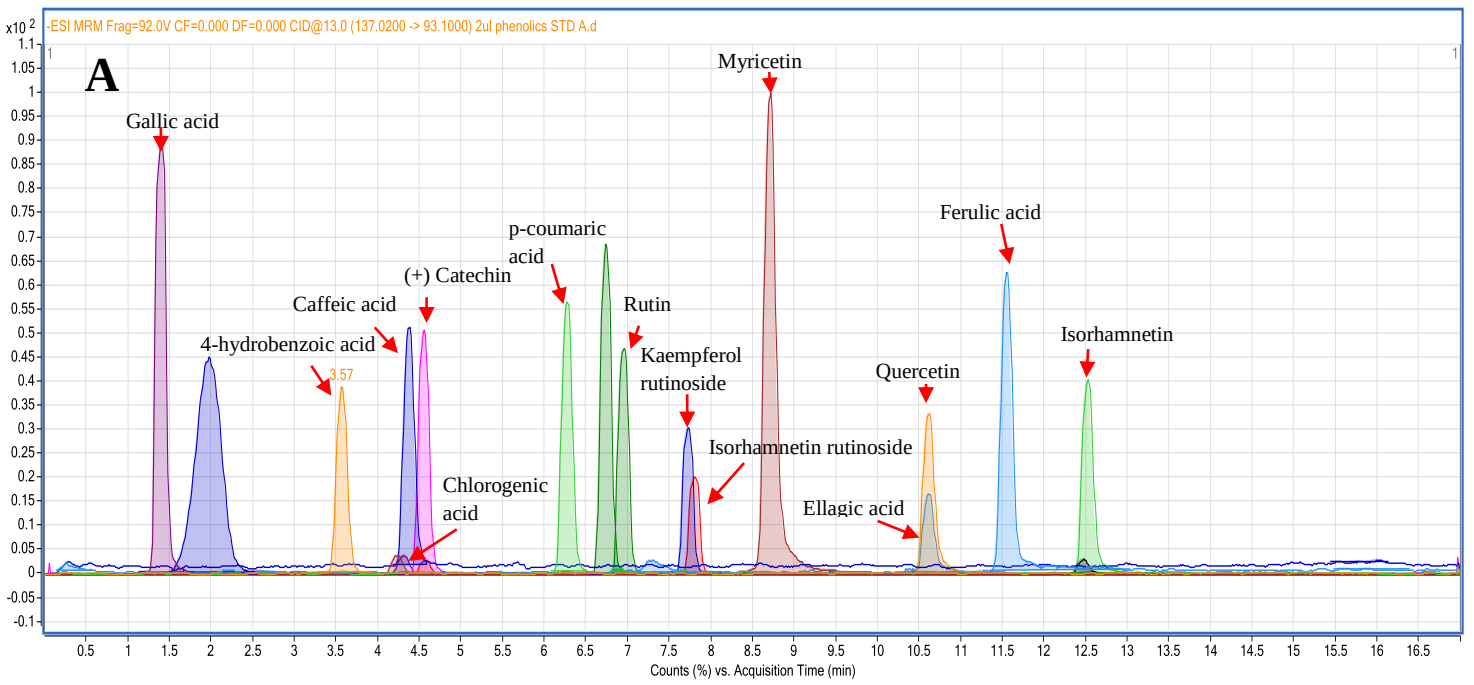


Figure 18 Early chromatograms of MRMs of 5 anthocyanins (A), and the mobile phase gradient and back pressure (B)



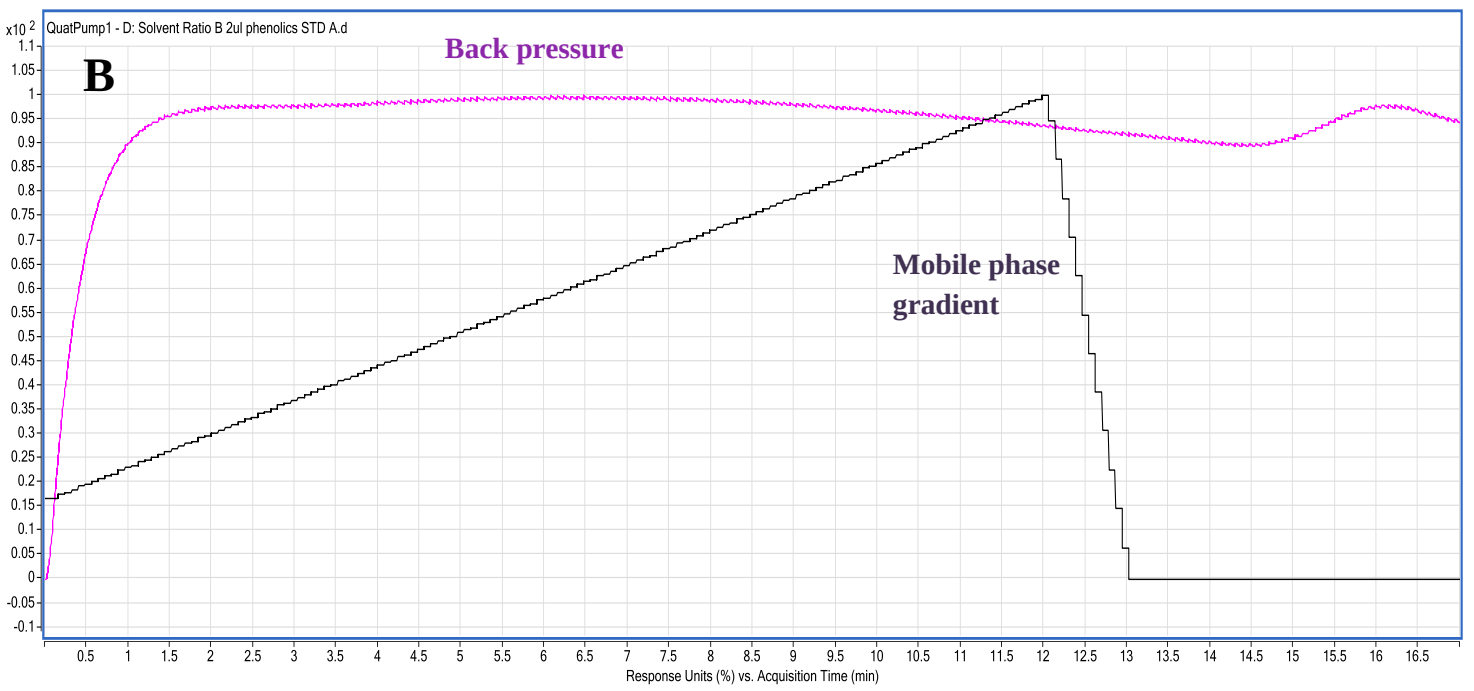


Figure 19 Early chromatograms of MRMs of polyphenolics (A), and the mobile phase gradient and back pressure (B)

The gradient program for anthocyanins was: 3% B at 0 – 3 min, 12% B at 3 – 18 min, 80% B at 18 – 19.4 min, and back to 3% B at 19.4 - 20.5 min. For polyphenolics, the gradient program used was: 97% at 0 – 3 min, 92% at 3 – 4 min, 90% at 13 min, and 85% at 13 – 13.5 min, 83% at 13.5 – 18.0 min, 79% at 18 – 23 min, 70% at 23 – 28 min, 10% at 28 – 29 min, and 97% at 29 – 31 min. The total run times were 20.5 min and 31.0 min for anthocyanins and polyphenolics respectively. Autosampler temperature was maintained at 4 ^{o}C and injection volume was 10 µl. The MMI source parameters were: gas temperature of 325 ^{o}C (anthocyanin method) and 300 ^{o}C (polyphenolic method). For both anthocyanin and polyphenol methods the same parameters for the following were used: APCI heater of 200 ^{o}C, gas flow of 5 L/min, nebulizer pressure of 60 psi, and capillary voltage of 2500 volts.

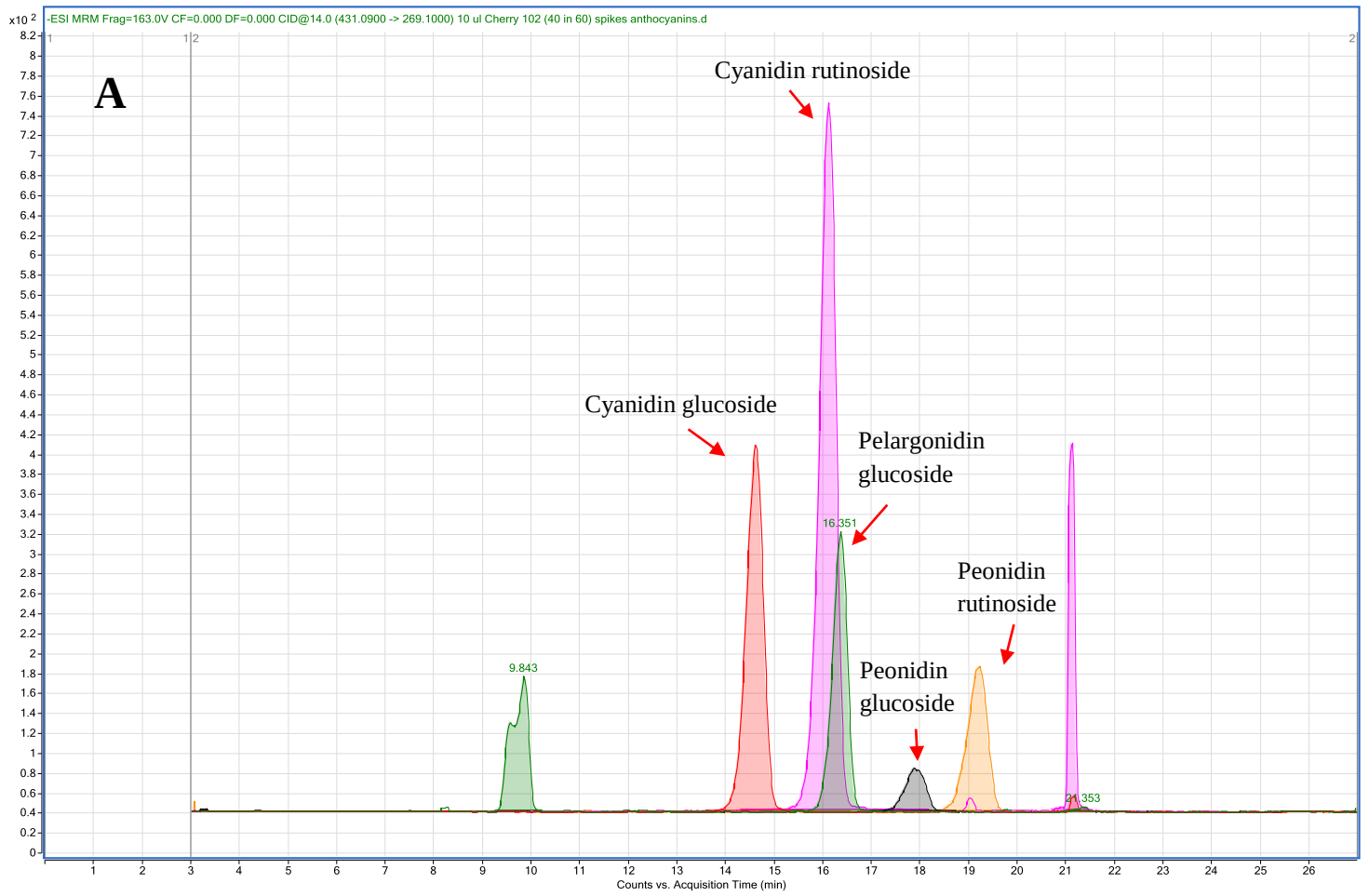
The actual gradient elution used for anthocyanin and polyphenolics methods are presented in Table 16 and 17. The MRMs together with their back pressure and mobile phase gradients are shown in Figure 20 and Figure 21.

Table 16 Gradient elution for anthocyanin compounds

Time, minutes	% B (MeCN, 0.1% formic acid)	% C (MilliQ, 1.0% formic acid)
0.01	3.0	97.0
3.00	3.0	97.0
18.00	12.0	88.0
19.00	80.0	20.0
19.40	80.0	20.0
20.50	3.0	97.0

Table 17 Gradient elution for polyphenolic compounds

Time, minutes	% A (MilliQ, 0.1% formic)	% B (MeCN, 0.1% formic)
0.01	97.0	3.0
3.00	97.0	3.0
4.00	92.0	8.0
13.00	90.0	10.0
13.50	85.0	15.0
18.00	83.0	17.0
23.00	79.0	21.0
28.00	70.0	30.0
29.00	10.0	90.0
31.00	97.0	3.0



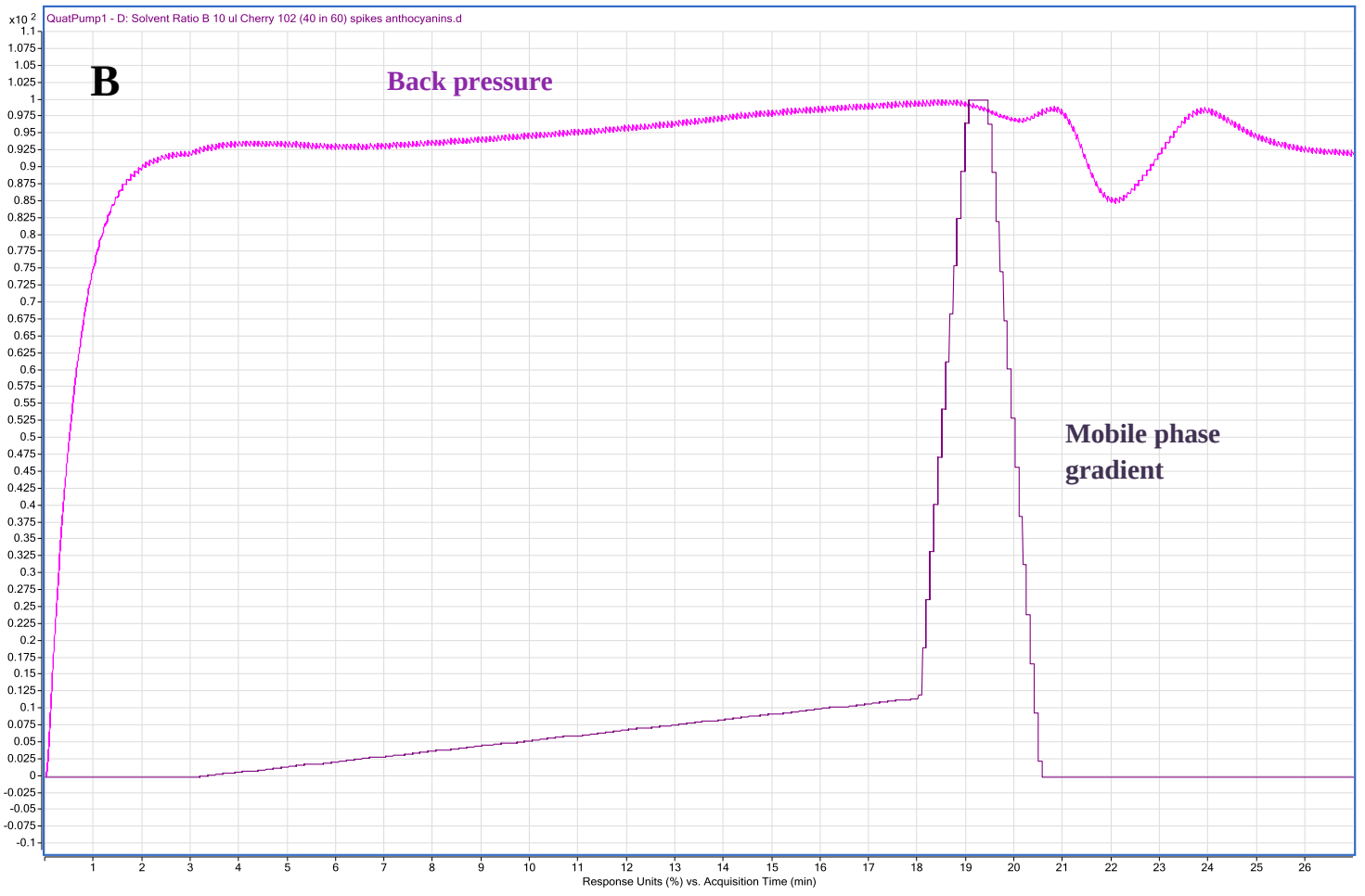


Figure 20 (a): MRMs of the 5 anthocyanins (A), and the back pressure and the mobile phase gradient (B)

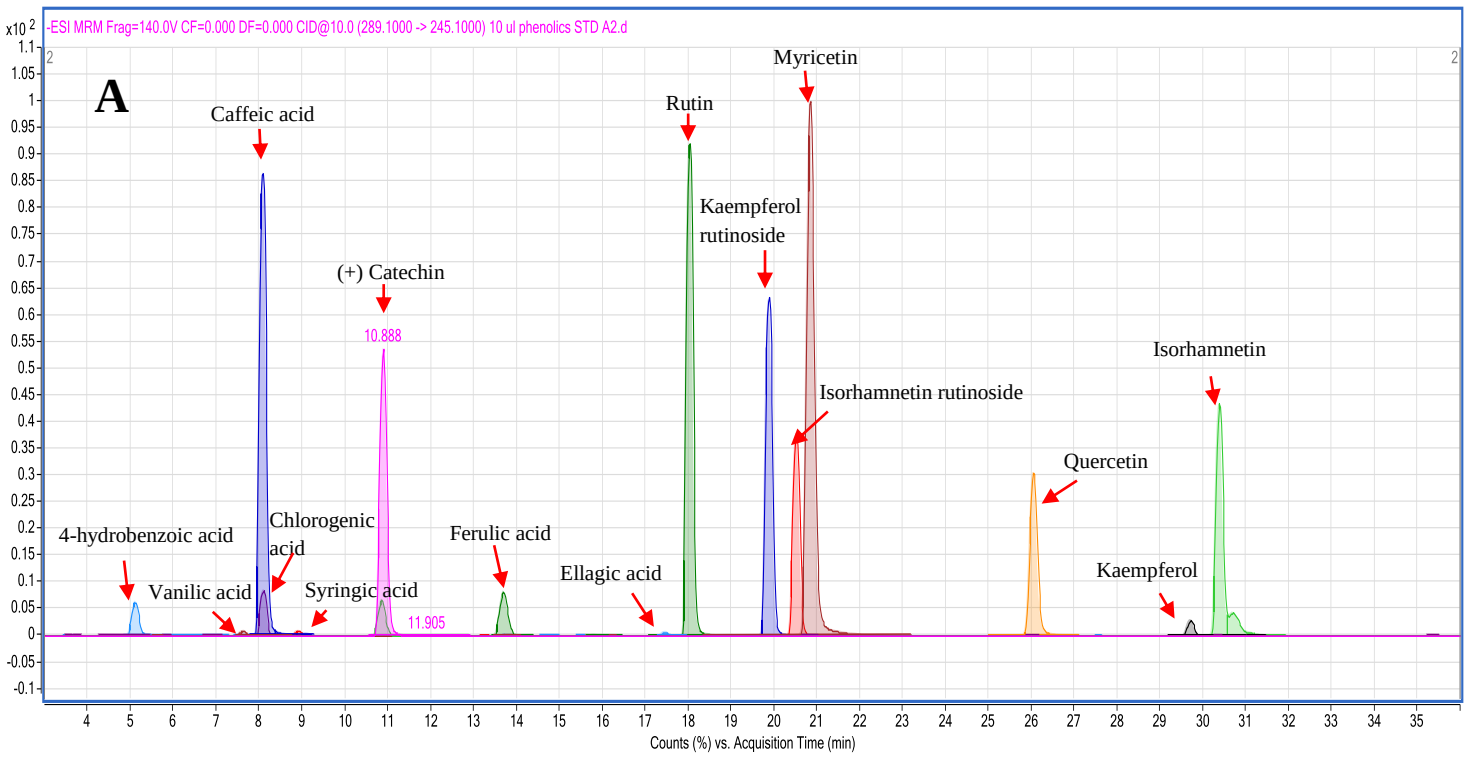


Figure 21 MRMs of the polyphenolics (A), and the back pressure and the mobile phase gradient (B)

3.5.1.2.3 Linearity and sensitivity

The linearity of the standard curves was determined for method validation. Calibration curve standards for both anthocyanins and polyphenolics were constructed (Figure 22). Linearity of the method was determined by using

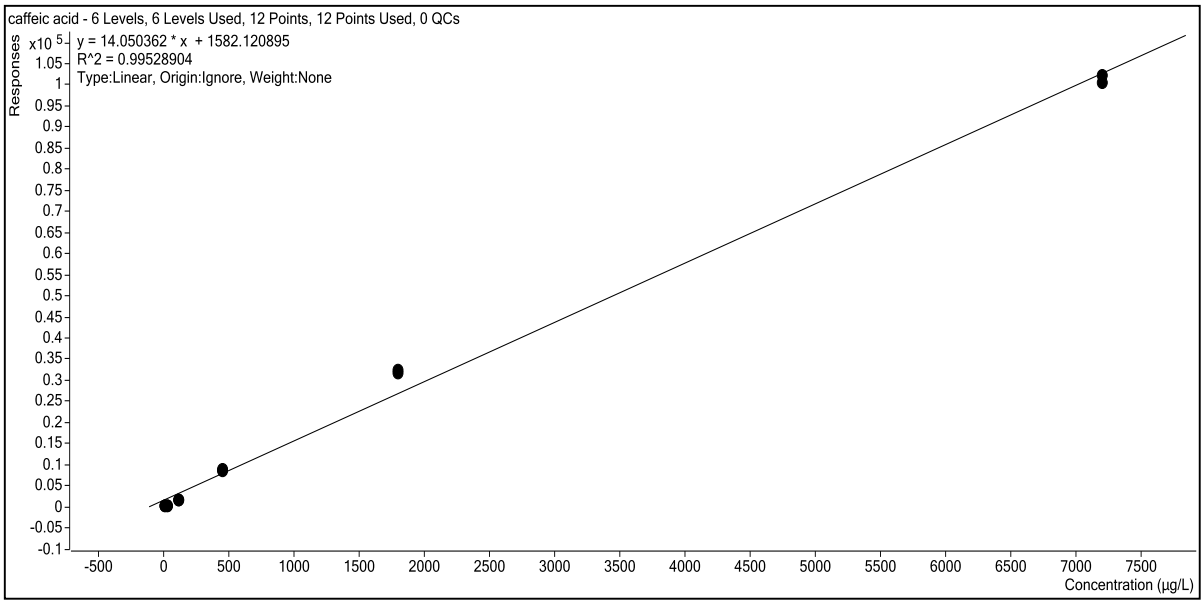


Figure 22 Standard curve of caffeic acid showing linearity

standard plots associated with 6 levels and 12 points for each standard, in which the concentration of the calibration curve standards ranged from 4 to 7200 ug/L. Concentrations of the analytes lower than the lowest range of standard curve were not determined. An example of a standard curve of caffeic acid is shown in Figure 17 and a summary of the standard curves for each compound with their associated regression equations (slopes: m and y-intercepts: b) and correlation coefficients (R^2) are presented in Table 18. Almost all analytes showed good linearity ($R^2 > 0.99$), except for four analytes that had correlation coefficients ranging from 0.9390 to 0.9868.

Table 18 Standard curves of each of the targets with their regression equation values (slopes: m and y-intercepts: b) and correlation coefficients (R^2)

Anthocyanins	**m**	**b**	**R^2**
Cyanidin glucoside	76.1341	64703.756	0.9555
Cyanidin rutinoside	87.4995	33037.541	0.9390
Pelargonidin glucoside	69.5536	658.7409	0.9935
Peonidin glucoside	19.5478	9437.4738	0.9962
Peonidin rutinoside	38.3212	3384.75	0.9985
Polyphenolics			
4-hydroxybenzoic acid	1.9331	237.3332	0.9948
(+) catechin	16.3802	2110.2491	0.9852
Vanillic acid	0.2233	74.2575	0.9917
Syringic acid	0.2439	33.7227	0.9868
Ellagic acid	0.1596	0.8276	0.9942
Chlorogenic acid	2.5694	186.1013	0.9941
Caffeic acid	14.0504	1582.1209	0.9953
P-coumaric acid	1.3138	207.1963	0.9944
Ferulic acid	1.2727	549.9466	0.9926
Rutin	26.725	727.3784	0.9989
Kaempferol rutinoside	19.2193	28.2073	0.9974
Isorhamnetin rutinoside	11.5625	68.6	0.9973
Myricetin	18.3243	639.0136	0.9975
Quercetin	6.5533	126.7604	0.9996
Kaempferol	0.7478	92.5469	0.9955
isorhamnetin	11.0976	639.656	0.9935

3.5.1.2.4 Spike and recovery tests

To further validate and investigate the methods used, spike and recovery tests were carried out. However, this experiment was not successful because of the low recoveries. There were not enough standards to work with for use as spiked material given the fact

that these standards were very expensive. Moreover, the efficiency of the recoveries will need to be determined first for these tests because of the variability from day to day analysis.

3.5.1.2.5 Extraction

Several extraction techniques were tested prior to actual analysis to determine which will give the best results in terms of concentrations of target compounds. These techniques are outlined in the following sections.

3.5.1.2.5.1 Initial extraction methods

Prior to extraction of bioactive compounds, cherry samples were frozen in liquid nitrogen and ground using an analytical grinding mill. Bioactive compounds (anthocyanins and polyphenolics) from frozen powdered cherry samples (200 mg) were extracted by adding 1.4 ml of extraction solvent (methanol acidified with 5% formic acid) in a 4-ml Eppendorf tube. The mixture was vortex-mixed at 2000 rpm for 10 sec and then incubated at 4^{o}C overnight. The next day, the mixture was vortex-mixed again at 2000 rpm for 10 sec and homogenates were centrifuged at 9500 xg for 5 min at room temperature. The supernatant liquid (1 ml) was pipetted into a new Eppendorf tube. This supernatant was then concentrated to evaporate the methanol using Centrivap (Degassex, Phenomenex, model number: DG-4400) at room temperature. The concentrated supernatant was resuspended with 1 ml of 5% acetonitrile (MeCN), and then vortex-mixed (2000 rpm, 10 sec) and centrifuged (9500 xg, 5min). Then, 100 µl of supernatant was pipetted into a new amber vial with inserts ready for LC-MS injections. When using this method, several problems were encountered when the extracts were analysed in the LC-MS. The concentrations of most target compounds decreased and some were lost due to solvent evaporation step. In addition, the evaporation of approximately 1 ml of methanol using Centrivap (Degassex, Phenomenex, model number: DG-4400) at room temperature usually would take up to 1 – 2 hours. This long evaporation time might intensify the temperature within the concentrator. Although conducted at room temperature, the equipment also generates heat that will then affect properties of the analyte.

Due to the difficulties encountered, an alternative method for extraction was developed. This method was adapted from the study of Yilmaz and others (2015). Their method used the optimised extraction conditions of 51% ethanol concentration, 49% water with 0.1% hydrochloric acid, 75 ^{o}C extraction temperature, 7- 8 min extraction time, and 12 ml/g solvent to solid ratio. All extractions were carried out in an amber glass bottles within a shaker incubator. The quantitative data obtained for anthocyanins, however, was not substantially better than the initial extraction method described above.

3.5.1.2.4.2 Extraction using Liquid-Liquid Extraction (LLE) procedures

3.5.1.2.4.2.1 Development of liquid-liquid extraction method

In this study, an entirely new extraction was tested, which would not require removal of the extraction solvent, thereby avoiding issues of target losses during evaporation in the CentriVap. Several studies used the evaporation of extraction solvents during the extraction of phenolics. Different evaporation techniques used included evaporation to dryness using the Buchi rotary vapour under reduced pressure (Perrin & Meyer, 2002; Yu et al., 2005), and evaporation under vacuum (Yu et al., 2005; Krygier, Sosulski, & Hogge, 1982). However, these authors did not report loss of analytes during evaporation. In this study, a solvent extraction method using isopropanol, milliQ and formic acid was developed, followed by liquid-liquid extraction to remove the majority of organic solvent from the aqueous extract. This extract could then be further diluted for direct injection into the LC-MS without further clean-up using this new solvent combination. Earlier extraction procedures employed organic solvents (Nawaz, Shi, Mittal, & Kakuda, 2006). According to Dai and Mumper (2010), solvents such as methanol, ethanol, acetone, ethyl acetate, and their combinations with different water proportions are often used for phenolics extraction from plant materials. Hexane (Santos-Buelga et al., 1995), ethanol-benzene combinations (Kofujita et al., 2009), and sulphur dioxide (Cacace and Mazza, 2002) have also been used as extraction solvents.

Following solvent extraction using isopropanol, milliQ water and formic acid, a second, non-water-miscible solvent was added to draw the extraction solvent out of the aqueous phase. Methanol, ethanol, and isopropyl alcohol (IPA) were further evaluated to maximize anthocyanin extraction together with milliQ water and formic acid. Quantitative data showed that IPA yielded better concentrations and recovery than other

alcohols. Sample cherry extracts using the ethanol-formic acid, methanol-formic acid, and IPA-formic acid are presented in Figure 23. Based on this figure, it is obvious that coloured anthocyanins were better extracted using the IPA solvent. For the non-coloured polyphenolics, higher concentrations were extracted as well using IPA compared to ethanol and methanol.

Chloroform, toluene, and dichloromethane were further tested for their ability to drive phase-separation of the aqueous and organic components and to draw the extract solvent out of the aqueous phase. Phenolics in the toluene extracts were greater in concentration compared to the others. Hence, toluene was selected for use in the final extraction method.

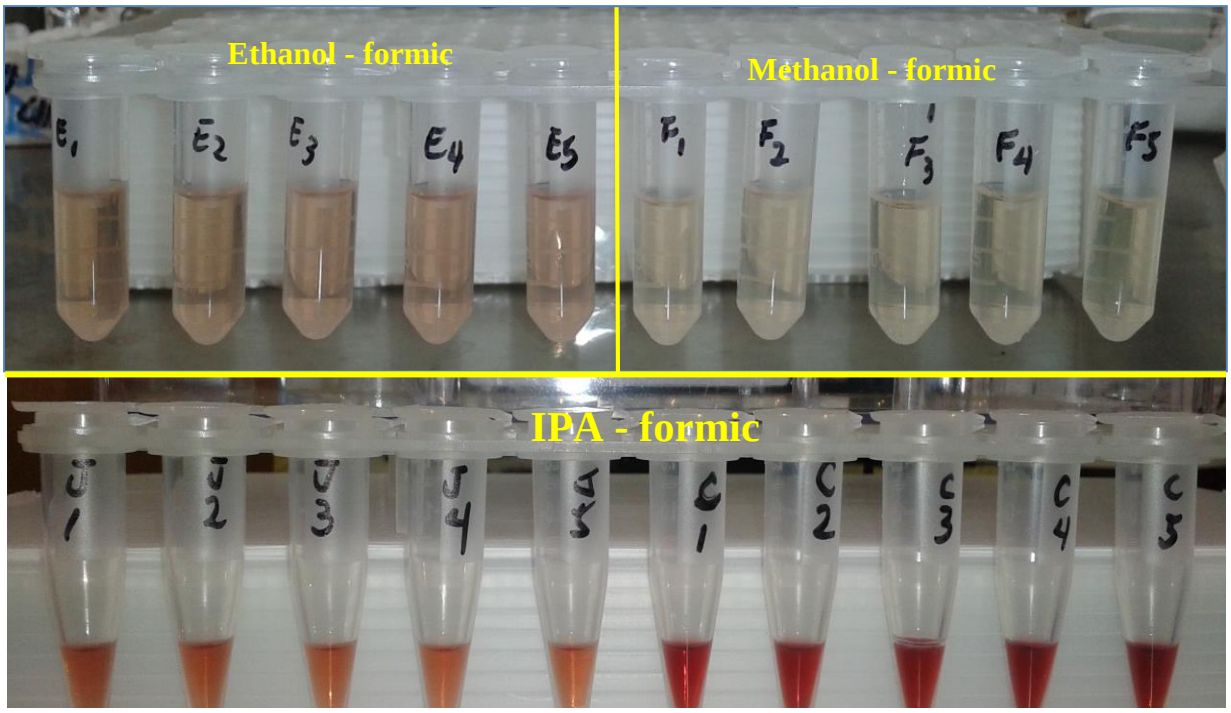


Figure 23 Cherry extracts obtained from three different solvents including ethanol, methanol, and IPA

3.5.1.2.4.2.2 Liquid – liquid extraction method for anthocyanin and polyphenolics analyses

Based on results obtained from method development of liquid-liquid extraction (LLE), the final mixture of extraction solvents used in this study included formic acid, milliQ water, IPA, and toluene. The extraction of anthocyanin and polyphenolic compounds from cherry samples was carried out using LLE also known as ‘solvent extraction’ or solvent partitioning. Samples were prepared by extracting anthocyanins and

polyphenolics from the frozen powdered cherry samples (0.2 g) in a 2-ml screw thread amber bottle (Thermo Fisher Scientific, Auckland, New Zealand) using extraction solvents: 50 ul LC/MS grade 99.5 % formic acid (Fisher Scientific, Ma 02451, USA), 200 ul of LC/MS grade isopropanol (IPA), and 100 ul of milli Q. Afterwards, the mixture was vortex mixed (Corning, MA, USA) at 2000 rpm for 10 seconds, and sonicated at 50 ^{o}C for 20 minutes using an Quantrex 90H Ultrasonic cleaner (L&R, New Jersey, USA). After sonication, 1 ml of LC/MS grade toluene was added for liquid separation. The mixture was vortex mixed again at 2000 rpm for 10 seconds, followed by centrifugation of homogenates using the Eppendorf centrifuge (Thermo Scientific, Hamburg, Germany; model: 5810-R) for 10 minutes at 9500 xg. Extracts (Figure 24) were carefully pipetted out (approximately 60-80 ul) and transferred to a new Eppendorf tube. Then, centrifuge homogenates again for 5 minutes at 9500 xg using the Hermle centrifuge (Hermle Labortechnik GmbH, Wehingen, Germany). The lower aqueous phase (40 ul) was collected and 60 ul of ultrapure (Milli-Q) was added in a 0.1 ml glass insert (Thermo scientific, Auckland, New Zealand) in a 1.5 ml amber bottle (Interlab Limited, Wellington, New Zealand) for LC-MS injections.

Using the developed extraction method however revealed that the internal standards were not recovered. As there was time limitation to examine new internal standards, the analysis of the samples was carried out using replicated standard calibration curves for quantitative determination of results.

Figure 24 Liquid-liquid extraction showing the extracts in between layers

3.5 Flavour Analysis

3.5.1 GC-MS coupled with automated SPME analysis of flavour volatiles

3.5.1.1 Extraction of volatiles using Headspace Solid-Phase Microextraction (HS-SPME)

The volatile extraction in cherry samples using HS-SPME was carried out according to the modified method of Sun and others (2010). Ground frozen cherry samples were thawed out at a room temperature, weighed 3.0 ± 0.1 g, and placed in a 20 mL flat bottom headspace vials fitted with a magnetic, PTFE/Silicone red septum and magnetic crimp cap (GERSTEL, Linthicum, MD, USA). Sample in the headspace vial was spiked with 10 μL of thiophene in methanol (0.01 ppm), which was used as an internal standard. To ensure proper distribution of internal standard in the sample, the vial with the sample was mixed using a vortex mixer (Corning, MA, USA).

For sample preparation, the headspace vial was heated at 35 °C for 5 min using an incubator equipped with an agitator, which was set at a speed of 250 rpm for better extraction. The volatile components in the sample were absorbed onto a gray, notched, 50/30 μm layer of divinylbenzene-carboxen-polydimethylsiloxane (Supelco Co., Bellefonte, PA, USA) fibre on a Stable Flex fiber 23 gauge (OD = 0.63 mm) that was exposed to the sample headspace for 30 min and desorbed for 120 sec. at 200 °C desorption temperature.

3.5.1.2 *GC-MS Analysis*

The Trace GC Ultra (Thermo Scientific, Waltham, MA, USA) GC-MS equipment was used in this study. It was equipped with a DSQ single mass spectrometer, which uses electron impact ionization as ionization source (Thermo Scientific). The GC-MS was installed with a VF-5 ms low bleed/MS fused-silica capillary column (5%-phenyl-95%-dimethylpolysiloxane phase, 30 m × 0.32 mm × 0.50 μm) (Agilent Technologies, Santa Clara, CA, USA). Helium was the carrier gas with a constant flow rate of 1.5 mL/min in the GC-MS. The mode of injection was splitless, and inlet temperature for the injection port was set to 200 °C with 50 mL/min split flow and 2 min splitless time. Chromatographic conditions were as follows: the oven was held for 3 min at 35 °C, then

raised to 170 °C at a rate of 5 °C/min and held for 2.0 min, then finally heated up to 250 °C and held for 3 min at this temperature. The mass spectrometer was operated in the electron impact mode (EI) with a source temperature of 200 °C, an ionising voltage of 70 eV, and transfer line temperature of 250 °C. The mass spectrometer scanned masses from 50 to 650 *m/z* at a rate of 0.8170 scan/s with a total scan time of 1.22 s.

3.5.1.3 Multi-Purpose Sampler (MPS)

An automated sample preparation and sample introduction was employed using the Gerstel MultiPurpose Sampler (MPS). This autosampler uses the Gerstel MAESTRO software, which controlled and set the method and sequence of the analysis. The "Prep Ahead" function was used in the analysis wherein the sample preparation steps were performed during the analysis of the preceding sample.

3.5.2 Identification of Volatile Compounds

Peak identification of unknown compounds 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, USA Version 2.0a, 2002, USA), or NIST web book (Aguilo-Aguayo et al., 2009a) .To confirm the identity of each volatile compound, retention indices (RIs) were calculated for each volatile compound using the retention times of a homologous series of C_7 to C_{30} *n*-alkanes (1,000 µg/mL in hexane from Supelco), and comparing the RI with compounds analysed under similar conditions in previous literature. The approximate quantities of the volatiles were estimated by comparison of their relative peak areas with that of the thiophene internal standard using a response factor of 1.

3.6 Microbiological Analysis

Microbiological analysis of control and PEF-treated cherries were conducted to determine if the use of low intensity PEF treatment could induce the growth of beneficial microorganisms like probiotic microorganisms (MO). Lactic acid bacteria (LAB) was used as health-related MO and was inoculated to the cherry samples.

3.6.1 Lactic acid bacteria

Lactic acid bacteria (LAB) bacteria are Gram-positive bacilli and cocci that are accountable for the metabolization of carbohydrates through fermentation process, which then yield lactic acid as the end product of this reaction (Salminen, Deighton, Gorbach, & Wright, 1993; Axelsson, 2004). For centuries, LAB has been used mainly for food preservation and later on in other areas of the food industry. LAB is typically used for production of probiotic foods.

Lactobacillus acidophilus ATCC 1643 was obtained from Fonterra Research Centre (Palmerston North, New Zealand).

3.6.2 Media, chemicals, and other reagents

Lactobacilli Difco MRS (de Man, Rogosa and Sharp) agar and Lactobacilli Difco MRs broth (Difco Laboratories Inc, Detroit MI) were purchased from Fort Richards Ltd, Auckland. 1M sodium hydroxide (NaOH) were obtained from Fort Richards Ltd, Auckland.

3.6.3 Preparation of culture media

MRS agar or MRS broth was prepared according to the manufacturer's instructions. The media were sterilized by autoclaving 121^{o} C for 15 minutes.

3.6.4 Preliminary growth experiment

This experiment was done to determine if *L. acidophilus* would grow in non-nutrient supplemented cherry puree with and without pH adjustment to pH 6.0. The pH was measured as described in Section 3.3.4 using a bench top pH meter (AOAC 251.58, AOAC International 2002). A 5 g of frozen powdered cherry prepared from freezing in liquid nitrogen and ground using an analytical grinding mill was inoculated with a colony of LAB without pH adjustment. The pH of one other cherry puree (5 g) was adjusted to pH 6.0 using 1.0 M of NaOH. The inoculated cherry purees were incubated in a CO_2 incubator at 37 ^{0}C for 72 hours. The LAB concentrations at 0 hr and 48 hrs

were determined using viable plate count in MRS agar. The plate count method was carried out based on The Compendium of Methods for Microbiology of Foods (CMMF) (Downes, Itō, & American Public Health, 2001) and AOAC.

The viable numbers of *L. acidophilus* were expressed as log CFU/ml and calculated using the following formula:

$$\text{Viable number} = \text{Log}\frac{\text{Number of CFU}}{\text{Volume plated (ml)x total dilution used}} \quad (7)$$

3.6.5 Growth of LAB in PEF- treated cherries

Colonies of *L. acidophilus* (Figure 25) were used to inoculate MRS broth (100 ml). The MRS broth culture was incubated anaerobically in a CO_2 incubator (Sanyo CO_2 incubator MCO-18AIC, Sanyo Electric Biomedical Co., Ltd., Osaka, Japan) (Figure 26) at 37^0C for 48 hours to obtain a cell concentration at OD_{650nm} = 0.75. One ml of this culture was used to inoculate each PEF treated cherry sample (5 grams, pH 6.0) contained in 100 ml plastic jars. The inoculated cherries were incubated anaerobically for 48 hours (Figure 27).

Figure 25 Lactobacillus acidophilus inoculated in MRS broth

Figure 26 CO2 Incubator

The 0 hr and 48 hour bacterial concentrations were determined by viable count using ten-fold serial dilutions and pour plating (Figure 28) in MRS agar. Colonies were counted after the plates were incubated in a CO2 incubator at 37^0C for 72 hours. All samples were plated in triplicates.

Figure 27 Plates with the samples in CO2 incubator (0 hour)

Figure 28 Pour plating

3.7 Statistical Analyses

All data were collated using Microsoft Office Excel 2007 and subjected to statistical analysis using the XLSTAT MX software release version 2012 (Addinsoft, USA).

3.*7.1 Physicochemical analysis and microbiological analysis*

Mean values from three independent experiments were reported with standard deviations (mean ± standard deviation). The significant differences observed among treatments were evaluated using one way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) comparison test for multiple comparisons. Significance was defined at 5% level with 95% confidence level.

3.7.2 Flavour analysis and bioactive compound analysis

One-way analysis of variance (ANOVA) was carried out on flavour volatile compounds and bioactive compounds (anthocyanins and polyphenolics) for each sample followed by Fisher's (LSD) multiple comparison tests to assess difference between sample means.

Chapter 4. Results and Discussions

4.1 Effect of PEF on the physicochemical properties of cherries

The physical properties of cherries included the size range and average fruit weight. In addition, the effect of different PEF processing parameters on the physicochemical properties of sweet cherry samples (juice yield or ratio of fruit to juice, conductivity, pH, colour, titratable acidity, total soluble solids, and moisture) are reported and discussed.

4.1.1 Size Range and average fruit weight

The size range, average fruit weight, and flesh to seed ratio of the Stella cherries used in this study are shown in Table 19. The size and weight of fruit are two of the most useful parameters to determine visual liking of sweet cherries (Romano, Cittadini, Pugh, & Schouten, 2006; Crisosto et al., 2003). The measured fruit weight in this study was slightly lower than that reported by Girard and Kopp (1998) for Stella cultivar, which was reported to be 13.7 g. In most sweet cherry cultivars, the fruit weight is influenced by crop load and stage of maturity (Gonçalves et al., 2006; Usenik et al., 2008; Drake & Elfving, 2002; Serrano et al., 2009; Usenik et al., 2010). Fruit volume is another important parameter as it plays a role in product quality evaluation following numerous technological processing (Vursavus et al., 2006). The fruit volume of cherry samples in our study was similar to that reported by Vursavuş, Kelebek, & Selli (2006) in sweet cherries such as Van, Noir De Guben and 0-900 Ziraat cultivars (4.53 to 6.26).

Table 19 Size range, fruit weight and volume, and flesh-to-seed ratio of Stella cherries (Mean ± Standard Deviation)

Parameters	Range	Mean ± S.D.
Total fruit weight (g)	8.37 - 10.49	10.10 ± 1.57
Length (mm)	20.89 - 24.82	23.44 ± 2.21
Width (mm)	22.83 - 26.65	24.86 ± 1.92
Diameter (mm)	21.52 - 25.96	24.16 ± 2.33
Fruit volume (cm^3)	5.22 - 9.16	7.52 ± 2.05
Flesh/seed ratio	7.37 - 10.43	9.10 ± 1.57

4.1.2 Ratio of fruit to juice and juice yield

The ratio of fruit to juice and juice yield before and after PEF processing were recorded and are presented in Table 20. The ratio of fruit to juice ranged from 1.32 to 2.42. There was a significant increase in juice yield for samples incubated for 24 hours after PEF and samples immediately after PEF compared to the control sample as seen in Figure 20. The increase in juice yield using moderate intensity PEF (0.3 to 2.5 kV/cm electric field intensity) in cherries in our study is in agreement with other researches (Schilling et al., 2007; Grimi et al., 2009; Jaeger et al., 2012). Schilling and others (2007) reported an apple juice yield increase from 1.7 to 7.7 % compared to the control samples in apple with increasing field intensities (1 to 3 kV/cm). Jaeger and others (2012) reported an increase of juice yield of 0 to 11 % and 8 to 31 % for apple mash and carrot mash respectively after PEF treatments using 3 kV/cm electric field intensity. In addition, grape juice yield increases of between 67% to 75% were reported in the study of Grimi and others (2009) after the application of 0.4 kV/cm electric field intensity. These increase in juice yields are due to the enhancing mass transfer rates by electroporation of plant cell membranes by PEF (Angersbach, Heinz, & Knorr, 2000; Grimi et al., 2009).

Table 20 Juice yield (%) and ratio of cherry fruit to juice

Sample codes	PEF Intensity kV/cm	Juice yield %	Ratio of fruit to juice
Control	0	0	2.26 ± 0.17 [a]
S2P1	0.30	0.74 ± 1.48 [b]	1.48 ± 0.06 [bcde]
S2P2	0.70	0.83 ± 1.35 [b]	1.50 ± 0.07 [bcde]
S2P3	1.00	0	1.53 ± 0.02 [bcde]
S2P4	1.40	0	1.59 ± 0.04 [bcd]
S2P5	1.70	0	1.53 ± 0.08 [bcde]
S2P6	2.10	0	1.68 ± 0.04 [b]
S2P7	2.50	0	1.63 ± 0.09 [bc]
S3P0	0	3.85 ± 0.69 [a]	1.37 ± 0.07 [de]
S3P1	0.30	1.76 ± 0.53 [b]	1.46 ± 0.08 [cde]
S3P2	0.70	1.47 ± 0.68 [b]	1.37 ± 0.07 [e]
S3P3	1.00	2.94 ± 0.97 [ab]	1.45 ± 0.07 [cde]
S3P4	1.40	2.13 ± 0.60 [ab]	1.38 ± 0.05 [de]
S3P5	1.70	1.66 ± 0.78 [b]	1.44 ± 0.03 [cde]
S3P6	2.10	0.96 ± 0.96 [b]	1.45 ± 0.05 [cde]
S3P7	2.50	1.67 ± 0.58 [b]	1.43 ± 0.04 [cde]
F value		8.224	26.71
Pr > F		0.0001	0.0001

Mean ± S.D: Standard deviation based on three independent samples and treatments.
S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. Means within the same column not bearing common superscripts differ ($p < 0.05$; one-way ANOVA with Fisher's LSD). Nd: not determined.

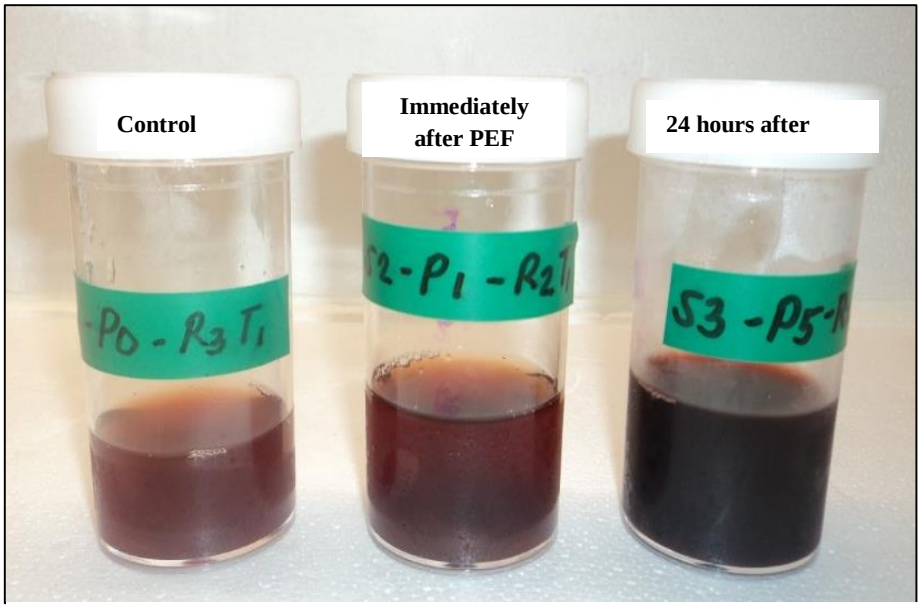


Figure 29 Juice extracts of the control, samples immediately after PEF, and samples 24 hours after PEF

4.1.3 Conductivity

Another main parameter for PEF processing is the conductivity of the solution, which is also a function of temperature (Reitler 1990; Toefpl, 2006). Conductivity is the inverse of resistivity and the unit for this is Siemens per unit length (S/m) (Toefpl, 2006). Electric conductivity, which is characteristic of a solution influences PEF treatment in terms of electric field strengths output (Vega-Mercado et al., 1996; Aronsson and Rönner, 2001). As temperature increases, the conductivity of the solutions will also change. This is due to the decrease of resistivity with increase of temperature (Dunn, 2001). One importance of conductivity is it influences the actual measurement of the electric field strengths (Toefl, 2006). Thus, to achieve a consistent output of electric field strengths, the temperature and conductivity of a product must be monitored. As seen in Table 21, there were no significant changes in temperature. Hence there was little change in conductivity of the samples in this study. The only significant change was observed for PEF sample after 24 hours storage (S3P1), which was higher in conductance compared to all samples. This was unusual because the change in temperature of this sample was not significant.

Table 21 Conductivity values of control and PEF-treated cherries

		Conductivity, uS/cm					
Samples	Electric Field Strength (kV/cm)	Before PEF	Immediately after PEF	24 hours after PEF	After 24 hours incubation (w/o PEF)	Change in Temperature [A] ΔT (°C)	Change in Conductivity of Chunks in Solution
Control	-	2.71 ± 0.30	ND	ND	ND	ND	ND
S2P1	0.30 ± 0.06	3.08 ± 0.31	4.59 ± 0.72 [abc]	ND	ND	1.90 ± 0.82 [a]	1.51 ± 1.03 [ab]
S2P2	0.70 ± 0.00	3.13 ± 0.24	4.73 ± 0.29 [ab]	ND	ND	4.10 ± 1.04 [a]	1.60 ± 0.51 [ab]
S2P3	1.00 ± 0.06	5.31 ± 2.95	4.85 ± 0.41 [ab]	ND	ND	0.73 ± 0.42 [a]	0.46 ± 3.30 [ab]
S2P4	1.40 ± 0.00	3.67 ± 0.63	4.35 ± 0.84 [abc]	ND	ND	0.47 ± 0.87 [a]	0.68 ± 1.27 [b]
S2P5	1.70 ± 0.06	4.06 ± 0.66	4.84 ± 0.24 [ab]	ND	ND	0.60 ± 1.70 [a]	0.77 ± 0.74 [b]
S2P6	2.10 ± 0.00	3.41 ± 0.59	3.53 ± 0.98 [abc]	ND	ND	0.10 ± 0.52 [a]	0.12 ± 0.46 [b]
S2P7	2.50 ± 0.06	3.22 ± 0.83	4.24 ± 0.95 [abc]	ND	ND	0.33 ± 1.92 [a]	1.02 ± 1.23 [b]
S3P0	-	2.97 ± 0.17	ND	ND	ND	ND	ND
S3P1	0.30 ± 0.06	2.98 ± 0.41	5.39 ± 0.46 [a]	6.57 ± 0.73 [a]	ND	1.30 ± 1.25 [a]	3.59 ± 0.53 [a]
S3P2	0.70 ± 0.00	3.55 ± 0.73	4.36 ± 0.31 [abc]	6.12 ± 0.38 [a]	ND	0.87 ± 1.46 [a]	0.81 ± 0.43 [b]
S3P3	1.00 ± 0.06	2.53 ± 0.28	2.79 ± 1.39 [c]	6.30 ± 0.23 [a]	ND	0.40 ± 4.35 [a]	0.26 ± 1.55 [b]
S3P4	1.40 ± 0.06	2.71 ± 0.27	3.45 ± 0.39 [bc]	6.18 ± 0.43 [a]	ND	2.10 ± 2.81 [a]	1.63 ± 0.60 [b]
S3P5	1.70 ± 0.06	2.87 ± 0.39	4.72 ± 0.36 [ab]	6.10 ± 0.44 [a]	ND	0.17 ± 1.10 [a]	1.85 ± 0.49 [ab]
S3P6	2.10 ± 0.00	2.94 ± 0.56	4.87 ± 0.36 [ab]	6.27 ± 0.24 [a]	ND	1.23 ± 0.68 [a]	1.93 ± 0.24 [ab]
S3P7	2.50 ± 0.06	2.72 ± 0.73	3.48 ± 0.49 [bc]	6.40 ± 0.41 [a]	ND	1.47 ± 2.90 [a]	0.76 ± 0.24 [b]
F value		1.768	19.929	370.875			
Pr > F		0.086	< 0.0001	< 0.0001			

Mean ± S.D: Standard deviation based on three independent samples and treatments. S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. [A] Changes in temperature due to PEF treatment. The initial temperature of cherry chunks in solution sample prior subjected to PEF treatment averaged at 20.4 ± 0.00 °C. Means within the same column not bearing common superscripts differ ($p < 0.05$; one-way ANOVA with Fisher's (LSD) multiple comparisons tests. Nd: not determined.

4.1.4 pH, titratable acidity, total soluble solids, colour and moisture content

The physicochemical characteristics measured in this study for all cherry samples (control and PEF-treated) included pH, colour, titratable acidity, total soluble solids, and moisture content. Cherry samples subjected for physicochemical analysis were cherry chunks separated from the juice after processing, frozen in liquid nitrogen and ground using analytical grinding mill. The results are presented in Table 22.

4.1.4.1 pH values

The pH values of samples (Table 22) ranged from 3.92 to 4.09. These values corresponded to the values obtained and reported in cherries in other literature (Girard & Kopp, 1998; Ballistreri et al., 2013). Based on the results, control sample (untreated) was significantly lower than all samples incubated for 24 hours after PEF except for S3P7. There were no significant differences in pH between samples immediately after PEF and control. This is in agreement with the study of Schilling and others (2007) who reported that the pH of apple juice after the application of 1 to 5 kV/cm electric field strengths did not significantly differ with the untreated control. In addition, Geulen and others (1994) reported no significant difference in the pH values of carrot juice samples after PEF application of 0.6 to 2.6 kV/cm electric field intensities.

4.1.4.2 Titratable acidity (TA)

Titratable acidity is the measure of malic acid (predominant acid) in cherries. The values of % malic acid in the samples ranged from 0.37 to 0.61. These values are within the range reported in cherries in other literature (Usenik et al., 2008).

Table 22 Physicochemical attributes of Stella Cherry Cultivar processed using PEF equipment (Mean ± Standard Deviation)

Codes	PEF Intensity	Energy Input	Storage Time	pH	Colour			Titratable Acidity	Total Soluble solids	Moisture content
	kV/cm	kJ/kg	hours		**L**	**a***	**b***	% Malic acid	^{o}Brix	%
Control	0	0	0	3.92 ± 0.02 ^{d}	18.57 ± 1.71	16.19 ± 1.57	6.92 ± 2.16	0.50 ± 0.05 ^{abcde}	14.61 ± 0.77 ^{abcde}	84.13 ± 1.58 ^{bcde}
S2P1	0.3	40	0.5	4.04 ± 0.05 ^{abcd}	18.88 ± 2.10	14.52 ± 1.37	6.50 ± 2.09	0.55 ± 0.04 ^{abc}	14.70 ± 0.30 ^{abcde}	83.18 ± 0.50 ^{cde}
S2P2	0.7	55	0.5	3.95 ± 0.03 ^{cd}	17.30 ± 2.58	15.28 ± 1.71	6.24 ± 1.33	0.53 ± 0.02 ^{abcd}	15.84 ± 0.31 ^{abcd}	84.72 ± 0.53 ^{abcde}
S2P3	1	48	0.5	4.03 ± 0.05 ^{bcd}	17.61 ± 0.61	14.74 ± 1.05	5.61 ± 1.09	0.60 ± 0.06 ^{a}	17.10 ± 0.85 ^{abc}	83.67 ± 1.42 ^{de}
S2P4	1.4	35	0.5	4.00 ± 0.04 ^{abcd}	17.82 ± 0.73	13.72 ± 1.22	5.72 ± 1.14	0.60 ± 0.04 ^{a}	19.08 ± 0.51 ^{a}	81.60 ± 1.22 ^{e}
S2P5	1.7	43	0.5	4.06 ± 0.00 ^{abcd}	18.35 ± 1.06	13.41 ± 1.04	5.76 ± 1.23	0.61 ± 0.05 ^{a}	17.10 ± 0.72 ^{abc}	82.23 ± 0.32 ^{e}
S2P6	2.1	42	0.5	4.01 ± 0.02 ^{abcd}	18.84 ± 1.46	14.66 ± 1.46	6.46 ± 1.12	0.56 ± 0.06 ^{ab}	19.27 ± 0.52 ^{a}	84.22 ± 2.69 ^{bcde}
S2P7	2.5	45	0.5	4.01 ± 0.04 ^{abcd}	18.97 ± 1.10	13.47 ± 0.51	5.98± 0.42	0.61 ± 0.03 ^{a}	17.56 ± 0.43 ^{ab}	82.40 ± 1.62 ^{e}
S3P0	0	0	24	4.02 ± 0.03 ^{abc}	19.68 ± 0.50	14.36 ± 0.91	6.87± 0.94	0.44 ± 0.07 ^{bcde}	11.62 ± 1.40 ^{de}	85.94 ± 0.67 ^{abcd}
S3P1	0.3	30	24	4.01 ± 0.03 ^{abcd}	20.47 ± 1.52	14.72 ± 1.52	7.25 ± 1.07	0.41 ± 0.06 ^{cde}	13.61 ± 0.96 ^{bdce}	86.74 ± 1.26 ^{abcd}
S3P2	0.7	34	24	4.09 ± 0.04 ^{a}	20.13 ± 2.36	14.29 ± 0.48	7.15 ± 1.66	0.37 ± 0.03 ^{e}	9.46 ± 0.34 ^{e}	87.90 ± 0.27 ^{a}
S3P3	1	34	24	3.96 ± 0.01 ^{abc}	19.55 ± 2.14	14.63 ± 1.05	7.24 ± 1.08	0.44 ± 0.00 ^{bcde}	11.90 ± 0.39 ^{cde}	86.72 ± 0.08 ^{abcd}
S3P4	1.4	31	24	4.04 ± 0.02 ^{abc}	20.28 ± 2.27	13.92 ± 0.14	7.31 ± 1.52	0.39 ± 0.04 ^{de}	11.74 ± 1.15 ^{de}	86.92 ±1.36 ^{abcd}
S3P5	1.7	43	24	3.99 ± 0.01 ^{ab}	19.89 ± 1.78	13.80 ± 1.00	6.86 ± 1.44	0.39 ± 0.05 ^{de}	11.62 ± 0.48 ^{de}	87.34 ± 0.37 ^{abc}
S3P6	2.1	48	24	4.05 ± 0.05 ^{abc}	19.94 ± 1.09	14.23 ± 0.65	6.66 ± 0.72	0.40 ± 0.04 ^{cde}	9.97 ± 1.01 ^{e}	87.93 ± 0.67 ^{a}
S3P7	2.5	45	24	4.01 ± 0.05 ^{abcd}	21.65 ± 0.68	14.46 ± 0.70	8.12 ± 1.49	0.44 ± 0.09 ^{bcde}	11.30 ± 0.43 ^{de}	87.54 ± 0.82 ^{ab}
F value				4.903	1.554	1.182	0.78	9.876	9.942	10.181
Pr > F				< 0.0001	0.144	0.333	0.689	< 0.0001	< 0.0001	< 0.0001

Mean ± S.D: Standard deviation based on three independent samples and treatments. S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. Means within the same column not bearing common superscripts differ ($p < 0.05$; one-way ANOVA with Fisher's LSD). Nd: not determined.

Significant differences were only found between titratable acidity of PEF-treated samples. Samples immediately after PEF (S2 samples) such as S2P3, S2P4, S2P5, and S2P7 were significantly higher than 24 hours after PEF samples (S3 samples). This means PEF treatments resulted in production of more organic acids (malic acid) in cherries immediately after PEF. The mechanism of PEF on the release of certain organic compounds is attributed to the so-called 'electroporation' or 'electropermeabilization' (Weaver and Chizmadzhev, 1996; Pakhomov et al., 2010). This occurs with application of electric fields (typically below 10 kV/cm) that induce electroporation of cell membranes, thereby improving the diffusion of solutes (Puertolas et al., 2013). The lower TA values in S3 samples may be due to the release of some components that may be neutral or alkali that might neutralize the acid present in cherries. Girard & Kopp (1998) reported that pH and TA were related and associated with acidity. As seen in Table 22, S3P2 sample that had the highest pH value had the lowest TA value.

4.1.4.3 Total soluble solids (TSS)

The total soluble solids or ^{o}Brix is the measure of the amount of sugars in a given sample that can dictate sweetness perception (Guyer et al., 1993). In cherries, these values would account for the amount of fructose (predominant sugar), followed by glucose (Martinez-Romero, 2006). Based on Table 22, the TSS values of cherry samples ranged from 9.46 (S3P2) to 19.27 (S2P6). Samples immediately after PEF (S2 samples) such as S2P4, S2P6, and S2P7 were found to be significantly higher in TSS values with increasing electric field intensities of 1.4, 2.1, and 2.5 kV/cm than most PEF – treated samples incubated for 24 hours (except S3P1 which had the lowest electric field intensity). Hence PEF was able to release more sugars for samples immediately after PEF (S3). The application of PEF has been demonstrated to be effective in extracting intracellular compounds that involves the electroporation or 'electropermeabilisation of plant cell membrane. The cell membrane of fruits or any plants act as a physical barrier holding the intracellular substances such as solutes, water, and juices (Lamanauskas et al., 2015). To promote and accelerate mass transport processes, the use of PEF can be employed (Lamanauskas et al., 2015). The permeabilization effect of PEF induces a reversible pore formation or the local rupture of the cell membrane in the tissues of foods, which permits the free flow of intracellular and valuable compounds including sugars, bioactive compounds, and juices (Knorr, 1999; Barsotti et al., 1999, Pataro et al., 2011). This mechanism depends mainly on the

electric field strengths applied. For reversible pore formation however, mild or moderate intensity must be applied (Lamanauskas et al., 2015). Several studies have demonstrated the use of mild PEF intensities in the effective extraction of sugars. The application of mild intensity PEF on apple juices improved and increased the juice quality in terms of Brix value (Bazhal and Vorobiev, 2000; Praporscic et al., 2007). In addition, the optimised electric field strengths of 0.6 and 0.26 kV/cm were able to extract sugar from sugar beet effectively (Vorobiev, & Lebovka, 2012).

4.1.4.4 Colour measurements

The colour measurements of the control and PEF-treated samples are reported in Table 22. The results of this study showed that L*, a*, and b* values ranged from 17.30 to 21.65, 13.41 to 16.19, and 5.61 to 7.25 respectively. However, no significant differences in L*, a*, and b* values were found between control and PEF-treated samples.

4.1.8 Moisture Analysis

Based on Table 22, samples incubated for 24 hours after PEF (S3) were significantly higher in moisture content than control (S1) and samples immediately after PEF (S2) at electric field intensities of 1.4 kV/cm electric field and above. This is again due to the electropermeabilization effect of PEF, that aside from the release of valuable compounds, water was also released out of the cell membrane of the fruit (Lamanauskas et al., 2015). This results could also be related to the amount of total soluble solids in cherries. A higher moisture content results in lower total soluble solids. Correspondingly, the amount of TSS in S2 samples were significantly higher compared to the control and S3 samples.

4.2 Effect of PEF on anthocyanins and polyphenolics

4.2.1 Anthocyanins

The effects of PEF treatments on the individual anthocyanin content of cherry samples are presented in Table 23. A total of five anthocyanins were identified and quantified in all cherry samples using LCMS. Anthocyanins are deposited in the skins of cherries and in flesh for some cultivars (Chaovanalikit, 2003). The high amounts of these compounds in the skins are due to their photoprotective agents functions and seed dispersal attractants. The content of these anthocyanins in cherry samples ranged from 0.15 to 2.39 microgram per gram. Cyanidin glucoside was the most abundant anthocyanin in cherries, followed by cyanidin rutinoside, peonidin glucoside, peonidin rutinoside, and pelargonidin rutinoside. Studies had reported that cyanidin-3-rutinoside was the most abundant anthocyanins in sweet cherries followed by cyanidin-3-glucoside in sweet cherry cultivars like Bing, Royal Ann, and Ranier (Chaovanalikit, 2003), Van, Navalinda, Pico Negro Limón, Ambrunés, Pico Negro, and Pico Colorado (Gonzalez-Gomez et al., 2010), and Bing, Lambert, Sam, Stella, Summit, Sylvia, and Van (Gao and Mazza, 1995).To our knowledge, this is the first time that cyanidin-3-glucoside has been reported as the first and main anthocyanin.. This might be due to the different anthocyanin extraction method used. Chaovanalikit (2003) used acetone and chloroform as extraction solvents; Gonzales-Gomez and others (1995) used a methanol solution with 0.2% hydrochloric acid and; Gao and Mazza (1995) used a combination of methanol, formic, and water in extracting both anthocyanin and polyphenolic compounds. In this study, the mixture of extraction solvents used included formic acid, milliQ water, IPA, and toluene.

Significant differences were found in the anthocyanin composition of PEF treated cherry samples. Samples incubated for 24 hours after PEF (S3 samples) had significantly higher cyanidin glucoside content compared to samples immediately after PEF (S2 samples). This suggests that anthocyanins needed time to be released after electroporation by PEF. Vallverdu-Queralt and others (2012) reported an increase in secondary plant metabolites such as polyphenols after the application of moderate intensity PEF (0.4 to 2.0 kV/cm electric field intensity) in tomato fruit after 24-hour

refrigeration at 4 ^{o}C. In their study, a significant increase in phenolic compounds with a maximum overall level of bioactive compounds obtained at 1 kV/cm electric field intensity. However, at 2.0 kV/cm electric field intensity, no increase in phenolics was observed. The application of 0.4 kV/cm PEF intensity increased the content of polyphenols in apple in the study of Grimi and others (2010). Moreover, Balasa and others (2006) reported an increase in the anthocyanin content in grapes after the application of PEF with 1 and 3 kV/cm electric field intensities. Another study by Bobinaitė and others (2014) reported a significant increase was found in the anthocyanin content and total phenolics of blueberry fruits when 1 kV/cm field intensity was applied. However, a slight decrease was observed when the most intense PEF treatment was applied (5 kV/cm). Based on this result, intensifying the treatment conditions does not enhance the release of phenolic compounds. The results of these investigations supported that PEF at mild intensities can improve the extraction of polyphenols. This could be attributed to the electropermeabilization or electroporation effect of PEF at low intensity electric field strengths and incubation for 24 hours. The mechanism of electroporation at low intensity field that promote survival of cell involves a reversible pore formation inside the plants membrane, which increases permeability (Dunn, 2001; Weaver and Chizmadzhev, 1996; Pakhomov et al., 2010). The PEF's impact on biomaterials is described by the loss of membrane barrier functions, which permits the selective extraction of biomaterials through reversible pore formation.

With cherry samples immediately after PEF, only S2P7 sample that the highest electric field intensity (2.50 kV/cm) in this study had significantly higher cyanidin glucoside than S2P6, S2P2, and S2P1 that have lower electric field intensity of 2.1, 1 0.7, and 0.3 kV/cm respectively. Hence for samples, immediately after PEF, only high energy intensity applied increased anthocyanin content of chaerry samples immediately after PEF to similar high levels observed in samples after 24 hours incubation. In summary, cyanidin glucoside was the only main anthocyanin compound present that increased in all S3 samples. The storage or incubation period suggests that bioactive compounds required time to be released. PEF induces transmembrane potential difference across plant cell membrane, When this reaches the breakdown potential or the critical value, a localized electrical breakdown will occur and an increase of cell permeability increases (Zimmerman et al., 1974), improving mass transfer rates during the extraction of plant

metabolites such as anthocyanins and polyphenols (Toepfl et al., 2006; Vorobiev & Lebovka, 2006).

Table 23 Anthocyanin compounds (ug/g wet basis) PEF-treated cherries (immediately after PEF and 24 hours after PEF)

Sample codes	PEF Intensity (kV/cm)	Cyanidin glucoside	Cyanidin rutinoside	Pelargonidin glucoside	Peonidin glucoside	Peonidin rutinoside
Control	0	1.95 ± 0.035 ^{cde}	1.37 ± 0.65	0.16 ± 0.01	1.00 ± 0.02	0.39 ± 0.10
Samples immediately after PEF						
S2-P1	0.30 ± 0.06	1.91 ± 0.35 ^{e}	1.37 ± 0.62	0.15 ± 0.00	0.98 ± 0.03	0.40 ± 0.12
S2-P2	0.70 ± 0.00	1.94 ± 0.33 ^{de}	1.44 ± 0.64	0.15 ± 0.01	0.99 ± 0.02	0.38 ± 0.10
S2-P3	1.00 ± 0.06	1.98 ± 0.29 ^{bcde}	1.63 ± 0.36	0.16 ± 0.01	1.00 ± 0.01	0.39 ± 0.07
S2-P4	1.40 ± 0.00	1.95 ± 0.33 ^{cde}	1.32 ± 0.61	0.16 ± 0.00	1.00 ± 0.01	0.39 ± 0.08
S2-P5	1.70 ± 0.06	1.98 ± 0.44 ^{bcde}	1.73 ± 1.34	0.16 ± 0.01	0.99 ± 0.03	0.46 ± 0.19
S2-P6	2.10 ± 0.00	1.94 ± 0.39 ^{de}	1.30 ± 0.64	0.16 ± 0.00	1.00 ± 0.05	0.38 ± 0.09
S2-P7	2.50 ± 0.06	2.34 ± 0.03 ^{abc}	2.02 ± 0.02	0.15 ± 0.01	1.02 ± 0.02	0.46 ± 0.02
Samples 24 hours after PEF						
S3-P0	0	2.36 ± 0.04 ^{ab}	2.03 ± 0.05	0.16 ± 0.02	1.03 ± 0.02	0.47 ± 0.02
S3-P1	0.30 ± 0.06	2.33 ± 0.04 ^{abcd}	2.03 ± 0.05	0.16 ± 0.01	1.02 ± 0.02	0.47 ± 0.02
S3-P2	0.70 ± 0.00	2.33 ± 0.06 ^{abcd}	2.04 ± 0.06	0.14 ± 0.01	1.00 ± 0.00	0.46 ± 0.02
S3-P3	1.00 ± 0.06	2.33 ± 0.01 ^{abcd}	2.00 ± 0.02	0.15 ± 0.00	1.01 ± 0.00	0.46 ± 0.02
S3-P4	1.40 ± 0.00	2.33 ± 0.02 ^{abcd}	2.03 ± 0.04	0.15 ± 0.01	1.01 ± 0.01	0.48 ± 0.04
S3-P5	1.70 ± 0.06	2.39 ± 0.03 ^{a}	2.18 ± 0.21	0.16 ± 0.00	1.04 ± 0.01	0.50 ± 0.01
S3-P6	2.10 ± 0.00	2.34 ± 0.04 ^{abc}	2.04 ± 0.03	0.15 ± 0.01	1.02 ± 0.02	0.45 ± 0.01
S3-P7	2.50 ± 0.06	2.32 ± 0.02 ^{abcd}	1.99 ± 0.05	0.15 ± 0.01	1.01 ± 0.00	0.45 ± 0.02
F value		2.126	1.254	0.929	1.780	0.842
Pr > F		0.036	0.286	0.544	0.084	0.628

Mean ± S.D: Standard deviation based on three independent samples and treatments. S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. Means within the same column not bearing common superscripts differ ($p < 0.05$; one-way ANOVA with Fisher's (LSD) multiple comparison test).

4.2.2 Polyphenolics in cherries

As reported, sweet cherries are also rich in polyphenolic compounds (Liu et al., 2011; Usenik et al., 2010). They are equally important as anthocyanins because they also contribute to the colour of cherry fruits through co-pigmentation with anthocyanins (Mazza & Brouillard, 1990). The skins of cherry fruits contain the highest concentrations of hydroxycinnamates, which are considered the major classes of phenolic in sweet cherries. These compounds are grouped as neochlorogenic and *p*-coumaroylquinic acids, which include chlorogenic, rutin, p-coumaric acid, glucosides such as kaemfperol and isorhamnetin, catechin, and epicatechin (Chaovanalikit, 2003).

The effects of PEF treatments on the polyphenolic content of cherry samples in our study are presented in Table 24. Only four polyphenolic compounds were significantly different in terms of storage and PEF treatments. These included rutin, 4-hydrobenzoic acid, isorhamnetin rutinoside, and myricetin. Overall, concentration of rutin, 4-hydrobenzoic acid and isorhamnetin rutinoside were higher immediately after PEF (S2 samples) compared to after 24 hours incubation (S3 samples). Hence storage time affected the release of these three compounds. Significantly higher contents were obtained immediately after PEF treatments that then substantially decreased upon storage for 24 hours. In addition, there was a decrease in these three polyphenols with increasing electric fields. Only the application of lower PEF intensity in fruits induced a higher content of polyphenols. Wiktor and others (2014) conducted a study to determine the impact of PEF treatments (1.85, 3, 5 kV/cm electric field intensities) on bioactive compound particularly polyphenols in apple tissues. Electric field intensity of 1.85 kV/cm significantly increased the total polyphenolic content (TPC), whereas 5.0 kV/cm decreased the amount of the TPC. Similarly, Brianceau and others (2014) reported that a mild electric field intensity of 1.2 kV/cm increased the total polyphenols in grapes pomace.

The concentration of rutin in S2 samples (S2P3, S2P4, and S2P5) was significantly higher compared to S3 samples (except for S3P4 and S3P5 sample). S2P3 and S2P5 (1.0 and 1.7 kV/cm electric field intensity respectively) had significantly higher rutin content than samples treated with higher intensity (S2P7: 2.5 kV/cm). There was a decrease in rutin content as PEF intensity increased. A similar trend was found for S3

samples. S3P6 sample with 2.1 kV/cm electric field intensity had significantly lower rutin content than samples treated with lower electric field intensities (S3P5 and S3P4).

The concentration of 4-hydrobenzoic acid was significantly higher in all S2 samples except S2P6 than all S3 samples. Within S2 samples, S2P3 (1.0 kV/cm electric field intensity) yielded higher content of hydroxybenzoic acid than samples treated with higher electric field intensities (S2P4: 1.4 kV/cm; S2P6: 2.1 kV/cm; S2P7: 2.5 kV/cm). As for S3 samples, the 4-hydrobenzoic acid content was not affected by PEF electric fields. Isorhamnetin rutinoside was also significantly higher for S2 samples (S2P3, S2P4, S2P5, and S2P7) than S3 samples (S3P1, S3P2, S3P3 and S3P6). Within S2 samples, the content of isorhamnetin rutinoside increased significantly as the intensity of PEF increased from 0.30 to 1.7 kV/cm in sample S2P1, S2P2 and S2P5) but decreased significantly when higher intensities were applied (2.1 to 2.5 kV/cm) in samples S2P6 and S2P7. Only myricetin was significantly higher in all S3 samples than all S2 samples. At present, there are no PEF studies that reported results related to the compounds we found in sweet cherries.

Table 24 Polyphenolic compounds (ug/g wet basis) in control and PEF-treated cherries (immediately after PEF and 24 hours after PEF)

Sample codes	PEF Intensity (kV/cm)	4-hydroxybenzoic acid	Vanillic acid	Chlorogenic acid	Rutin	Kaempferol rutinoside	Isorhamnetin rutinoside	Myricetin	Quercetin
Control	0	1.84 ± 0.39 ^{ab}	0.13 ± 0.18	0.34 ± 0.08	7.77 ± 0.31 ^{a}	3.36 ± 1.00	0.40 ± 0.08 ^{ab}	0.31 ± 0.10 ^{bc}	0.17 ± 0.16
Samples immediately after PEF									
S2-P1	0.30 ± 0.06	1.72 ± 0.34 ^{ab}	0.08 ± 0.13	0.13 ± 0.06	6.29 ± 1.42 ^{abc}	1.56 ± 0.55	0.27 ± 0.03 ^{bcde}	0.25 ± 0.10 ^{c}	0.14 ± 0.04
S2-P2	0.70 ± 0.00	1.75 ± 0.32 ^{ab}	0.09 ± 0.09	0.18 ± 0.08	6.26 ± 1.35 ^{abc}	2.69 ± 0.99	0.26 ± 0.07 ^{bcdef}	0.27 ± 0.10 ^{c}	0.81 ± 1.16
S2-P3	1.00 ± 0.06	2.03 ±0.06 ^{a}	0.03 ± 0.05	0.17 ± 0.15	8.14 ± 1.60 ^{a}	2.56 ± 0.56	0.37 ± 0.13 ^{abc}	0.26 ± 0.09 ^{c}	0.05 ± 0.09
S2-P4	1.40 ± 0.00	1.52 ± 0.26 ^{bc}	0.02 ± 0.02	0.14 ± 0.12	7.41 ± 0.76 ^{ab}	3.39 ± 1.10	0.39 ± 0.06 ^{abc}	0.25 ± 0.09 ^{c}	0.08 ± 0.08
S2-P5	1.70 ± 0.06	1.56 ± 0.55 ^{abc}	0.07 ± 0.06	0.67 ± 0.45	7.86 ± 1.02 ^{a}	2.49 ± 1.24	0.44 ± 0.07 ^{a}	0.24 ± 0.09 ^{c}	0.15 ± 0.03
S2-P6	2.10 ± 0.00	1.35 ± 0.41 ^{bcde}	0.01 ± 0.00	0.08 ± 0.07	6.49 ± 0.19 ^{abc}	2.61 ± 0.60	0.28 ± 0.07 ^{bcde}	0.24 ± 0.10 ^{c}	0.03 ± 0.05
S2-P7	2.50 ± 0.06	1.45 ± 0.55 ^{bcd}	0.06 ± 0.06	0.13 ± 0.13	5.04 ± 1.03 ^{bcd}	2.32 ± 0.52	0.30 ± 0.14 ^{abcd}	0.25 ± 0.08 ^{c}	0.19 ± 0.07
Samples 24 hours after PEF									
S3-P0	0	1.20 ± 0.23 ^{cdef}	0.04 ± 0.06	0.35 ± 0.10	4.13 ± 2.63 ^{cd}	1.27 ± 0.91	0.16 ± 0.09 ^{defg}	0.43 ± 0.02 ^{a}	0.57 ± 0.67
S3-P1	0.30 ± 0.06	0.86 ± 0.17 ^{ef}	0.12 ± 0.20	0.17 ± 0.06	4.29 ± 0.95 ^{cd}	1.07 ± 0.43	0.14 ± 0.09 ^{efg}	0.40 ± 0.02 ^{ab}	0.35 ± 0.20
S3-P2	0.70 ± 0.00	0.86 ± 0.22 ^{ef}	0.14 ± 0.24	0.26 ± 0.24	4.22 ± 2.83 ^{cd}	2.15 ± 2.21	0.12 ± 0.12 ^{fg}	0.39 ± 0.04 ^{ab}	0.25 ± 0.23
S3-P3	1.00 ± 0.06	0.80 ± 0.20 ^{f}	0.06 ± 0.08	0.22 ± 0.22	4.25 ± 2.21 ^{cd}	1.57 ± 1.01	0.15 ± 0.11 ^{efg}	0.39 ± 0.04 ^{ab}	0.24 ± 0.16
S3-P4	1.40 ± 0.00	0.82 ± 0.08 ^{f}	0.03 ± 0.05	0.43 ± 0.24	5.85 ± 1.55 ^{abc}	2.27 ± 1.09	0.30 ± 0.10 ^{abcd}	0.40 ± 0.02 ^{ab}	0.10 ± 0.09
S3-P5	1.70 ± 0.06	1.00 ± 0.14 ^{def}	0.09 ± 0.09	0.36 ± 0.28	5.76 ± 0.58 ^{abc}	2.39 ± 1.70	0.25 ± 0.08 ^{cdef}	0.42 ± 0.01 ^{ab}	0.20 ± 0.11
S3-P6	2.10 ± 0.00	0.93 ± 0.17 ^{ef}	0.08 ± 0.09	0.60 ± 0.74	3.03 ± 2.72 ^{d}	2.51 ± 0.23	0.10 ± 0.10 ^{g}	0.41 ± 0.02 ^{ab}	0.29 ± 0.20
S3-P7	2.50 ± 0.06	0.77 ± 0.20 ^{f}	0.05 ± 0.07	0.21 ± 0.12	4.75 ± 0.78 ^{cd}	1.96 ± 0.20	0.16 ± 0.03 ^{defg}	0.42 ± 0.02 ^{ab}	0.13 ± 0.14
F value		5.911	0.981	1.275	2.862	1.176	4.45	3.78	0.528
Pr > F		0.000	0.360	0.273	0.006	0.338	0.000	0.001	0.946

Mean ± S.D: Standard deviation based on three independent samples and treatments. S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. Means within the same column not bearing common superscripts differ ($p < 0.05$; one-way ANOVA with Fisher's least significant difference (LSD) multiple comparison tests).

4.3 Effect of PEF on the flavour quality

4.3.1 Identification of volatile compounds

The effect of PEF treatments and storage conditions on cherry samples is shown in Table 25. A total of 33 compounds were identified in all cherry samples. Most of the compounds have been reported previously to be characteristic of sweet cherry flavour from previous studies (Mattheis et al., 1997). These volatiles largely comprised 15 aldehydes, 10 alcohols, four esters, two terpenes, one acid and one alkane. Aldehydes and alcohols were the dominant volatile groups in all cherry samples, followed by alkane, esters, terpenes, and acid. In the aldehyde and alcohol groups, (E)-2-hexenal, benzaldehyde, hexanal, (Z)-2-hexen-1-ol, and benzyl alcohol were among the highest. These results were similar to other researches (Girard & Kopp, 1998; Zhang et al., 2007; Sun et al., 2010). However, application of PEF treatments significantly increased the volatile concentrations of hexanal, (E)-2-hexenal, benzaldehyde, (Z)-2-hexen-1-ol and benzyl alcohol (Table 25). C6 compounds and aromatic compounds were among the most significant class of compounds according to their ratio to the internal standard, i.e., hexanal (36.65–209.69), (E)-2-hexenal, (129.91–1109.53), benzaldehyde (49.33–882.79), (Z)-2-hexen-1-ol (2.69–26.09), and benzyl alcohol (17.46–129.45).

Table 25 Volatile compounds (expressed as ratio to the internal standard) in control and PEF-treated cherries (immediately after PEF and 24 h after PEF)

No.	Volatile Compounds	RI [α]	Identification [β]	Treatment *														
				S1 (Control)	S2P1	S2P2	S2P3	S2P4	S2P5	S2P6	S2P7	S3P0	S3P1	S3P2	S3P3	S3P4	S3P5	S3P6	S3P7
	Aldehydes																		
1	Butanal	<653	MS	0.08 b	ND	0.40 ab	0.23 ab	0.34 ab	0.30 ab	0.30 ab	ND	ND	ND	0.34 ab	0.32 ab	1.00 a	1.00 a	ND	0.09 b
2	Pentanal	666	MS+RI	0.86 abc	ND	1.14 abc	0.52 abc	0.71 abc	0.46 bc	0.73 abc	1.69 a	0.30 c	1.48 ab	ND	0.21 c	ND	0.02 c	ND	ND
3	2-Pentenal, (E)-	756	MS+RI	ND	ND	ND	0.04 b	0.01 b	0.14 ab	ND	ND	ND	0.06 b	0.05 b	ND	ND	0.20 a	0.06 b	ND
4	Hexanal	804	MS 85%	50.91 b	87.29 b	134.60 ab	73.97 b	149.37 ab	119.94 ab	83.04 b	81.91 b	36.65 b	85.12 b	123.48 ab	55.51 b	209.69 a	74.75 b	104.66 ab	117.46 ab
5	2-Hexenal, (E)-	858	MS 85%	310.92 bc	243.13 bc	613.11 abc	253.49 bc	1109.53 a	585.49 abc	321.77 bc	282.74 bc	129.91 c	402.29 bc	646.29 abc	176.06 bc	672.14 ab	295.66 bc	593.69 abc	521.42 bc
6	Heptanal	906	MS+RI	0.19 c	0.59 bc	1.24 bc	0.50 bc	0.77 bc	1.02 bc	2.73 a	1.23 bc	0.51 bc	0.82 bc	1.26 bc	0.99 bc	1.32 bc	1.55 ab	1.31 bc	1.59 ab
7	2,4-Hexadienal, (E,E)-	927	MS 85%	3.29 abc	2.32 abc	4.35 abc	2.95 abc	6.20 a	5.61 ab	0.53 c	2.89 abc	1.78 bc	ND	5.53 ab	3.72 abc	5.71 a	3.50 abc	5.97 a	4.42 abc
8	2-Heptenal, (Z)-	963	MS+RI	0.07 b	ND	ND	0.18 b	0.01 b	0.04 b	ND	0.17 b	ND	ND	ND	ND	0.47 b	0.28 b	1.90 a	0.17 b
9	Benzaldehyde	968	MS 85%	166.78 bc	125.02 bc	153.17 bc	108.97 bc	179.31 bc	154.14 bc	363.65 b	882.79 a	49.33 c	102.60 bc	140.80 bc	65.86 c	192.09 bc	66.73 c	184.00 bc	114.72 bc
10	2,4-Nonadienal, (E,E)-	992	MS 85%	ND	ND	ND	0.19 b	ND	ND	ND	ND	0.11 b	ND	ND	ND	1.25 b	3.28 a	ND	0.24 b
11	Octanal	1007	MS 85%	0.30 b	0.37 b	0.22 b	0.29 b	0.23 b	0.74 b	ND	1.36 b	0.68 b	1.06 b	0.60 b	5.20 a	1.02 b	1.39 b	0.84 b	0.70 b
12	2-Octenal, (E)-	1034	MS 85%	0.61 c	ND	0.75 bc	0.41 c	0.14 c	ND	0.90 bc	0.81 bc	1.33 bc	ND	ND	1.76 abc	4.41 a	3.43 ab	2.97 abc	1.18 bc
13	Nonanal	1109	MS 85%	0.88 b	1.69 b	3.17 b	ND	ND	2.18 b	ND	6.10 b	3.32 b	4.47 b	2.95 b	16.10 a	3.45 b	3.70 b	4.79 b	4.88 b
14	2-Nonenal, (E)-	1166	MS+RI	0.39 b	ND	0.02 b	ND	ND	0.88 a	ND	ND	0.31 b	ND	0.29 b	0.24 b	ND	0.02 b	1.25 a	ND
15	Decanal	1211	MS 85%	0.22 c	0.41 bc	1.50 ab	0.16 c	0.29 bc	0.56 abc	ND	1.28 abc	0.42 bc	1.83 a	0.34 bc	0.37 bc	0.74 abc	0.94 abc	0.60 abc	0.66 abc

Table 25. *Cont.*

No.	Volatile Compounds	RI [α]	Identification [β]	Treatment *														
				S1 (Control)	S2P1	S2P2	S2P3	S2P4	S2P5	S2P6	S2P7	S3P0	S3P1	S3P2	S3P3	S3P4	S3P5	S3P6	S3P7
	Alcohols																		
16	3-Pentanol	*<653*	MS	3.56 [a]	ND	ND	ND	ND	ND	ND	ND	0.27 [b]	ND	ND	ND	ND	ND	ND	ND
17	1-Penten-3-ol	661	MS+RI	0.34 [b]	ND	ND	ND	ND	ND	0.82 [b]	ND	ND	ND	ND	0.23 [b]	ND	2.24 [a]	0.48 [b]	0.45 [b]
18	2-Hepten-1-ol, (Z)-	701	MS+RI	0.05 [d]	0.24 [c]	0.99 [ab]	0.36 [bc]	0.18 [c]	0.60 [bc]	0.77 [bc]	1.52 [a]	ND	0.17 [c]	ND	0.32 [bc]	ND	0.16 [c]	0.16 [c]	0.42 [bc]
19	3-Buten-1-ol, 3-methyl-	733	MS+RI	0.64 [b]	0.67 [b]	1.51 [ab]	0.65 [a]	1.22 [ab]	1.05 [b]	0.66 [b]	2.45 [a]	0.44 [b]	ND	0.48 [b]	0.34 [b]	0.69 [b]	0.75 [b]	1.00 [b]	0.75 [b]
20	2-Hexen-1-ol, (Z)-	871	MS+RI	2.69 [d]	11.45 [bcd]	11.68 [abcd]	16.72 [abcd]	26.09 [a]	ND	7.58 [cd]	22.62 [abc]	12.27 [a]	11.05 [bcd]	20.57 [a]	15.17 [a]	22.52 [a]	13.26 [d]	24.49 [ab]	11.47 [bcd]
21	2-Buten-1-ol, 3-methyl-	781	MS+RI	0.69 [abc]	0.56 [abc]	0.92 [abc]	0.65 [abc]	1.29 [a]	1.05 [ab]	0.89 [abc]	0.60 [abc]	0.50 [bc]	ND	0.76 [abc]	0.34 [ab]	0.69 [ab]	0.31 [c]	1.23 [ab]	0.73 [abc]
22	2-Nonen-1-ol, (E)-	947	MS+RI	0.01 [b]	ND	ND	ND	ND	ND	2.77 [a]	ND	ND	ND	ND	0.16 [b]	ND	ND	ND	ND
23	3-Heptanol	957	MS+RI	0.15 [b]	0.68 [b]	0.67 [b]	0.37 [b]	0.52 [b]	1.08 [b]	ND	2.55 [a]	0.16 [b]	0.20 [b]	0.42 [b]	1.16 [b]	0.51 [b]	ND	ND	0.41 [b]
24	Benzyl Alcohol	1044	MS 85%	59.17 [bc]	46.17 [bc]	32.94 [bc]	27.03 [bc]	95.72 [ab]	72.82 [abc]	52.02 [bc]	129.45 [a]	22.74 [c]	46.22 [bc]	43.67 [bc]	17.46 [c]	65.15 [abc]	25.94 [c]	78.42 [abc]	44.50 [bc]
25	2-Hexadecanol	1401	MS+RI	0.07 [b]	0.19 [ab]	0.74 [ab]	0.18 [ab]	0.02 [b]	0.18 [ab]	1.46 [ab]	0.79 [ab]	1.18 [ab]	0.15 [ab]	ND	ND	0.55 [ab]	1.08 [ab]	0.20 [ab]	1.75 [a]

Table 25. *Cont.*

No.	Volatile Compounds	RI [α]	Identification [β]	Treatment *														
				S1 (Control)	S2P1	S2P2	S2P3	S2P4	S2P5	S2P6	S2P7	S3P0	S3P1	S3P2	S3P3	S3P4	S3P5	S3P6	S3P7
	Esters																		
26	Butanoic acid, methyl ester	723	MS+RI	0.03 [b]	0.06 [b]	0.30 [b]	0.20 [b]	0.20 [b]	0.14 [b]	ND	ND	0.14 [b]	2.99 [a]	0.19 [b]	0.08 [b]	1.28 [b]	0.25 [b]	1.29 [b]	0.16 [b]
27	Acetic acid, 2-phenylethyl ester	916	MS+RI	ND	ND	0.91 [a]	ND	ND	0.68 [a]	ND	ND	ND	0.67 [a]	ND	ND	ND	ND	ND	ND
28	Benzoic acid, 2-hydroxy-, methyl ester	1201	MS+RI	0.46 [b]	ND	0.74 [b]	0.72 [b]	2.52 [b]	ND	ND	ND	0.56 [b]	ND	1.25 [b]	22.25 [a]	1.82 [b]	ND	1.62 [b]	ND
29	Decanoic acid, methyl ester	1326	MS+RI	3.31 [b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	15.58 [a]	ND	ND	ND	ND
	Terpenes																		
30	Menthol	1186	MS 85%	0.05 [b]	ND	ND	ND	ND	ND	11.26 [a]	ND	ND	ND	ND	ND	ND	0.21 [b]	0.19 [b]	0.13 [b]
31	Geranyl vinyl ether	1259	MS 85%	0.25 [ab]	0.36 [ab]	0.80 [ab]	0.34 [ab]	0.75 [ab]	0.67 [ab]	0.12 [b]	0.45 [ab]	0.29 [ab]	ND	0.74 [ab]	ND	0.90 [a]	ND	0.66 [ab]	0.60 [b]
	Acids																		
32	n-Decanoic acid	1312	MS 85%	0.02 [b]	ND	ND	ND	ND	0.30 [ab]	ND	0.04 [b]	0.10 [b]	ND	ND	ND	0.58 [a]	ND	0.25 [b]	0.13 [b]
	Alkanes																		
33	Undecane	1101	MS 85%	4.69 [ab]	2.90 [b]	6.13 [ab]	4.90 [ab]	6.86 [ab]	5.81 [ab]	ND	4.65 [ab]	ND	ND	6.57 [ab]	10.72 [ab]	6.18 [ab]	13.10 [a]	7.49 [ab]	5.02 [ab]

[a,b,c] Different letters within the same row (different treatment for the same volatile compounds) differ significantly using Fisher's least significant difference ($p < 0.05$).
* Ratio to internal standard; S1 = control, S2 = PEF, S3 = 24 h incubation at 4 °C after PEF P 0, 1, 2, 3, 4, 5, 6, 7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 $kV \cdot cm^{-1}$. ND: not detected.
[α] RI on a VF-5MS column, was calculated in relation to the retention time of n-alkane (C7–C30) series. [β] MS, tentative identification by comparison of mass spectrum with NIST library spectrum (over 85%); MS + RI, mass spectrum identified using NIST mass spectral database and RI agree with literature values (Sun et.al 2010).

According to Sun and others (2010) and Zhang and others (2007), the highest content of hexanal and (E)-2-hexenal were present in the Stella cultivar with relative peak areas of 21.0 and 6.81, respectively. These values were very low compared to the values obtained after the cherries were processed for both samples immediately after PEF and 24 h after PEF (Table 25).

The C6 aldehydes and alcohols are generated by the consecutive action of the enzymes lipoxygenase and alcohol dehydrogenase on polyunsaturated fatty acids (Salinas et al., 2004). In the present study, it was found that production of C6 aldehydes and alcohols was enhanced by the electroporation effects of PEF application. Studies have reported that C6 aldehydes are produced from enzymatic reactions, which are attributed to the quantities of precursor molecules already present in the fruit (Bernalte et al., 1998; Mattheis et al., 1997). Hexanal and (E)-2-hexenal are products of fatty acid oxidation (linoleic and linolenic acid oxidation) in the presence of lipoxygenase, while (Z)-2-hexen-1-ol is a secondary compound from these oxidation reactions (Paillard & Rouri, 1984). Benzaldehyde is produced from the hydrolysis of amygdalin present in cherries (Zhang et al., 2007). Fatty acid and lipid oxidation reactions can also be attributed to the mechanism of electropermeabilisation by PEF. When an external electric field is applied on biological cells (animal, plant or microbial), disruption of the cell membrane occurs (Toefpl et al., 2006).This results in the increase of enzymes that react to produce and increase the volatile compounds found in this study.

The C6 compounds are known to have desirable odours. Hexanal and (E)-2-hexenal have a characteristic green leaf-odour, while benzaldehyde, benzyl alcohol, and (Z)-2-hexen-1-ol are described as having almond-like, floral, and vegetable odour, respectively (Sun et al., 2010; Serradilla et al., 2010). These important volatiles have also been reported by Sun and others (Sun et al., 2010) to have high flavour dilution factors. According to them, the significant aroma compounds that greatly contribute to the aroma profile of Stella cultivar that had the highest flavour dilution value of >64 were hexanal, (E)-2-hexenal, following were (Z)-3-hexenal and benzaldehyde which had 32 flavour dilutions (FD) values, while octanal, 2–4-nonadienal, and (Z)-3-hexen-1-ol had an FD value of 16.

A significant increase in the level of benzaldehyde was found after the application of 2.5 kV/cm (S2P7) immediately after PEF. This was probably due to the

electropermeabilization effect of the high electric field strength, which increased and stimulated the metabolic activity of enzymes present in the plant cell (Vallverdú-Queralt et al., 2012). No significant differences were found between samples except for the S2P7. Majority of the samples after 24 hours decreased compared to S2 samples immediately after PEF, but the decrease was not significant from the control. Aldehydes namely butanal, octanal, (E)-2-octenal, and nonanal with concentrations between 0.01 to 16.1 increased significantly after 24 hours of incubation except for pentanal, which decreased after 24 hours of incubation. Aldehydes such as (E)-2-pentenal, (Z)-2-heptenal, (E,E)-2,4-nonadienal, 2-nonenal were noticeably not present in most of the samples immediately after PEF but were released after 24 hours of incubation. Two of the S3 samples (S3P4 and S3P5) had a higher concentration of (E,E)-2, 4-nonadienal compared to a previous study of Sun and others (2010). The compound (E,E)-2,4-nonadienal, which has a fatty odour, was determined to be one of the aroma-active compounds that greatly contributed to the aroma of Stella cultivar (Sun et al., 2010). The concentration of hexanal increased after PEF treatments both immediately after PEF and 24 hours after PEF. At 1.4, 2.1, and 2.5 kV/cm electric field strengths an increase in concentration was observed after 24 hours but the increase was only significant in S3P4 samples at 1.4 kV/cm electric field strength. The concentration of heptanal increased after PEF treatments. Sample S2P6 with 2.1 kV/cm electric field strength was observed to be significantly different in heptanal among S2 samples.

(Z)-2-Hexen-1-ol was detected in all samples except for the S2P5 sample. There was a significant increase ($p < 0.05$) in the concentration of (Z)-2-hexen-1-ol for S2 (S2P4 and S2P7) and most S3 (S3P2, S3P3, S3P4, and S3P6) samples compared to control sample, immediately after PEF and after 24 h incubation. Benzyl alcohol was present in the majority of samples immediately after PEF. However, it was only significantly higher in S2P7 (2.5 kV/cm electric field strength) after 24 hours.

4.3.2 Volatile Analysis of Cherry Samples Untreated and Treated with Different PEF Energy Intensities

4.3.2.1 Headspace Volatile Compounds of PEF-Treated Cherries

Based on the data in Table 25, PEF samples treated with the low PEF intensity produced higher concentrations of volatile compounds. This is in agreement with the findings of Toepfl and others (2006) who studied the permeabilization of cell membranes in food by PEF reported that low intensity PEF can potentially induce stress

reactions in plant systems or cell cultures, so that bioproduction of certain compounds are enhanced and stimulated. As seen in Table 25, more flavour volatiles were released in S3 samples after 24 h storage, and S2 samples immediately after PEF treatment at the highest electric field intensities. These samples were S3P4, S3P5, S3P6, S3P7, S2P5, S2P6, and S2P7 with electric field strengths of 1.40 to 2.50 kV/cm, and specific energy input of 30.0 to 47.0 kJ/kg.

A total of fourteen volatile compounds were associated with S3 samples (S3P4, S3P5, S3P6, S3P7 with electric field strength of 1.4, 1.7, 2.1, 2.5 kV/cm respectively) with the highest concentrations being mostly of aldehydes such as butanal, hexanal, (*E*)-2-hexenal, heptanal, (*E*,*E*)-2,4-hexadienal, (*E*)-2-pentenal, (*E*)-2-octenal, (Z)-2-heptenal, decanal; three alcohols such as 1-penten-3-ol, 2-hexadecanol, 3-methyl-3-buten-1-ol, one terpene—geranyl vinyl ether—and one acid—*n*-decanoic acid. Most of these volatiles have been reported in previous studies on cherries (Sun et al., 2010; Wen et al., 2014). Green and grassy were the most intense odour characteristic of cherries. This attribute, as mentioned earlier, is due to the C_6 aldehydes (hexanal and (*E*)-2-hexenal). (*E*,*E*)-2,4-Hexadienal also produces a sweet, green, and fruity aroma in cherries. 2-Octenal was responsible for the green and nutty flavour, while 2-heptanal has been described as almond-like in odour (Sun et al., 2010; Wen et al., 2014). The compound decanal has been reported to have citrusy, orange, and green attributes (Sun et al., 2010; Wen et al., 2014). However, according to Wen and others (2014), decanal has not previously been described as an important aroma compound in sweet cherries.

S2 samples namely S2P5, S2P6, S2P7 having electric field strength of 1.7, 2.1, and 2.5 kV/cm respectively have the highest concentrations of aldehydes namely, (*E*)-2-nonenaland benzaldehyde; alcohols such as (*E*)-2-nonen-1-ol, 3-methyl-2-buten-1-ol, (*Z*)-2-hepten-1-ol, (*Z*)-2-hexen-1-ol, and benzyl alcohol; esters such as butanoic acid methyl ester and acetic acid, 2-phenylethyl ester; and one terpene, which is menthol. It is worth noticing that samples immediately after PEF treatment with higher energy intensities produced the highest level of benzaldehyde and benzyl alcohol. Benzaldehyde is said to be the primary contributor to the characteristic flavour of sweet cherry fruit and benzyl alcohol is its deramification product (Zhang et al., 2007). Benzaldehyde and benzyl alcohol are responsible for the almond and floral odour in cherries (Sun et al., 2010; Wen et al., 2014) while it is also reported that (*E*)-2-nonenal and (*E,Z*)-2,6-nonadienal impart a green attribute described as fresh cucumber.

Similarly, green and vegetable attributes may be due to (*Z*)-2-hexen-1-ol, while minty attributes may originate from menthol (Sun et al., 2010).

Two S3 samples namely S3P2 and S3P3 treated with 0.7 and 1.0 kV/cm electric field strength respectively resulted in the highest concentrations of a total of eight volatile compounds, namely nonanal, octanal, 2-hydroxybenzoic acid methyl ester, decanoic acid methyl ester, 3-heptanol, and undecane. Nonanal contributed to the citrusy (orange-like) and green aroma in cherries while octanal was reported to have a fruity (lemon-like) and green aroma as well (Sun et al., 2010; Wen et al., 2014).

Finally, control (S1 and S3P0) and PEF treated samples (S2 samples: S2P1, S2P2, S2P3, S2P4; S3 sample: S3P1) with the lowest energy intensities (S2 samples: 0.3, 0.7, 1.0, and 1.4 kV/cm respectively; S3 sample: 0.3 kV/cm) had the least volatiles. However, these samples had the highest concentrations of pentanal and 3-pentanol. The impact of electroporation on the samples 24 h after PEF treatment resulted in significantly increased concentrations of most of the aldehydes. This suggested that volatile compounds after PEF processing required time to be released. When low or moderate PEF is applied, electropermeabisation occurs, which creates reversible process of pore formation. Through this process, volatile compounds are released following the reactions of lipoxygenase enzymes. Guderjan, Töepfl, Angersbach and Knorr (2005) reported that after the application of low intensity PEF (0.6 kV/cm electric field strength and 0.62 kJ/kg energy input) to maize germ oil, the phytosterol concentration increased up to 32% after a subsequent incubation time of 24 h. The incubation according to them, increased stress response of the plant "bioreactor". Similarly, Vallverdú-Queralt and others (2012a) reported that after application of moderate PEF treatment on tomato juice significantly increased the content of polyphenols than the untreated samples. According to them, the increase could be attributed to the defense response of plants to the moderate intensity PEFs (MIPEF) being applied. MIPEF treatment not only provides potential to induce stress reactions in tomato fruits after 24 h of refrigeration by enhancing metabolic activity and accumulating secondary metabolites, but also can increase permeability of the cellular membrane making the extraction of the bioactive constituent more efficient (Vallverdú-Queralt et al., 2012b).

However, other samples (S3P2 and S3P3) analysed 24 h after PEF treatments produced less volatiles. This may be due to the lower energy intensities applied to them (0.7–1.0

kV/cm electric field strengths; 34 kJ/kg energy input). In the present study, all volatiles were similar to those reported in Stella cherries (Sun et al., 2010).

The increasing moderate energy intensities (1.4 to 2.5 kV/cm electric field strengths and 31 to 47 kJ/kg energy inputs) of PEF applied to the samples in this study were able to extract more C_6 aldehydes and aromatic alcohols. This result is in agreement with the study of Vallverdu-Queralt and others (2013). They reported that low or moderate intensity PEF induced sublethal stress to cells by permeabilizing tissue structures that in turn increased extraction of more volatiles and bioactive constituents. The highest electric field intensity in our study actually corresponded to moderate PEF intensity in other studies of Vallverdu-Queralt and others (2013). The energy intensities used in this study were definitely much lower compared to the intensities used in commercial juice pasteurisation. The intensities used in commercial products are typically about 35–60 kV/cm (Aguilo-Aguayo et.al 2010). In summary, samples with the lowest energy intensities (0.30–1.0 kV/cm, 30–54 kJ/kg) in both S3 and S2 treatments and control samples had the least amount of all the major volatiles.

4.4. Effect of PEF processing of cherry samples on the growth of *L. acidophilus* (LAB) bacteria.

4.4.1 Preliminary growth experiment

This experiment was done to determine if *L. acidophilus* would grow in non-nutrient supplemented cherry puree with and without pH adjustment to pH 6.0.
The term 'initial pH' used in this experiment refers to the pH of cherry puree before adjustment to pH 6.0. The initial pH of cherry puree was 3.9. Results show (Table 26) that after 48 hours, there was a decrease in viable cell number of LAB in non-nutrient supplemented cherry without pH adjustment.

Table 26 Growth of LAB in cherries at 0 and 48 hours with pH adjustments

	0 hour		48 hour	
Sample	c.f.u/ml	log c.f.u/ml	c.f.u/ml	log c.f.u/ml
Initial pH,				
pH 3.9	1320000	6.12	46000	4.66
pH 6	530000	5.72	131000000	8.12

In contrast, growth was observed in samples with pH adjusted to pH 6.0. The acidity of the matrix environment is one of the factors that influence the viability of probiotic bacteria (Shinde, 2012). The optimum pH for growth of most strains of *Lactobacillus* is between pH 5 to 6. Hence at low pH, its survival decreases (Von Wright & Axelsson, 2011; Kailasapathy & Chin, 2000). Hence samples were adjusted to pH 6.0 in subsequent LAB growth experiments.

4.4.2 LAB growth in PEF treated Cherries

The initial viable counts of all samples (control and PEF-treated) before incubation (0 hour) were all in the range of 1 x 10E7 cfu/ml. After 48 hours of incubation, the number of viable cells in the majority of samples decreased significantly except for S2P1, S2P2, S2P4, and S2P5. The viable LAB counts of control and different PEF treated samples are shown in Table 27, Figure 30, and Figure 31. Results showed that the counts in control sample after 48 hours were significantly lower than the PEF-treated ones, particularly in samples immediately after PEF (S2) except for S2P6 and S2P7 with the highest energy intensities applied. Both the control samples (control and S3P0) had significantly lower LAB counts than S2P1 (0.30 kV/cm / 39.92 kJ/kg), S2P2 (0.70 kV/cm / 55 kJ/kg), S2P3 (1.0 kV/cm / 48.31 kJ/kg), S2P4 (1.40 kV/cm / 35 kJ/kg), and S2P5 (1.70 kV/cm / 43 kJ/kg).

In most PEF treated samples after 48 hours incubation, numbers of LAB decreased. The effect of PEF treatments on the growth of LAB however significantly increased for S2P1, S2P4, and S2P5 samples. This suggests that increased growth was observed at mild electric field intensities between 0.30 kV/cm and 1.70 kV/cm compared to higher electric field intensities of 2.1 and 2.5 kV/cm. The growth of LAB on S2 samples might be due to more nutrients being released and made available for bacteria growth. This could be correlated with the results of PEF applications on the bioactive compounds, wherein, significantly higher contents of polyphenols (4-hydrobenzoic acid, rutin, and isorhamnetin rutinoside) were found in S2 samples. Nutrients refer to compounds such as sugars, small proteins, and other phytochemicals including phenolics, anthocyanins, and organic acids. These nutrients impart varied effects on the growth of LAB and in this case were found to be stimulatory.

Sutherland and others (2009) reported that sugars, small proteins, and other food-specific phytochemicals including phenolics and organic acids were able to promote the growth of some specific probiotic bacteria in fruits such as blueberry and strawberry. Similarly, Hap (2009) reported that the extracts of blueberry and strawberry exerted a significant growth enhancement of probiotic bacteria. Although the exact mechanism of the growth-enhancing effect of compounds fruit extracts is not well understood, their presence could either serve as an additional source of energy or as an antioxidant. Several studies suggest that lactic acid bacteria utilize phenolic compounds in some fruits for growth (Molan et al., 2009; Mullen et al., 2002; Vuorinen et al et al., 2000). Probiotic bacteria have the ability to metabolize phenolics which increase their growth rates (Alberto et al., 2001). Anthocyanins in fruits can also stimulate growth of LAB according to Werlein and others (2005). The mechanism involved here could be attributed to the conversion of anthocyanins into other compounds with different bioavailability and bioactivity on the enzymes present in probiotic bacteria (Avila et al., 2009). Sugars such as glucose, fructose, and sucrose have also been known to increase the growth of LAB (Von Wright & Axelsson, 2011). In our study, we found a significant increase in TSS values for S2 samples. However the actual changes in sugar composition were not determined in this study and would be worth investigating to further explain the increase in LAB growth.

Different results were obtained with samples incubated for 24 hours after PEF (S3 samples) at 4^{O}C. The numbers of LAB for all S3 samples, except for S3P3 which was contaminated, decreased after 48 hours, and the reduction in number increased as the intensity of the applied electric field increased. However, the mechanism of the inhibitory effect of these compounds remains unclear. The inhibitory effects could be due to the complex composition of the compounds released. The high concentrations of phenolics and organic acids and the interactions between these compounds could result in an inhibitory effect (Hap & Gutierrez, 2012). In this study, there were significant increases in the values of titratable acidity for S2 than S3 samples. The electroporation effect of PEF treatment on the samples incubated for 24 hours after processing may account for the increased release of inhibitory compounds.

Table 27 Aerobic plate counts of LAB in cherries for 0 hour and 48 hours of incubation (mean ± standard deviation)

Sample Codes	PEF Intensity	Calculated Energy	Count, log c.f.u./mL	
	kV/cm	kJ/kg	0 hour	48 hour
Control	0	0	7.74 ± 0.06 $^{b\ (x)}$	7.12 ± 0.11 $^{ef\ (y)}$
		Samples Immediately after PEF		
S2P1	0.30 ± 0.06	39.92 ± 0.10	7.22 ± 0.05 $^{i\ (x)}$	7.41 ± 0.06 $^{c\ (y)}$
S2P2	0.70 ± 0.00	54.75 ± 6.16	7.44 ± 0.00 $^{def\ (x)}$	8.22 ± 0.12 $^{a\ (y)}$
S2P3	1.00 ± 0.06	48.31 ± 1.37	7.59 ± 0.10 $^{c\ (x)}$	7.30 ± 0.00 $^{cd\ (y)}$
S2P4	1.40 ± 0.00	34.63 ± 1.41	7.37 ± 0.04 $^{efg\ (x)}$	7.90 ± 0.13 $^{b\ (y)}$
S2P5	1.70 ± 0.06	43.22 ± 1.06	7.45 ± 0.01 $^{de\ (x)}$	8.20 ± 0.28 $^{a\ (y)}$
S2P6	2.10 ± 0.00	41.86 ± 2.16	7.31 ± 0.04 $^{ghi\ (x)}$	7.12 ± 0.10 $^{ef\ (y)}$
S2P7	2.50 ± 0.06	45.30 ± 1.71	7.28 ± 0.01 $^{ghi\ (x)}$	7.05 ± 0.06 $^{fg\ (y)}$
		Samples 24 hour after PEF		
S3P0	0	2.97 ± 0.17	7.26 ± 0.08 $^{hi\ (x)}$	6.89 ± 0.01 $^{g\ (y)}$
S3P1	0.30 ± 0.06	29.80 ± 0.09	7.35 ± 0.05 $^{efgh\ (x)}$	7.24 ± 0.06 $^{de\ (x)}$
S3P2	0.70 ± 0.00	34.43 ± 3.51	7.34 ± 0.07 $^{fgh\ (x)}$	7.11 ± 0.10 $^{ef\ (y)}$
S3P3*	1.00 ± 0.06	34.26 ± 4.87	7.36 ± 0.06 $^{efg\ (x)}$	7.59± 0.20 $^{a\ (y)}$
S3P4	1.40 ± 0.06	30.82 ± 2.26	7.51 ± 0.11 $^{cd\ (x)}$	7.11 ± 0.10 $^{ef\ (y)}$
S3P5	1.70 ± 0.06	42.91 ± 2.13	8.70 ± 0.02 $^{a\ (x)}$	7.09 ± 0.08 $^{ef\ (y)}$
S3P6	2.10 ± 0.00	47.52 ± 2.57	7.53 ± 0.10 $^{cd\ (x)}$	6.90 ± 0.05 $^{g\ (y)}$
S3P7	2.50 ± 0.06	45.46 ± 4.70	7.48 ± 0.05 $^{d\ (x)}$	6.92 ± 0.03 $^{g\ (y)}$
F			96.285	81.864
Pr > F			< 0.0001	< 0.0001

Mean ± S.D: Standard deviation based on three independent samples and treatments.
Samples expressed as: S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. Means within the same column and row not bearing common superscripts $^{a, b, c, d, e, f,g,h,i}$ and $^{x, y}$ respectively differ ($p < 0.05$; one-way ANOVA with Fisher's LSD comparison test). *: Contaminated

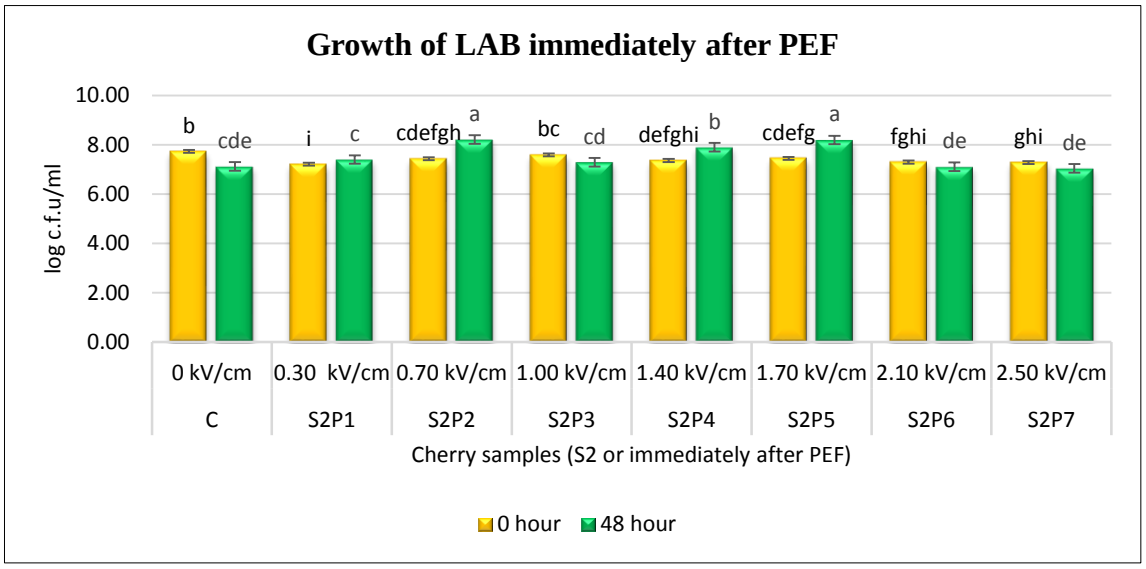


Figure 30 Total lactic acid bacteria count (LAB) in cherries immediately after PEF treatments

a-i Different letters represent significant differences between treatments (P < 0.05)
Samples expressed as: S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. Superscripts ($^{a, b, c, d, e, f,g,h,i}$) differ ($p < 0.05$; one-way ANOVA with Fisher's LSD comparison test). *: Contaminated

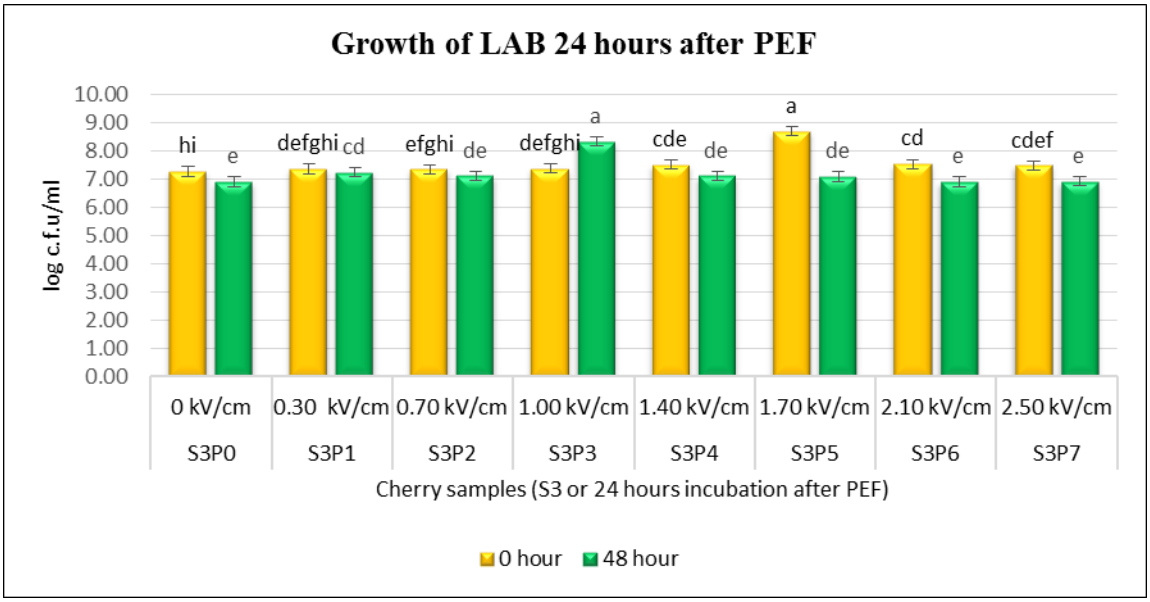


Figure 31 Total lactic acid bacteria count (LAB) in cherries 24 hours after PEF treatments

a-i Different letters represent significant differences between treatments (P < 0.05)
Samples expressed as: S1 = control, S2= PEF, S3 = 24 h incubation at 4 °C after PEF P 0,1,2,3,4,5,6,7 = 0, 0.3, 0.7, 1.0, 1.4, 1.7, 2.1, 2.5 kV•cm−1. Superscripts ($^{a, b, c, d, e, f,g,h,i}$) differ ($p < 0.05$; one-way ANOVA with Fisher's LSD comparison test). *: Contaminated

Overall, the growth of LAB in cherries was affected by PEF treatments. The permeabilization effect of PEF in food and the application of low to moderate PEF intensity could enhance and stimulate bioproduction of certain compounds leading to either stimulatory effects for S2 samples or inhibitory effects for S3 samples on the growth of lactic acid bacteria.

Chapter 5. Conclusion

This research was primarily carried out to determine the effects of pulsed electric field processing on the flavour, physicochemical and microbiological properties of Stella sweet cherries. Sweet cherry is an essential stone fruit that is gaining popularity in the world not only because of its balanced sweetness and enticing flavor, but also its health-benefiting nutrients and unique antioxidants content. Since cherries are seasonal, a large part of fresh cherry fruits are processed into other products such as brined, canned, dried, frozen jams, jellies, and concentrates that have extended shelf life. Frozen fruits are generally used as an intermediate raw material or incorporated in foods like jams, jellies, and smoothies. There is increasing demand for high quality processed cherry products that retain natural flavour, vitamins and minerals, and bioactive properties.

Different technologies have been used in the processing of cherry fruits. The common methods include dehydration, pasteurization, and canning. Despite the advantages and beneficial effects of thermal processing particularly on preservation of foods, their effects could be undesirable too, which compromise especially the flavour and nutritional aspects of the product. Pulsed electric field (PEF) is a promising and emerging technology because its non-thermal properties prevent the detrimental effects of heat on valuable nutrients. Moreover, the use of mild or moderate intensity PEF on cherries have recently been explored for the possibility of extracting and stimulating biosynthesis of secondary metabolites such as anthocyanins and polyphenolics (Vallverdu-Queralt et.al 2012). The research questions or specific objectives along with the findings in this study are outlined below:

Specific Objective One:

To determine the basic physical properties such as the size range and average fruit weight of cherry samples. Findings revealed that the size range and average fruit weight of the Stella cherries used in this study were similar to that reported in literature.

To determine the effect of different PEF treatments on the physico-chemical properties of cherry samples such as juice yield, conductivity, pH, colour, titratable acidity, total soluble solids, and moisture. In our study, cherry samples were processed using PEF at mild or moderate electric field intensities (0.3, 0.7, 1.0, 1.4, 2.1, and 2.5 kV/cm). The

physicochemical characteristics of cherry samples were not significantly affected by PEF treatments in general. As for conductivity, only a slight change was observed in PEF-treated samples. The change however did not affect the target electric field strengths output because there was no significant change in the temperature after PEF treatment as well.

PEF treated samples were however significantly different to control sample in terms of juice yield, pH, titratable acidity, total soluble solids and moisture content. In terms of juice yield, a significant increase was observed in all PEF-treated samples compared to the control sample. The pH values of samples incubated for 24 hours after PEF (S3 samples) significantly increased compared to control and samples immediately after PEF treatment (S2 samples). In contrast, the TA and TSS values of most S3 samples after PEF significantly decreased compared to control and S2 samples. As the intensity of PEF treatments increased, the TSS value also increased for S3 samples. Moisture content on the other hand was significantly higher in S3 samples than S2P5 and S2P6 (processed with 1.7 and 2.1 kV/cm electric field intensity respectively), and control samples.

Specific Objective Two:

To determine the effects of different PEF treatments and storage conditions on the volatile profile of cherry samples. Samples incubated for 24 h after PEF treatment (S3) generated higher concentrations of volatiles than samples immediately after PEF treatments (S2). Overall, the effect of moderate intensity PEF treated samples induced higher amounts of volatile compounds characteristic of cherry flavour. Moreover, no undesirable compounds were detected for all samples because of the low energy intensities applied.

Specific Objective Three:

To perform method development for the optimum extraction of cherry samples for LCMS analysis and to determine the effects of different PEF treatments and storage conditions on the extraction or release of bioactive compounds including the anthocyanins and polyphenols on PEF-treated samples. An optimized extraction method for the extraction of anthocyanins and polyphenolics on cherry samples and LCMS method development were established prior to analysis. The use of liquid-liquid

extraction method with a new solvent combination was employed followed by separate LCMS method, which included a new gradient program for anthocyanins and polyphenolics to obtain an improved elution of each compound prior to analysis.

Among the anthocyanins, the cyanidin glucoside was significantly affected by PEF treatments. The samples incubated for 24 hours after PEF (S3) had significantly higher cyanidin glucoside content compared to most samples immediately after PEF (S2 samples). For polyphenols, a contrasting trend to anthocyanins was observed. Only four compounds, namely rutin, 4-hydroxybenzoic acid, isorhamnetin rutinoside, and myricetin, were significantly affected by storage and PEF treatments. Samples immediately after PEF (S2) generated significantly higher content of rutin, 4-hydroxybenzoic acid and isorhamnetin rutinoside compared to samples that were incubated for 24 hours (S3). Myricetin on the other hand, was the only compound that was significantly higher in S3 samples than S2 samples. Interestingly, polyphenol increased significantly as the electric field intensities decreased. This suggests that the effect of mild PEF intensities generated higher concentrations of polyphenolics.

Specific Objective Four:

To determine the growth of probiotic bacteria specifically the lactic acid bacteria on PEF-treated cherry samples. The growth of LAB in cherries was affected by the different PEF treatments overall. The effects of PEF on samples immediately after PEF treatment (S2 samples) were stimulatory (more nutrients were released after the application of PEF), which positively increased the LAB count, while inhibitory effects were observed for samples incubated for 24 hours after PEF (S3 samples), which resulted in a decrease in LAB counts.

Conclusion

From our findings in this research, it can be concluded that the application of mild or moderate PEF intensities influenced chemical, microbiological and flavour characteristics of cherries. With regards to methodology, this study has also demonstrated the use and capability of PEF processing in terms of extracting valuable components of cherries that included flavour volatiles and bioactive compounds. Based on our findings, food processors would actually have a technological edge using a non-thermal processing method like PEF at mild or moderate electric field intensities in

meeting the current consumer demand for minimally-processed and fresh-like cherry products.

5.1 Limitations

Inevitably, a study like this also faces limitations and compromises. The main limitation of this research was the lack of time to perform further optimization of the polyphenol extraction. In this study, the extraction methods for anthocyanins were successfully optimized. The extraction method developed through liquid-liquid extraction improved the extraction of anthocyanins but not polyphenolics. A different liquid matrix or different solvent combinations for extraction of polyphenols could be further tested to optimize extraction of polyphenolics.

5.2 Recommendations/Further Research

Analysis of other heat labile bioactive compounds. The effect of different PEF treatments on the retention of other heat labile bioactive compounds like Vitamin C, lutein, zeaxanthin and beta carotene content would also be important.

Sugar analysis. In this study, only the total soluble solids of PEF treated samples were determined. Although, this analysis would account for the main sugar in cherries (fructose and glucose), it would be worth investigating changes in the fructose, glucose, and sorbitol content of cherries with mild or moderate PEF treatments.

Sensory analysis. The current study only presented the volatile profile of the cherry samples treated by PEF. It would be good to further carry out sensory analysis of samples that can be further correlated with the flavour results from this study.

Texture analysis and Scanning Electron Microscopy (SEM) analysis. Physical analyses to determine how PEF treatment affects the texture and structure of cherry cellular tissues are also important. Texture analysis would help determine the effect of PEF on textural changes in cherry tissues due to permeabilization of mild intensity PEF. SEM would further reveal structural changes induced by PEF treatments. Changes in structure might support our findings on the release of volatile and bioactive compounds due to the reversible pore formation (resealing of cells) mechanism of PEF.

In-vivo and in-vitro analyses. It would be interesting to investigate in-vitro effects on PEF treated cherries with enhanced bioactive compounds to determine the protective effects of these bioactive compounds (anthocyanins and polyphenols) as antioxidants using cancer cell lines techniques.

Method validation. Fundamental parameters for assay validation would be very useful such as, linearity, precision, accuracy, and sensitivity. In this study, only the accuracy and sensitivity of the bioactive compounds were not established. The accuracy could be determined by calculating the percent recovery of each compound (Bilbao et al., 2007). This was not achieved in the study because of the insufficient chemical standards to work with as spiked materials. Determining the limit of detection (LOD) and limit of quantification (LOQ) could indicate the sensitivity of the assay. LOD was defined as the amount of analyte that gives a peak with a signal-to-noise ratio of 3, whereas LOQ was the lowest amount of analyte with a signal-to-noise ratio of 10 (Bilbao et al., 2007). Spike and recovery experiments could further validate the LCMS method for determination of polyphenolic compounds.

The use of a liquid matrix more suitable for polyphenolics extraction could be used. For instance, the use of a less acidic pH in the polyphenol extraction procedures is worth investigating. According to White and others (2010) and Arranz and others (2009), alkaline treatments have been found to be effective in releasing bound phenolics in phenolic extractions.

List of Publications

Journal Publication

Sotelo, K.A.G., Hamid, N., Oey, I., Gutierrez-Maddox, N., Ma, Q. L., Leong, S.Y (2014). Effect of Pulsed Electric Fields on the flavour profile of red-fleshed sweet cherries (Prunus avium var. Stella). Manuscript submitted for Molecules Open Access Journals which belongs to the special issue "Aromas and Volatiles of Fruits" by the Molecular Diversity Preservation International (MDPI) Journal. Received: 25 November 2014 / Revised: 25 February 2015 / Accepted: 10 March 2015 / Published: 23 March 2015

Oral Presentations

Sotelo, K.A.G., Hamid, N., Oey, I., Gutierrez-Maddox, N. (2014). Effect of Pulsed Electric Field (PEF) processing on the Physicochemical and Flavour Quality of Sweet Cherry Fruit Chunks. Oral presentation session presented at Postgraduate Conference 2014. November 21, 2014, Auckland University of Technology, Auckland, New Zealand.

Poster Presentations

Sotelo, K.A.G., Hamid, N., Oey, I., Gutierrez-Maddox, N. (2014). Effect of Pulsed Electric Field Processing on the Physicochemical and Flavour Quality of Cherry Fruit Chunks in Solution. Poster session presented at NZIFST Conference 2014. July 1-3, 2014: Challenges into Opportunities. Wigram, Christchurch, New Zealand.

Sotelo, K.A.G., Hamid, N., Oey, I., Gutierrez-Maddox, N. (2014). Effect of Pulsed Electric Field Processing on the Volatile Profile of Cherry Fruit Chunks in Solution. Poster session presented at 8th Annual AUT Postgraduate Symposium 2014. August 22, 2014 Auckland University of Technology, Auckland, New Zealand. Awarded best poster presenter.

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Appendix

Appendix A. Pulsed Electric Field Processing Operating Parameters Checklist

Pulsed Electric Field Processing of Cherry Fruit Chunks in Solution

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 (^{o}C)			
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			