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COLLEGE OF SCIENCE, ENGINEERING AND FOOD SCIENCE

SCHOOL OF FOOD AND NUTRITIONAL SCIENCES

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ANTIOXIDANT ACTIVITY OF PLANT-DERIVED EXTRACTS AS ASSESSED THROUGH DIETARY SUPPLEMENTATION OF CHICKEN DIETS AND VIA ACTIVE PACKAGING APPLICATIONS EMPLOYING COMMERCIAL CHICKEN PACKAGING FORMATS

THESIS

Presented by

Noorul Syuhada Mohd Razali

For the degree of

DOCTOR OF PHILOSOPHY

In Food Science and Technology

March 2019

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Declaration

I hereby declare that this thesis is my own work and contains no material that has been accepted for the award of any degree in University College Cork or elsewhere.

Certified by: _____

Noorul Syuhada Mohd Razali

Date: **8/3/2019**

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Abstract

Phenolic compounds, antioxidant activity (*in vitro* and in chicken muscle homogenates) and antimicrobial activity of grape seed (GS) extract and two mixtures of grape seed, olive leaf and sweet chestnut bark ($GS/OL/CB_1$ and $GS/OL/CB_2$) extracts were examined. Both extract mixtures contain diverse phenolic compounds and possessed greater ($p<0.05$) antioxidant activities than GS alone. Both extract mixtures exhibited moderate antimicrobial activities against Gram positive bacteria.

Chicken diets were supplemented with GS extracts (100, 200 and 300 mg/kg) and extract mixtures (50 mg/kg) for 35 days pre-slaughter. Quality parameters of fresh chicken breast meat stored in aerobic and modified atmosphere packaging (MAP) (40% $C0_2$: 60% N_2) at 4°C were compared to controls. GS-enriched diets inhibited lipid oxidation in chicken breast (300 mg/kg) and thigh (100 mg/kg) muscle homogenate systems. Supplementation did not influence the antioxidant status of blood plasma, lipid stability of chicken organs, proximate composition of chicken breast and thigh muscles and the majority of shelf-life parameters of chicken breast meat. GS extract (300 mg/kg) improved the sensorial attributes of chicken breast meat in both packaging condition.

The effect of GS-enriched diets on the shelf-life parameters of cooked chicken breast and thigh meat patties stored in aerobically and in MAP (40% CO_2 : 60% N_2) at 4°C was evaluated. GS-supplementation reduced the redness of cooked chicken breast and thigh patties stored in both packaging treatments. Dietary supplementation decreased the levels of lipid oxidation in cooked chicken patties and improved sensory qualities of cooked chicken patties stored in both packaging treatments.

The ability of both extract mixtures to improve the shelf-life parameters of cooked chicken breast stored in aerobic and MAP (40% CO_2 : 60% N_2) at 4°C, in comparison to tea catechin (TC) pads and films (positive control) was examined. TC, $GS/OL/CB_1$ and

GS/OL/CB_2 active pads (1.9 and 3.8 mg/cm^2) and films (0.7 and 1.5 mg/ml) were developed and characterised. Both active pads and films improved the lipid stability of cooked breast meat during refrigerated storage.

Dissemination

Submitted manuscript

Noorul-Syuhada, M.R., O'Grady, M.N. and Kerry, J.P. (2018). Characterisation of grape seed extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts for potential use as antioxidant and antimicrobial agents in poultry muscle (Submitted to *Food Chemistry*).

Manuscripts (in preparation)

Noorul-Syuhada, M.R., O'Grady, M.N. and Kerry, J.P. (2018). Supplementation of grape seed extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts in chicken diets: effects on quality and shelf-life characteristics of raw chicken meat (In preparation for submission to *International Journal of Poultry Science*).

Noorul-Syuhada, M.R., O'Grady, M.N. and Kerry, J.P. (2018). Effect of dietary supplementation of grape seed extract on the quality and shelf-life of cooked chicken patties stored in aerobic and modified atmosphere packaging conditions (In preparation for submission to *British Poultry Science*).

Noorul-Syuhada, M.R., O'Grady, M.N. and Kerry, J.P. (2018). The effect of plant extract-enriched 'active' pads on the oxidative stability of cooked chicken breast meat stored aerobically and in modified atmosphere packages (In preparation for submission to *Food Packaging and Shelf Life*).

Noorul-Syuhada, M.R., O'Grady, M.N. and Kerry, J.P. (2018). Use of plant extract-enriched 'active' gelatin films to enhance the oxidative stability of cooked chicken breast meat stored in aerobic and vacuum skin packaging conditions (In preparation for submission to *Food Packaging and Shelf Life*).

Poster presentation

Noorul-Syuhada, M.R., O'Grady, M.N. and Kerry, J.P. (2016). Antioxidant active packaging and the oxidative stability of cooked chicken. In: *Proceedings of the 18th IuFoST World Congress of Food Science and Technology,* Dublin, Ireland, 21 – 25 August 2016.

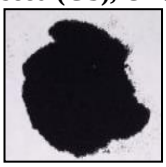
Schematic Overview of Thesis

Plant-derived extracts

Grape seed extract

(GS)

2 Mixes of:
Grape seed (GS), Olive leaf (OL) and Sweet chestnut bark (CB) extracts



(GS/OL/CB$_1$)

(GS/OL/CB$_2$)

Tea catechin extract

(TC)

GS
GS/OL/CB$_1$
GS/OL/CB$_2$

GS/OL/CB$_1$
GS/OL/CB$_2$
TC

ANTIOXIDANT AND ANTIMICROBIAL PROFILING

Chapter 2

- Identification of polyphenol compounds (LC-MS-TOF)
- *In vitro* antioxidant assays
- Lipid oxidation of chicken breast and thigh homogenates
- *In vitro* antimicrobial assay

FUNCTIONALITY IN CHICKEN DIETS

Chapter 3
EFFECT ON FRESH RAW CHICKEN

- Antioxidant status of chicken plasma and tissue systems
- Proximate composition of raw chicken breast and thigh
- Quality and shelf life of fresh raw chicken breasts (colour, pH, drip loss, cook loss, texture, microbiology, lipid oxidation and sensory)

GS

Chapter 4
EFFECT ON COOKED CHICKEN MUSCLES

- Quality and shelf life of cooked breast or thigh patties (colour, lipid oxidation, microbiology and sensory)

FUNCTIONALITY IN ACTIVE PACKAGING APPLICATIONS

Chapter 5
ANTIOXIDANT ACTIVE PADS

- *In vitro* antioxidant assays
- Development of active pads and proposed mechanism of action
- Quality and shelf life (colour, pH and lipid oxidation)

Chapter 6
ANTIOXIDANT ACTIVE FILMS

- Film characterisation (*in vitro* antioxidant activities, physical properties and structural profiles)
- Quality and shelf life (colour and lipid oxidation)

List of Abbreviations

*a**	Redness
ABTS	2,2'-azino-di-[3-ethyl- benzthiazoline sulphonate]
APLSR	ANOVA-Partial Least Squares Regression
*b**	Yellowness
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CB	Sweet chestnut bark extract
CFU	Colony forming unit
CO_2	Carbon dioxide gas
cm	Centimeter
cm^2	Centimeter square
DPPH	2,2-diphenyl-1-picrylhydrazyl
$DPPH^{\bullet}$	chromogen radical
DPPH-H	non-radical pale yellow hydrazine form
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
EQ	Ethoxyquin
EVOH	ethylvinylalcohol
$FeCl_3.6H_20$	ferric chloride hexahydrate
$FeCl_3$/Asc	ferric chloride/sodium ascorbate
Fe^{2+}-TPTZ	Ferrous-tripyridyltriazine complex
Fe^{3+}-TPTZ	Ferric-tripyridyltriazine complex
FRAP	Ferric reducing antioxidant power
FTIR	Fourier transform infrared
g	gram
GAE	Gallic acid equivalents
GCG	Gallocatechin-3-O-gallate
GP	Grape pomace
GPC	Grape pomace concentrate
GRAS	Generally recognised as safe
GS	Grape seed extract
GS100	Diet supplemented with 100 mg/kg grape seed extract
GS200	Diet supplemented with 200 mg/kg grape seed extract
GS300	Diet supplemented with 300 mg/kg grape seed extract
GSH	Reduced glutathione
GSPE	Grape seed proanthocyanidin extract
$GS/OL/CB_1$	Mixture of grape seed, olive leaf and sweet chestnut bark extracts no 1

GS/OL/$CB_1$50	Diet supplemented with GS/OL/CB_1 extract at 50 mg/kg
GS/OL/CB_2	Mixture of grape seed, olive leaf and sweet chestnut bark extracts no 2
GS/OL/$CB_2$50	Diet supplemented with GS/OL/CB_2 extract at 50 mg/kg
HAT	Hydrogen atom transfer
HCl	Hydrochloric acid
hr	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
H_3BO_3	Boric acid
KCl	Potassium chloride
kg	kilogram
kPa	Kilopascal
kV	Kilovolts
L	Litre
*L**	Lightness
LC-MS	Liquid chromatography mass spectroscopy
LDPE	Low-density polyethylene
MAP	Modified atmosphere packaging
MDA	Malondialdehyde
MIC	Minimum inhibition concentration
min	minute
mg	Miligram
MHB	Mueller Hinton Broth
ml	Mililitre
mM	Milimolar
mm	Milimeter
mm^2	Milimeter square
mmol	milimole
Mo^{6+}	molybdenum ions
Mo^{5+}	molybdenum ions
m/z	mass to charge ratio
N	Newton
N_2	Nitrogen gas
Na_2CO_3	sodium carbonate
NO	Nitrogen oxide
ng	nanogram
nm	nanometer
O_2	oxygen
$O_2^{\bullet-}$	Superoxide radical
$^{\bullet}OH$	Hydroxyl radical

OL	Olive leaf extract
OMWW	Olive oil mill waste water
OP	Oxygen permeability
ORAC	Oxygen reducing antioxidant capacity
PE	Polyethylene
PET	Polyethylene terephthalate
ppm	Part per million
PUFAs	Polyunsaturated fatty acids
PVC	polyvinylchloride
RH	Relative humidity
ROS	Reactive oxygen species
rpm	Rotation per minute
s	second
SCW	Sweet chestnut wood
SEM	Scanning electron microscope
SET	Single electron transfer
SOD	Superoxide dismutase
Spp.	Species
TAS	Total antioxidant status
TBA	2-thiobarbituric acid
TBARS	2-thiobarbituric acid-reactive substances
TC	Tea catechin
TCA	Trichloroacetic acid
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TOF	Time-of-flight
TPC	Total phenol content
TSA	Tryptic soy agar
TVC	Total viable count
UV	UV-Vis
WBSF	Warner-Bratzler shear force
WVP	Water vapour permeability
VSP	Vacuum skin packaging
ΔE^*	Total colour difference
%	Percentage
µm	Micrometer
µg	Microgram
µl	Microlitre
θ	Contact angle

CHAPTER 1

1 Literature Review

1.1 Introduction

Poultry refers to all domesticated species of birds including chickens, ducks, turkeys, quail, pigeons and ostrich (FAO 2003). Poultry meats are consumed worldwide due to their high nutritional value (high protein) and low price (Salmon and Audran 2016). In the poultry industry, broiler chickens are reared specifically for meat production due to their rapid growth rate, high feed conversion and quantity of breast meat obtained (SafeFood 2005; Nardoia 2016). Commercial broiler production involves chickens rearing chicken indoors in a controlled environment and feeding with a numbers of high nutrient diets (Connell and Lynch 2004). Broilers are typically fed nutrients which include; polyunsaturated fatty acids (PUFAs), vitamins, minerals (such as iron and copper) and enzymes (phytase). The focus is to supply broiler with sufficient essential nutrients and energy beneficial for well-being, growth performance, nutrient and energy utilisation (Wenk 2003). However, ingredients such as iron, copper and phytase can catalyse oxidation of lipids and other nutrients which affect storage stability of the feed materials.

Broilers are usually reared in cramped commercial units which increases susceptibility to infection by certain microorganisms, such as protozoan parasites, *Campylobacter* spp., *Salmonella* spp., *Escherichia coli*, and others. Microorganisms not only compromise the general health and well-being of broilers, but some also cause food-borne illness in humans upon consumption. Coccidiosis is an infection in poultry which is caused by protozoan parasites resulting in significant profit losses in the poultry industry (SafeFood 2005). Coccidiostats and histomonostats are used in chicken diets intentionally to protect chickens against parasitic-related diseases, but only limited numbers of these additives are authorised for use in European countries as listed in Regulation (EC) No 1831/2003 (European Commission 2014). However, concerns exist that such additives may

increase antimicrobial resistance in chickens and residual compounds may also be transferred to humans upon meat consumption.

Nutritional, pathological, physiological or environmental poultry husbandry factors during rearing can elevate the formation of free radicals debilitating health, overall performance and meat quality of broiler chickens (Salami *et al.* 2015). Diets containing polyunsaturated fatty acids (PUFAs) or highly oxidised fats, insufficient amount of nutrients and a high stocking density (cramped production unit) may induce oxidative stress in broilers (Morrissey *et al.* 1998; Salami *et al.* 2015). Oxidative stress increases the risk of oxidative rancidity in muscle tissues of living chickens and the risks may can remain after slaughter (Salami *et al.* 2015). In addition, the conversion of muscle to meat post-slaughter can also lead to the oxidation of the highly unsaturated phospholipid fractions in subcellular membranes (Morrissey *et al.* 1998).

The National Research Council (1994) recommends supplementation of vitamin E into poultry diets at a level of 10 mg all-rac-α-tocopheryl acetate/kg feed. This dose is only sufficient for prophylaxis and growth promotion purposes but insufficient to protect chickens under conditions of oxidative stress (Salami *et al.* 2015). In addition, vitamin E can be added into chicken diets to act as antioxidant agents for the protection of feed materials against oxidative deterioration throughout production, processing and storage of feeds and maintaining taste, appearance and palatability characteristics (European Commission 2003; Salami *et al.* 2016). Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ethoxyquin (EQ), have previously been used to perform the same function (Błaszczyk *et al.* 2013) however their use in poultry diets is no longer permissible in European countries as revised in Regulation (EC) No 1831/2003 (European Commission 2014).

Efforts have been made to remove or reduce the use of synthetic additives in chicken diets and chicken meat since such additives may impose a risk to public health and due to the increased public awareness and consumer demands for healthier and 'clean label' food products. Furthermore, research efforts aim to fully or partially replace the use of expensive vitamin E with plant extracts from various food and beverage industries. Plant-derived extracts contain polyphenols which possess numerous bioactive properties such as antioxidant, antimicrobial and antiviral activities. These extracts can perform similar functions to vitamin E and synthetic antioxidants delivering prophylaxis functionality enhanced health status and protecting chickens against oxidative stress and lipid oxidation (Surai 2014; Salami *et al.* 2015).

1.2 Plant materials as sources of bioactive compounds

Phenolic compounds or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom (Bravo 1998) and are present in materials such as grape seed, olive leaf, sweet chestnut bark and green tea. Phenolic compounds possess one or more aromatic rings with one or more hydroxyl groups and generally, are categorised as flavonoids, phenolic acids, stilbenes, coumarins, and tannins (Figure 1.1) (Liu 2004). Flavonoids share a common structure consisting of two aromatic rings (A and B) that are bound together by 3-carbon atoms which form an oxygenated heterocycle (ring C) (Figure 1.2) (Manach *et al.* 2004). Differences in the generic structure of the heterocycle C ring classify phenolic compounds as flavonols, flavones, flavanols (catechins and proanthocyanidins), flavanones, anthocyanidins and isoflavonoids (Figure 1.1) (Liu 2004; Manach *et al.* 2004). Non-flavonoids contain an aromatic ring with one or more hydroxyl groups including phenolic acids, stilbenes, saponins and other polyphenols (Surai 2014).

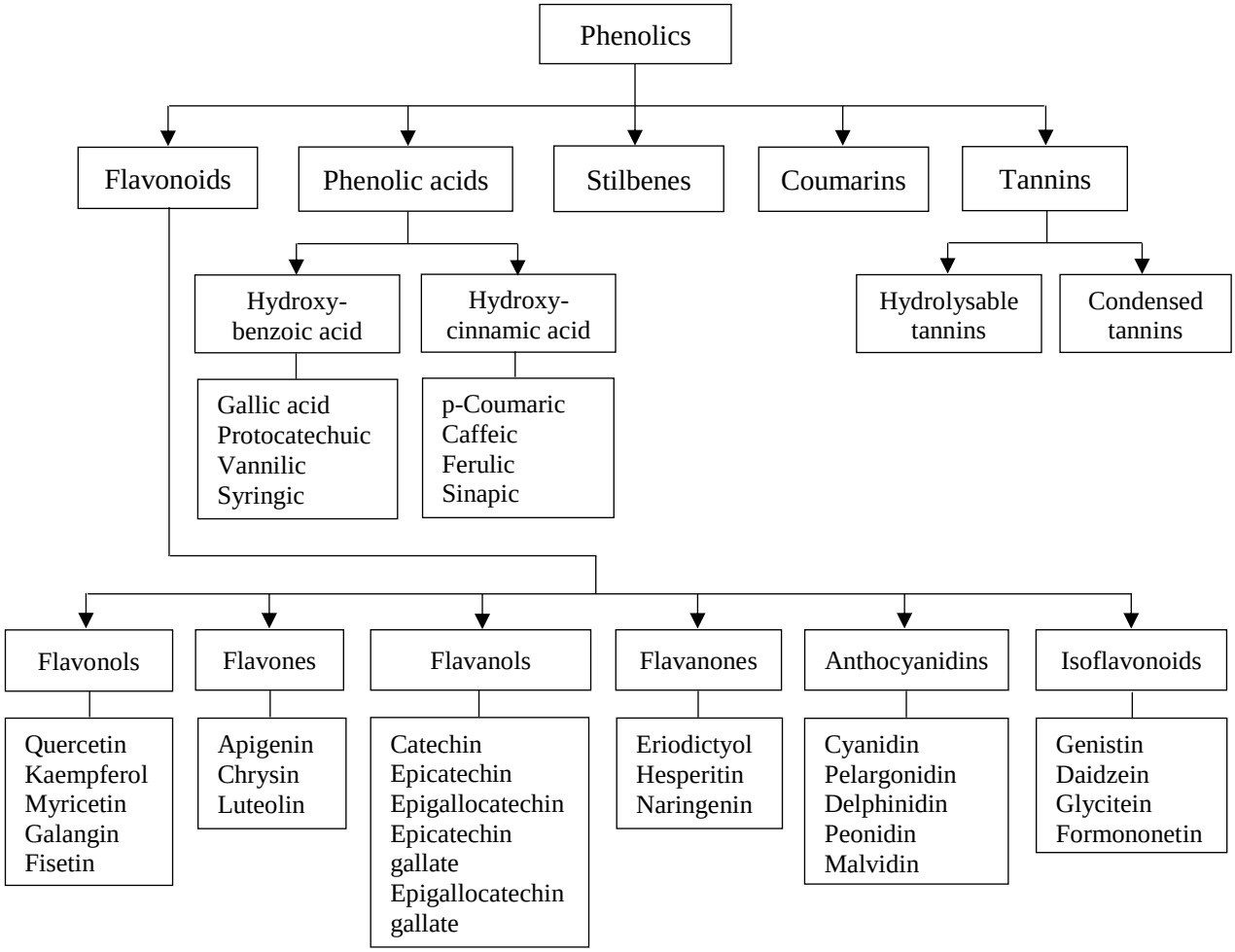


Figure 1.1 Classification of phenolics.
Source: Bravo (1998) and Liu (2004).

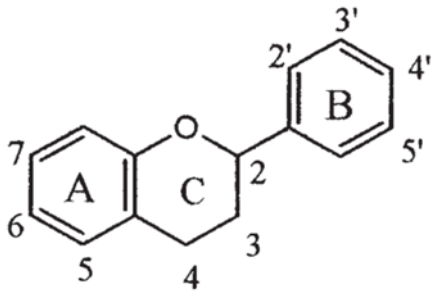


Figure 1.2 The generic structure of flavonoids.
Source: Liu (2004).

1.2.1 Grape seed extract

Grapes (*Vitis vinifera*) are one of the world's largest fruit crops with approximately 75 million tons produced annually (FAO-OIV 2016). Grape juice and wine industries generate large quantities of waste residue mainly solid by-product such as grape pomace (seeds, skin and stems) (Brenes *et al.* 2016). The seeds contain greater concentration of phenolic compounds as compared to the skin (Rockenbach *et al.* 2011). Grape seed represents 38 – 52% of grape pomace on dry matter basis and it can be separated, extracted, dried and purified to produce grape seed extract (Lau and King 2003; Brenes *et al.* 2016). The major composition of crude grape seed extract is summarised in Table 1.1 where total flavonoids represent approximately 41% of the compounds present.

Table 1.1 Chemical composition of grape seed extract.

Constituent	**Composition (%)**
Total flavanols	40.9
Procyanidins	38.5
Monomeric flavanols	2.4
Total sugar	19.3
Glucose	7.8
Sucrose	trace
Fructose	8.9
Total organic acids	16.7
Citric acid	11.6
Malic acid	trace
Ash	5.0
Moisture	2.5
Protein	3.7

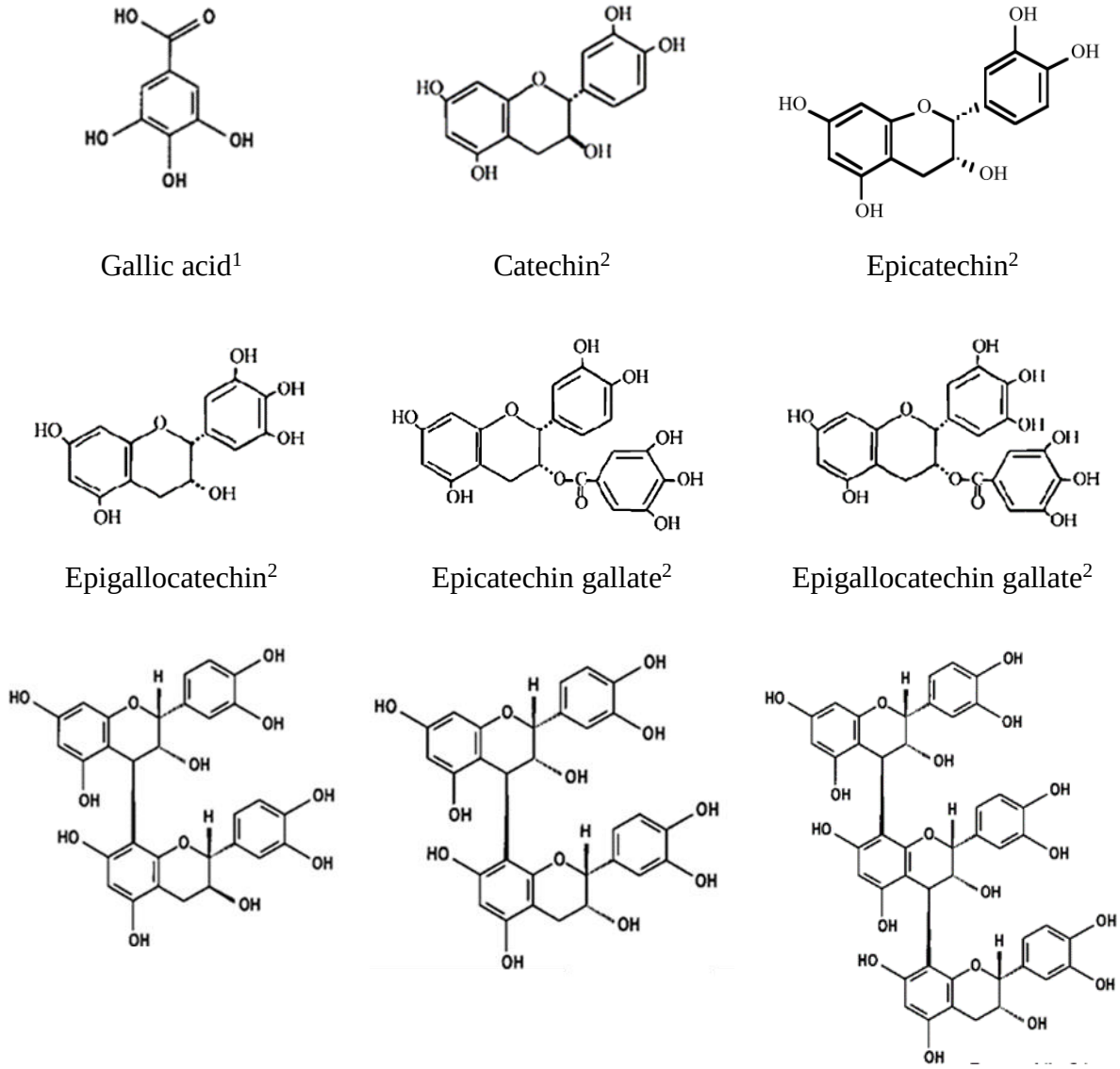
Source: Saito *et al.* (1998).

1.2.1.1 Bioactive compounds present in grape seed extract

Grape seed extract is rich in phenolic compounds including phenolic acids (gallic acid), chlorogenic acid, flavanols (catechin, epicatechin and epicatechin derivatives and proanthocyanidins B1, B2, B3, B4 and C1), flavonols (rutin, quercetin and kaempferol derivatives) and stilbenes (trans-resveratrol) (Escribano-Bailon *et al.* 1992; Rockenbach *et al.* 2011) as depicted in Figure 1.3. Monomeric (catechin and epicatechin) and proanthocyanidin polymers (epicatechin-3-*O*-gallate, procyanidin B2, B5, B2-3-*O*-gallate, B2-3'-*O*-gallate, C1 and two trimers) in grape seed extract scavenge superoxide ($O_2^{\bullet -}$) and hydroxyl ($^{\bullet}OH$) radicals (Da Silva *et al.* 1991). The superior antioxidant capacity (peroxyl radical scavenging ability) of grape seed extract is mainly attributed to its dimeric, trimeric, oligomeric, and/or polymeric procyanidins (Yilmaz and Toledo 2004). Xu *et al.* (2010) found that DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-di-[3-ethyl-benzthiazoline sulphonate]) radical scavenging abilities and ferric reducing antioxidant power (FRAP) of grape seed extract are highly correlated to the main phenolic compounds (total phenols, flavonoids, flavan-3-ols) present in the grape seed extract.

Grape seed extract exhibits effective antibacterial activity against Gram positive bacteria (*Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria innocua* and *Brochothrix thermosphacta*) compared to Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica*) (Jayaprakasha *et al.* 2003; Delgado Adámez *et al.* 2012). Gram negative bacteria have an outer membrane surrounding the cell wall which functions to restrict diffusion of compounds through a lipopolysaccharide layer (Vaara 1992) which may reduce the antimicrobial potency of the extracts (Delgado Adámez *et al.* 2012). The extent of the inhibitory effects of grape seed extract is attributed to the polyphenols composition and profile. Gallic acid is identified as the active compound responsible to inhibit the growth of *Escherichia coli* (Jayaprakasha *et*

al. 2003) where the three hydroxyl groups of gallic acid are responsible for antibacterial activity (Delgado Adámez *et al.* 2012).



Gallic acid[1] Catechin[2] Epicatechin[2]

Epigallocatechin[2] Epicatechin gallate[2] Epigallocatechin gallate[2]

Procyanidin B1[1] Procyanidin B2[1] Procyanidin C1[1]

Figure 1.3 Chemical structures of polyphenol compounds present in grape seed. Source: [1]Nakamura *et al.* (2003) and [2]Yilmaz (2006).

1.2.2 Olive leaf extract

Olive leaf, which is a by-products of olive tree farming, accumulates during the pruning of olive trees (*Olea europaea* L.). In the olive oil industry, olive leaf represents ~10% of the total weight of material present at an olive oil press (Paiva-Martins *et al.* 2009).

In recent times, olive leaf can be processed into olive leaf extract and both olive leaf and olive leaf extract have been utilised as animal feed, food additives and supplement due to its availability, low cost and minimal processing (Altiok *et al.* 2008; Govaris *et al.* 2010; Lee and Lee 2010). OL extract contains active compounds termed as olive biophenols which exert therapeutic properties such as antioxidant and antimicrobial properties (Benavente-García *et al.* 2000; Sudjana *et al.* 2009). Olive leaf extract contains a large amount of moisture and fibre and moderate to high level of phytochemicals (Table 1.2).

Table 1.2 The composition of olive leaf extract.

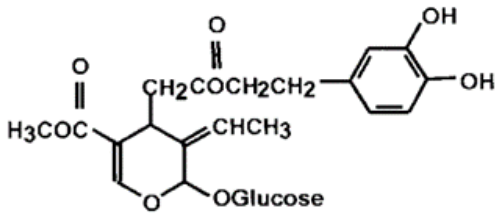
Constituent	**Composition (%)**
Moisture	48.80
Carbohydrate	6.60
Crude fat	24.08
Crude fibre	30.92
Ash content	7.60
Phytochemicals	**Level present***
Alkaloids	+++
Flavonoids	+++
Tannins	++
Saponins	+++
Terpenoids	++
Sterol and steroids	++
Glycosides	+++

*+++= high, ++ = moderate, + = low
Source: Rajappa and Shaji (2015).

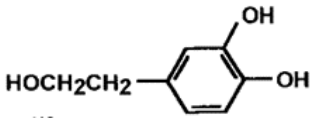
1.2.2.1 Bioactive compounds present in olive leaf extract

Benavente-García *et al.* (2000) reported that olive leaf extract contains five compound groups: oleuropeosides (oleuropein and verbacoside); flavones (luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7-glucoside, luteolin and deosmetin); flavonols (rutin); flavan-3-ols (catechin) and substituted phenols (tyrosol, hydroxytyrosol, vanillin, vanillic acid and caffeic acid). The most abundant compounds in olive leaf extract is

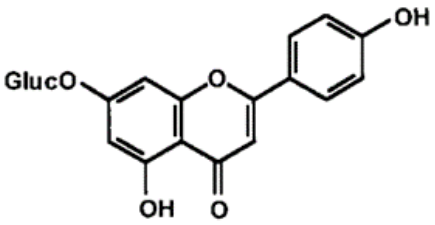
oleuropein and followed by hydroxytyrosol (precursor of oleuropein) (Benavente-García *et al.* 2000). Figure 1.4 shows the chemical structures of phenolics found in olive leaf.



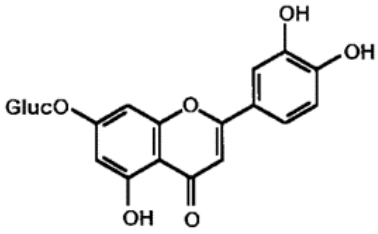
Oleuropein



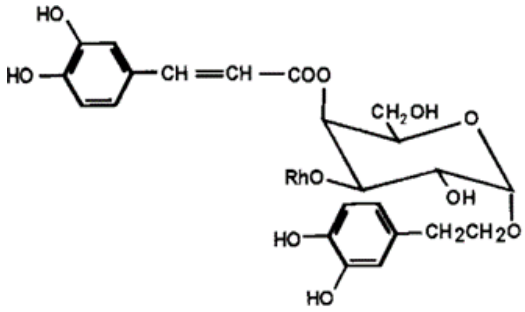
Hydroxytyrosol



Apigenin-7-glucoside



Luteolin-7-glucoside



Verbascoside

Figure 1.4 Chemical structures of phenolics present in olive leaf.
Source: Benavente-García *et al.* (2000).

Olive leaf extract has been shown to possess antioxidant activities including DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power (FRAP), oxygen reducing antioxidant capacity (ORAC) and β-carotene bleaching activity (Benavente-García *et al.* 2000; Hayes *et al.* 2011). The antioxidant activity of olive leaf extract is based on the ability of the phenolic compounds to transfer single electron (DPPH and FRAP

assays) and hydrogen atoms (ORAC) (Hayes *et al.* 2011). The flavonols, flavans-3-ols and flavones with a catechol structure are efficient olive phenolic compounds which quench the ABTS radical cation however activity depends on the number and position of free hydroxyl groups in their structure (Benavente-García *et al.* 2000; Hayes *et al.* 2011). Hydroxytyrosol has an antioxidant capacity similar (ABTS) to olive leaf extract due to the presence of a conjugated double bond with the catechol structure (3',4'-catechol B ring substitution pattern) (Benavente-García *et al.* 2000).

Olive leaf extract also has antimicrobial activities against Gram positive bacteria (*B. cereus*, *B. subtilis* and *S. aureus*), Gram negative bacteria (*P. aeruginosa*, *E. coli* and *Klebsiella pneumoniae*) and fungi (*Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton rubrum*, *Candida albicans* and *Cryptococcus neoformans*) (Markín *et al.* 2003; Pereira *et al.* 2007). The antimicrobial potencies of olive leaf extract is attributed to the ability of the biophenols (an o-diphenol system on the backbone structure of olive polyphenols) to penetrate to the cell membrane of Gram positive and Gram negative bacteria (Bisignano *et al.* 1999). Oleuropein is less toxic to bacterial cells than hydroxytyrosol possibly due to the fact that the glycosidic group prevents oleuropein from reaching the target site.

1.2.3 Sweet chestnut bark extract

Sweet chestnut (*Castanea sativa* Mill.), is a tree belong to the *Fagaceae* family, present in Mediterranean regions of Europe (Chiarini *et al.* 2013). The *Castanea sativa* Mill. species is predominant and mostly consumed in Portugal with an annual chestnut fruit production reaching 20,000 tons (Barreira *et al.* 2008). The bark and wood of sweet chestnut trees are important sources of tannins and other phenolic compounds (Chiarini *et*

al. 2013). The chemical composition of bark and wood from sweet chestnut trees is similar and tannins represent a major amount of chemical compounds present (~78%) (Table 1.3).

Table 1.3 Chemical compositions of sweet chestnut bark and wood extract.

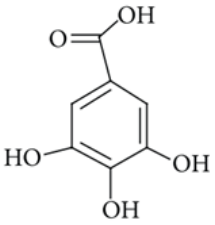
Constituent	**Bark extract[1] (%)**	**Wood extract[2] (%)**
Water	2.9	2.9
Tannin	77.8	77.8
Non-tannin (oligosaccharides, salt, vegetable resins and gums)	17.7	17.7
Insoluble	1.6	1.6
Crude fibers	0.24	-
Ash	1.7	-

Source: [1]Brizi *et al.* (2016), [2]Liu *et al.* (2009).

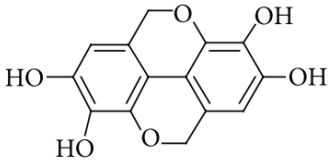
1.2.3.1 Bioactive compounds present in sweet chestnut bark extract

Tannins are classified as condensed tannins and hydrolysable tannins (Figure 1.1) (Schiavone *et al.* 2008). Condensed tannins (or proanthocyanidins) are composed of a group of polyhydroxy-flavan-3-ol oligomers and polymers linked by carbon-carbon bonds between flavanol subunits (Schofield *et al.* 2001). Hydrolysable tannins can be divided into gallotannins and ellagitannins having gallic acid and ellagic acid residues, respectively, attached to the hydroxyl group of glucose by ester linkage (Ekambaram *et al.* 2016).

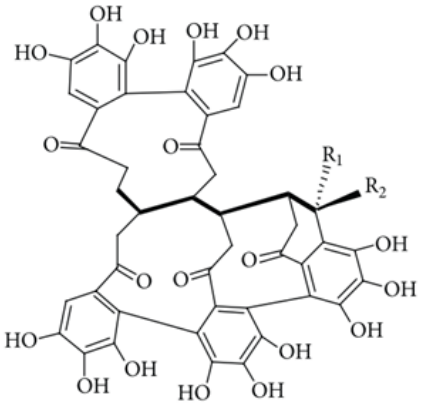
Sweet chestnut bark extract contains major component of vescalagin, castalagin, ellagic acid, vescalin, castalin and gallic acid (Figure 1.5) as well as minor compounds detected in trace levels; 5-*O*-galloylhamamelose, (3,5-dimethoxy-4-hydroxyphenol)-1-*O*-*β*-D-(6'-*O*-galloyl)-glucoside isomer, m-digallic acid, kurigalin isomer and chestanin (Chiarini *et al.* 2013; Comandini *et al.* 2014). Compounds such as castalin, vescalin, castalagin, vescalagin, kurigalin, 5-*O*-galloylhamamelose and chestanin are classified as ellagitannins (Comandini *et al.* 2014).



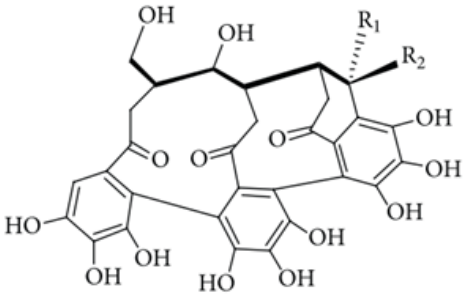
Gallic acid



Ellagic acid



Vescalagin (R_1=H, R_2=OH)
Castalagin (R_1=OH, R_2=H)



Vescalin (R_1=H, R_2=OH)
Castalin (R_1=OH, R_2=H)

Figure 1.5 Chemical structure major phenolics present in sweet chestnut bark. Source: Chiarini *et al.* (2013).

In a human neuroblastoma cell model, sweet chestnut bark extract exhibits *in vitro* antioxidant activity against oxidative stress, suggesting potential health benefits in the prevention of neuronal diseases (Brizi *et al.* 2016). Chiarini *et al.* (2013) demonstrated that sweet chestnut bark extract reduces the formation of reactive oxygen species (ROS) and improves cell viability following oxidative stress in cultured cardiomyocytes in a concentration-dependent manner, indicating cardioprotective activity. Antioxidant activities of sweet chestnut bark extract are attributed to the high phenolic and tannin content (Chiarini *et al.* 2013; Brizi *et al.* 2016). In addition, Vekiari *et al.* (2008) reported that sweet chestnut bark contains a significant concentration of ellagic acid. Ellagic acid exhibits good antioxidant activities (DPPH, ABTS, FRAP and ORAC) owing to the

presence of four phenolic hydroxyl groups with a fused benzofuran structure (Hayes *et al.* 2011).

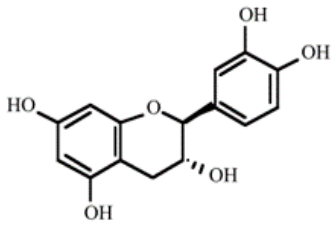
To date, the scientific literature contains no information on the antimicrobial properties of sweet chestnut bark extract. Chestnut wood extract contains hydrolysable tannins including gallic acid, ellagic acid, vescalagin, castalagin, castalin, roburin A-E and grandinin (Voljč *et al.* 2013) and displays inhibitory activity against the growth of *Salmonella gallinarum*, *Pasteurella multocida*, *Staphlycoccus aureus*, *Campylobacter jejuni*, *Salmonella enteritidis*, *Escherichia coli* and *Clostridium perfringens* in *in vitro* assays (Graziani *et al.* 2006). Chestnut wood extract also possesses *in vitro* antiviral activity against avian reovirus and avian metapneumovirus due to an interaction between tannins and viral proteins which inhibit the attachment and penetration of the virus into the cell membrane (Lupini *et al.* 2009). Since sweet chestnut bark extract contains a polyphenolic profile similar to sweet chestnut wood, it is plausible to assume that sweet chestnut bark extract would display antimicrobial activity and anti-viral activity in poultry.

1.2.4 Green tea catechin

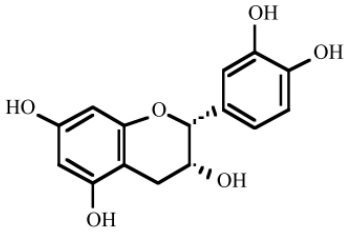
Green tea (*Camellia sinensis* L.) grows primarily in the tropical and temperate regions of Asia mainly China, India, Sri Lanka and Japan as well as in other countries such as Africa and South American (Senanayake 2013). Leaves of *Camellia sinensis* plant can be processed to produces green (unfermented), oolong (partially fermented) and black (fully fermented) teas (Chou *et al.* 1999; Senanayake 2013). Green tea has received great attention for its antioxidant properties due to the present of functional polyphenols, known as tea catechins (~86%) (Tang *et al.* 2002).

1.2.4.1 Bioactive compounds present in green tea catechins

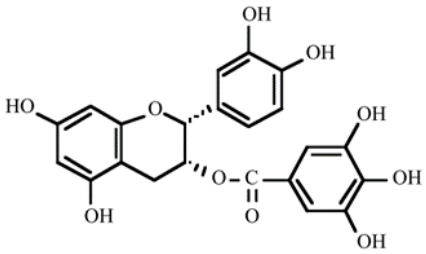
Green tea is one of the major sources of natural flavan-3-ol tannins (Hong *et al.* 1994) and isomers include: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) (Figure 1.6) (Tang *et al.* 2001a). The relative ratio of catechin, gallocatechin, EGC, ECG and EGCG in green tea is approximately 1.0 : 1.3 : 1.1 : 6.7 : 30.0 (Hong *et al.* 1994). Green tea contains greater amounts of EGCG, EGC, ECG and epicatechin compared to oolong and black teas because the fermentation process in tea leaf processing reduces the concentration of catechin in the end product (Zuo *et al.* 2002).



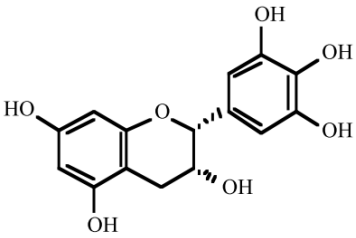
Catechin



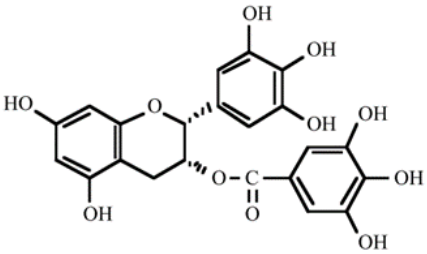
Epicatechin (EC)



Epicatechin gallate (ECG)



Epigallocatechin (EGC)



Epigallocatechin gallate (EGCG)

Figure 1.6 Chemical structure of catechins present in green tea.
Source: Shimamura *et al.* (2007)

Green tea catechin extract exhibits *in vitro* antioxidant activities (ORAC and DPPH and ABTS radical scavenging activities) (Tsai *et al.* 2008). Benzie and Szeto (1999) reported a strong correlation between the FRAP value of a green tea infusion and the total phenol content, indicating that the number of phenolic hydroxylic groups present in catechins are a major determinant of antioxidant power. The activity is primarily attributed to the combination of aromatic rings and hydroxyl groups assembling the chemical structure and capability of the hydroxyl groups to bind and neutralise free radicals (Senanayake 2013). ECG, EGCG and gallocatechin-3-*O*-gallate (GCG) compounds purified from tea demonstrated potency in inhibiting lipid oxidation in rat heart mitochondria due to the galloyl group in 3-*O*-position which increase the scavenger activity of flavan-3-ol tannins (Hong *et al.* 1994).

The concentration of catechins in green tea not only contributes to antioxidant potency but also give rise to antimicrobial activity. Green tea extract has demonstrated antimicrobial activities against the growth of Gram positive bacteria (*Streptococcus sanguinis*, *Staphlycoccus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*), Gram negative bacteria (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Escherichia coli*, *Proteus vulgaris* and *Salmonella* spp.) and fungi (*Candida albicans*) (Chou *et al.* 1999; Tsai *et al.* 2008; Archana and Abraham 2011).

1.3 Methodologies for characterising polyphenolic compounds and the bioactivity of plant-derived extracts

Plant-derived extracts, such as grape seed, olive leaf and sweet chestnut bark, contain numerous and complex phenolic compounds possessing bioactivities. Ignat *et al.* (2011) reviewed instrumental analytical techniques (such as spectrophotometric and chromatographic) employ for the separation, qualification and quantification of phenolic

compounds present in various plant extracts. Spectrophotometric methods are utilised for the determination of plant phenolics (such as total phenol and total flavonoids) due to simplicity and low cost, however such methods only provide an estimated result and do not separate or quantify individual compounds present (Ignat *et al.* 2011). The separation and determination of individual phenolics compounds in plant-derived extract is achieved using chromatographic techniques such as gas chromatography, high performance liquid chromatography (HPLC) or liquid chromatography (LC).

Chromatographic separation depends on the molecular weight, stereo-chemistry, polarity and secondary modification of the analytes, implying that specific methods are employed depending on the nature of the analyte (Domínguez-Rodríguez *et al.* 2017). The determination of polyphenols using gas chromatography requires derivatisation to volatile compounds (such as by methylation and trifluoroacetylation) (Ignat *et al.* 2011). A reverse phase HPLC system is a preferred analytical tool for the determination of the phenolics compounds using polarity-based elution methods. The sensitivity and detection limits of HPLC equipment limit the use of this technique for the determination of phenolics compounds due to the complexity of compounds present in plant-derived extracts, which may result in co-eluting or overlapping peaks (Ignat *et al.* 2011; Domínguez-Rodríguez *et al.* 2017).

Mass spectrometry (MS) is an analytical technique used to elucidate the chemical structures of molecules, such as polyphenols (Ignat *et al.* 2011). A mass spectrometer converts the eluted analytes into a charged (ionised) state, with subsequent analysis of the ions and fragment ions generated during the ionisation process, on the basis of their mass to charge ratio (*m/z*) (Pitt 2009). Detection and characterisation of plant-derived extracts in chapter 2 of this thesis utilised electrospray ionisation (ESI). ESI-MS can discriminate

among classes of polyphenol families and gather information on the glycosylation position (Domínguez-Rodríguez *et al.* 2017).

Previous studies reported the concentration of phenolic compounds present in grape seed and grape seed extracts using HPLC techniques with detectors, such as diode array detector (DAD) and UV-VIS detector as outlined in Table 1.4. The type and concentration of phenolic compounds present depends on many factors such as grape variety, environmental conditions, processing and chromatography techniques employed (level of detection, sensitivity etc.).

Table 1.4 Phenolic composition of grape seed and grape seed extract.

Phenolics compounds	**Grape seed**	**Grape seed extract**	**Grape seed extract**
	HPLC-UV-VIS[1]	**HPLC-DAD[2]**	**HPLC-DAD[3]**
	mg / 100 g	**%(w/w)**	**mg / 100 g**
Gallic acid		0.02 - 2.06	
Catechin	21 - 155	1.03 - 4.93	27.12 - 117.00
Epicatechin	23 - 193	0.61 - 2.83	17.78 - 47.50
chloeogenic acid			2.87 - 6.8
Procyanidins B1	3 - 62	0.7 - 1.73	
Procyanidins B2	9 – 106	0.66 - 1.54	
Procyanidins B3	2 – 71		
Procyanidins B1-3-O-gallate	3 - 74		
Procyanidins B2-3-O-gallate	2 – 26		
Procyanidins B2-3'-O-gallate	1 – 8		
Procyanidins C1	0 - 10	0.4 - 1.26	
Procyanidins T2	3 - 76		
Quercetin derivatives			2.35 - 3.68
Rutin			2.57 - 9.05
t-Resveratrol			1.11 - 1.42

Sources: [1](Fuleki and Da Silva 1997), [2](Nakamura *et al.* 2003), [3](Rockenbach *et al.* 2011).

Grape seed extract contains oligomers heptamers consisting of procyanidins and their galloylated derivatives. HPLC-MS/MS is utilised to obtain characteristic fragmentation data in the cases where copolymers occur (Lazarus *et al.* 1999). Authors also demonstrated the ability of the HPLC/MS technique to separate single and double linked procyanidins, prodelphinidins, and copolymer oligomers present in foods and beverage samples such as grape seed, grape juice and green tea.

HPLC-DAD was used to identified and quantified seven phenolic compounds present in olive leaf extract (Table 1.5) with oleuropein identified as the major component (Pereira *et al.* 2007). Recent research employing LC-MS/MS technique characterised twelve phenolics compounds (Table 1.5) of different cultivar of olive leaf and classify the cultivars according to their actual phenolic profile (Mohamed *et al.* 2018).

Table 1.5 The concentration of phenolic compounds presents in olive leaf extract.

Phenolic compounds	**Phenolic compounds (mg/kg)**	
	HPLC-DAD[1]	**LC-MS/MS[2]**
Caffeic acid	220.5	0.26 – 432.47
Vanillic acid		3.23 – 256.57
Vanillin		1.59 – 147.10
Catechin		0.00 – 37.60
Verbascoside	996.1	5.03 - 170.25
Hydroxytyrosol		5.40 – 290.19
Tyrosol		2.10 – 174.62
Oleuropein	26471.4	110.38 – 4741.62
Luteolin 7-glucoside	4208.9	
Luteolin		1.79 – 273.96
Rutin	495.9	25.55 – 825.36
Apigenin 7-O-glucoside	2333.1	9.44 – 2475.53
Luteolin 7-O-glucoside	1355.9	8.27 – 819.32

Sources: [1](Pereira *et al.* 2007), [2](Mohamed *et al.* 2018).

The phenolic composition of sweet chestnut bark extract was qualitatively and quantitatively determined using HPLC-DAD/MS (Table 1.6) based on retention time, molecular weight, spectroscopic properties and MS fragmentation characteristics. In addition, a recent HPLC-DAD/ESI-MS study quantified seven compounds i.e. vescalin, castalin, gallic acid, vescalagin, 1-O-galloyl castalagin, castalagin and ellagic acid, where 1-O-galloyl castalagin was identified for the first time in sweet chestnut bark extract (Table 1.6).

Table 1.6 Phenolics compounds present in sweet chestnut bark extract.

Phenolics compounds	**HPLC-DAD/MS[1]**	**HPLC-DAD/ESI-MS[2]**
	(g Gallic Acid Equivalent/100 g)	**(g/100 g)**
Vescalin	1.18	0.44 - 1.22
Castalin	1.47	0.31 - 1.00
Gallic acid	3.68	0.65 - 2.08
Vescalagin	5.01	0.29 - 4.08
1-O-Galloyl castalagin	-	1.58 - 5.39
Castalagin	4.96	1.05 - 3.80
Ellagic acid	3.64	0.43 - 0.93
Other compounds	4.07	

Sources: [1](Chiarini *et al.* 2013), [2](Comandini *et al.* 2014).

For quantification purposes, plant-derived extracts need to be analysed against a standard curve of individual standards compounds identified in the qualification stage or a suitable internal standard need to be included in the sample run. Since plant-derived extracts naturally contain diverse phenolic compounds, the quantification of individual compounds is problematic. Due to this limitation, *in vitro* antioxidant and antimicrobial assays are conducted to elucidate overall activities and properties of the extracts.

1.3.1 *In vitro* antioxidant assays

The antioxidant activity of plant-derived extracts can be assessed using various *in vitro* antioxidant tests based on chemical reaction assays. The assays can be based either on hydrogen atom transfer (HAT) or single electron transfer (SET) reactions. The HAT and SET-based methods employ a substrate, the colour of which changes upon reaction, thereby allowing antioxidant activity to be quantified spectrophotometrically (Huang *et al.* 2005).

The HAT-based method measures the ability of an antioxidant to quench free radicals by hydrogen donation to form stable compounds (Prior *et al.* 2005). The most commonly used HAT methods to assess the antioxidant activity or potential of plant-derived extracts include the oxygen reducing antioxidant capacity (ORAC), β-carotene bleaching and total radical trapping antioxidant parameter (TRAP) assays. SET-based assays determine the ability of antioxidants to transfer one electron to reduce any compounds (Prior *et al.* 2005). The total phenol content (TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, FRAP and Trolox equivalence antioxidant capacity (TEAC) assays are frequently employed SET-based assays used to determine potential antioxidant capacity of plant-derived extracts.

No single assay can accurately reflect antioxidant activity of an antioxidant because multiple reaction mechanisms are likely to be involved in such reaction since antioxidant agents deactivate radical in two major mechanisms i.e. HAT and SET (Prior *et al.* 2005; Hayes *et al.* 2011). Thus, various *in vitro* antioxidant assays are employed to fully elucidate antioxidant capacity of plant-derived extracts. Total phenol content assay measures the quantity of phenolic compounds of plant-derived extract by electron transfer mechanism (Ahmad and Abdullah 2013). Similarly, *in vitro* antioxidant assays (such as ferric reducing antioxidant power and DPPH radical scavenging activity) also measure antioxidant

activities of plant-derived extracts based on an electron transfer. A good correlation between total phenol content and antioxidant activity (such as ferric reducing antioxidant power and DPPH radical scavenging ability) of green tea was reported (Veljković *et al.* 2013). Phenolic compounds possess one or more aromatic rings with one or more hydroxyl groups and hydroxyl groups are considered to be important for radical scavenging activities (Bors *et al.* 1990; Liu 2004). In agreement to previous study which reported a good relationship between radical scavenging activities (FRAP, DPPH, and ORAC assays) of ellagic acid, sesamol, olive leaf extract and lutein (Hayes *et al.* 2011).

Therefore, both *in vitro* HAT and SET assays are preliminary methods use to evaluate the antioxidant properties of plant extracts. However, it is necessary to subject plant-derived extracts to advanced evaluation or screening techniques prior to their inclusion into foods or packaging systems. Section 1.3.1 will focus on the *in vitro* antioxidant assays utilised in the experimental chapters of the thesis:

1.3.1.1 Total phenol content (TPC)

Total phenol content (TPC) is used to evaluate antioxidant activity based on levels of polyphenolic compounds present in plant extracts. The principle of the TPC assay involves the reduction of Mo^{6+} (yellow) to Mo^{5+} (blue) when an antioxidant compound is present (Singleton and Rossi 1965) and the reaction is monitored spectrophotometrically at 750 nm. Results are measured against a standard curve of a known phenolic compound (such as gallic acid) and expressed in terms of equivalent measures of the known compound. However, the present of other components containing phenols such as sulphites, reducing sugars, amino acid and lipophilic compounds may interfere with the accuracy of this assay (Singleton *et al.* 1999). Previously published studies have reported TPC levels of 50.74 – 99.28 mg gallic acid equivalents (GAE)/g, 66 mg GAE/g and 561 mg GAE/g

for grape seed, olive leaf and sweet chestnut bark extract, respectively (Xu *et al.* 2010; Ahmad-Qasem *et al.* 2013; Comandini *et al.* 2014).

1.3.1.2 Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay involves a mechanism where colourless ferric-tripyridyltriazine complex (Fe^{3+}-TPTZ) is reduced to the ferrous form (Fe^{2+}-TPTZ) resulting in an intense blue colour measured spectrophotometrically at 593 nm (Benzie and Strain 1999). FRAP determination of the plant-derived extracts are measured against a standard curve of a known antioxidant (such as Trolox) and expressed in terms of equivalent measures of the known compound (Benzie and Strain 1999). However, this method specifically measures the reducing capability of a compound based upon ferric ion (Prior *et al.* 2005) but not the overall antioxidant activity of the test compound. In addition, the FRAP assay does not measure the thiol group antioxidants (such as glutathione) and is unable to detect slow reacting polyphenolic compounds (Prior *et al.* 2005). The FRAP levels reported for grape seed and olive leaf extracts are 0.08 – 0.15 mg Trolox equivalents (TE)/g and 102 mg TE/g, respectively (Xu *et al.* 2010; Ahmad-Qasem *et al.* 2013). However, no FRAP value for sweet chestnut bark extract is available in the scientific literature.

1.3.1.3 DPPH radical scavenging ability

The DPPH radical scavenging assay measures the ability of antioxidant compound to reduce the purple chromogen radical ($DPPH^{\bullet}$) into non-radical pale yellow hydrazine form (DPPH-H) (Brand-Williams *et al.* 1995). The colour change is monitored spectrophotometrically at 515 – 520 nm. In its radical form, $DPPH^{\bullet}$ absorbs at 515 nm, but the absorption disappears upon reduction by an antioxidant or a radical species (Brand-

Williams *et al.* 1995). The DPPH activity of the extracts can be expressed as an equivalent of a known antioxidant such as Trolox. However, DPPH can also be decolourised by reducing agents and hydrogen transfer which contributes to inaccurate interpretations of antioxidant capacity (Prior *et al.* 2005). Previous studies reported DPPH levels of 0.06 – 0.11 mg TE/g and 0.21 mg TE/g for grape seed and olive leaf extracts, respectively (Xu *et al.* 2010; Botsoglou *et al.* 2013). No DPPH level of sweet chestnut bark extract is available in the scientific literature. But the sweet chestnut fruit inner shell (having large quantity of polyphenols) has DPPH level of 39.99 – 43.70 mg TE/g (Ham *et al.* 2015).

1.3.2 *In vitro* antimicrobial assays

Antimicrobial susceptibility testing is used to investigate the potential inhibitory activity of plant-derived extracts against the growth of microorganisms. The most commonly employed methods are dilution and diffusion methods. Dilution methods are the most appropriate technique for determination of minimum inhibition concentration (MIC) of an antimicrobial agent against a specific microorganism using either agar (agar dilution) or broth medium (macrodilution or microdilution) (Balouiri *et al.* 2016). The agar dilution and macrodilution methods are tedious in terms of preparation and analysis and require large amounts of reagents which sometimes limit repetition of the analysis. In contrast, the microdilution assay (for example 96-well plate) minimises and simplifies the entire preparation and procedure but can be very costly.

The agar disc-diffusion and agar well diffusion are diffusion methods used to assess the ability or potential of an antimicrobial agent to inhibit the growth of a test microorganism (Balouiri *et al.* 2016). In some of the experimental sections of this thesis, the agar-disc diffusion assay is employed in order to screen for potential antimicrobial activity of plant-derived extracts against Gram positive bacteria (*Staphlycoccus aureus* and

Bacillus cereus), Gram negative bacteria (*Escherichia coli* and *Pseudomonas fluorescens*) and chicken microflora. Agar plates are inoculated with a standardized inoculum of test microorganisms, followed by the pressing of 6 mm discs-containing antimicrobial agents onto the surface of inoculated agars and subsequent incubation in appropriate conditions. The antimicrobial compounds diffuse into the agar and inhibit germination and growth of the test microorganisms (Balouiri *et al.* 2016). The diameters of inhibition growth zones demonstrate the antimicrobial activity of the test compounds. This assay is simple, low cost, suitable to test various antimicrobial agents and microorganisms and results can be easily interpreted. However, this method is inappropriate to determine the MIC of a test compound because it is impossible to quantify the actual amount of antimicrobial agent diffused into the agar medium (Balouiri *et al.* 2016).

1.4 Chicken meat – composition, quality and parameters

The production of poultry is expected to rise in 2017 due to the increase in export and domestic demand, low production cost, consumer preference and nutrient content (Salmon and Audran 2016). Chicken breast (*Pectoralis major*) muscle contains higher protein and lower fat levels compared to chicken thigh muscle *(Biceps femoris*) (Table 1.7).

Table 1.7 The proximate compositions of chicken breast and thigh muscles.

Composition	**Breast[1] (%)**	**Thigh[2] (%)**
Water	75.40	73.89 - 74.40
Protein	22.57	16.90 - 16.97
Fats	1.74	5.80 - 6.59
Ash	0.29	0.99 - 1.03

[1]Choo *et al.* (2014), [2]Jung *et al.* (2011).

Table 1.8 showed that chicken breast meat contains less mineral (except calcium) and monounsaturated fatty acid and polyunsaturated fatty acid (PUFA) but higher fraction of phospholipids compared to chicken thigh meat. The high levels of lipid, iron and PUFA in chicken thigh meat may render it susceptible to lipid oxidation compared to chicken breast meat.

Several quality and shelf-life parameters including colour, pH, drip loss, cook loss, texture, lipid oxidation, microbiological analysis and sensory properties can be influenced by chicken diets and packaging techniques. The nutrient content of chicken meat renders it susceptible to many deteriorating factors, such as microbial growth and lipid oxidation, which affects the nutritional, sensory and shelf-life properties (Cortinas *et al.* 2005). A number of quality and shelf-life determining factors are measured when assessing the shelf-life stability, safety and acceptability of muscle foods such as chicken.

Table 1.8 The composition of mineral, lipid fraction and fatty acid composition of chicken breast and thigh meat.

Composition	Breast	Thigh
	Trace mineral (mg/ 100 g meat)[1]	
Calcium	3.25	3.17
Iron	1.34	1.75
Sodium	37.79	59.82
Magnesium	22.27	26.81
	Lipid fraction (100%)[2]	
Non-phospholipids[a]	33.9	59.2
Phospholipids[a]	60.9	37.8
	Fatty acid composition (g100 g of total fatty acid)[1]	
Myristic acid (C14:0)	0.43	1.11
Myristoleic acid (C14:1)	-	0.32
Palmitic acid (C16:0)	24.03	25.04
Palmitoleic acid (C16:1)	4.35	6.18
Heptadecenoic acid (C17:1)	-	0.15
Stearic acid (C18:0)	10.73	6.12
Oleic acid (C18:1)	37.44	39.18
Linoleic acid (C18:2)	14.3	15.59
Alpha-Linoleic acid (C18:3)	1.35	1.70
Arachidic acid (C20:0)	-	1.06
Eicosanoic acid (C20:0)	0.38	0.11
Eicosaenoic acid (C20:1)	0.93	-
Eicosadienoic acid (C20:2)	-	0.17
Dihomo-gama-linolenic acid (C20:3)	1.16	0.22
Arachidonic acid (C20:4)	2.20	1.03
Eicosapentanoic acid (C20:5)	0.17	0.15
Docosahexaenoic acid (C22:6)	1.23	1.65
Tetracosaenoic acid (C24:1)	1.33	0.24
Total saturated fatty acid	35.57	33.32
Total monounsaturated fatty acid	43.94	46.17
Total polyunsaturated fatty acid	19.17	20.51

Source:[1](Ahmed *et al.* 2015), [2](Marion and Miller 1968).
[a]as a percentage of total lipid.

1.4.1 Methodologies for assessing the quality and shelf-life of chicken meat

1.4.1.1 Surface colour

The colour of raw poultry meat is a critical quality attribute influencing the consumer acceptability of raw and cooked poultry meat products. Chicken breast muscle is typically very pale and extremely low in myoglobin level (Faustman *et al.* 1996). Factors such as pH, total pigment concentration, myoglobin and iron content can affect the colour of chicken breast fillets (Boulianne and King 1998). The surface colour of raw breast meat can be evaluated by measuring the lightness (L^*), redness (a^*) and yellowness (b^*) using a colorimeter. The colour of raw chicken breast is as follows: L^*: 43.9 – 47.5, a^*: 1.5 – 2.0 and b^*: 0.8 – 2.3 (Fletcher 1999).

1.4.1.2 pH

The pH of raw chicken breast muscle is in the range 5.84 – 5.99 (Boulianne and King 1998; Fletcher *et al.* 2000). The ultimate pH of meat is dependent upon the amount of glycogen present in the muscle at the time of slaughter (Van Laack *et al.* 2000). Muscle glycogen deficiency (when birds under stress such as struggling and heat stress) in birds' results in limited glycolysis in the muscles after death and results in a high ultimate pH (Ali *et al.* 2008). Fletcher (1999) suggested a strong correlation between muscle pH and meat colour, dark-coloured muscle has a higher pH while light-coloured muscle has a lower pH. The pH of chicken meat can be measured by insertion of a pH probe into intact muscle or dipping the pH probe into a muscle sample which has been homogenised in distilled water.

1.4.1.3 Drip loss

Measurement of drip loss depicts the water binding properties of meat (Allen *et al.* 1998). Less drip loss value of chicken muscle reflects the integrity of the muscle in

retaining water within the muscle network. Drip loss can be determined using gravimetric measurements after a pre-determined duration in refrigerated storage and expressed as a percentage (%) of an initial muscle weight. The drip loss of chicken muscle is in the range 1.47 – 1.49% (Voljč *et al.* 2013). Drip from the muscle is presumably due to the action of gravity draining of the fluid in muscle fibres to the cut surfaces (Offer *et al.* 1989).

1.4.1.4 Cook loss

Similar to drip loss, cook loss also represents the water binding properties, or capacity of meat. Low cook loss is desirable as it depicts meat muscle capacity in holding water (Allen *et al.* 1998). The cook loss of meat can be determined by weighing the meat before and after cooking and usually is expressed as percentage of the raw sample weight . Chae *et al.* (2007) reported that the cook loss of chicken breast meat ranged between 15.5 and 18.6%.

1.4.1.5 Texture

Texture is one of the most important attributes perceived by the consumer in assessing the acceptability of cooked meat (Lyon and Lyon 1996). Fletcher (2002) explained two major contributors to meat tenderness: 1) maturity of connective tissue (involving chemical cross-linking of collagen which increases with age) and 2) contractile state of myofibrillar protein (depends on the biochemical state of muscle at slaughter, the rate and severity of rigor mortis development and handling of carcass and muscle during rigor development). The most common instrumental methods used to determine the tenderness of cooked poultry meat (whole muscle) are Warner-Bratzler or the Kramer Shear Press (Sams 2001). The basic principle of a shearing test measures the force required to cut or shear through the muscle fibres. It represents the force needed to bite through the

meat with teeth to illustrate meat tenderness or toughness (Lyon and Lyon 1991; Sams 2001). The force is recorded as a weight measurement (such as kilogram, kg) and can be converted to the force unit of Newton (N) (Sams 2001). The Warner-Bratzler shear force (WBSF) of cooked chicken breast meat typically ranged between 31.38 – 99.05 N (Lyon and Lyon 1996).

1.4.1.6 Lipid oxidation

Poultry meats contain high level of PUFA which increase susceptibility to lipid oxidation in both raw and cooked poultry meat (Higgins *et al.* 1998; Sheehy *et al.* 2014). Oxidation can occur in both raw and cooked meat but the severity of lipid oxidation is higher in processed or cooked meats (Sheehy *et al.* 2014). Processing (such as grinding, chopping or heating) disrupts meat structure and exposes the phospholipids to oxygen (O_2), enzymes, heme pigments and metal ions and also bring PUFA in close contact with catalysts, thereby catalysing lipid oxidation (Frankel 1998a). The rate and extent of lipid oxidation in chicken meat can be regulated successfully using synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Smet *et al.* 2008; Tavárez *et al.* 2011). The most widely employed method to determine levels of lipid oxidation is the 2-thiobarbituric acid (TBA) assay to measure the malondialdehyde (MDA) content expressed in mg MDA/kg meat. MDA formed via decomposition of certain primary and secondary lipid peroxidation products, which readily participate (at low pH and elevated temperature) in nucleophilic addition reaction with 2-thiobarbituric acid (TBA) to generate a red, fluorescent 1:2 MDA:TBA adduct (Janero 1990). The level of lipid oxidation in raw breast chicken meat can be as low as 0.07 – 0.12 mg MDA/kg meat (Tavárez *et al.* 2011).

1.4.1.7 Microbiological analysis

Poultry-derived pathogens, such as *Campylobacter* spp., *Salmonella* spp., *Escherichia coli*, *Staphylococcus* spp. and *Listeria monocytogenes*, can cause food borne illnesses in humans through the consumption of contaminated food products (Sams 2001). Pathogens growths negatively affects the colour and appearance of poultry products, thereby limiting their shelf-life. Traditionally, antimicrobial agents (such as potassium propionate and sodium benzoate) are utilised in poultry-based products to control the growth of pathogens which delay microbial spoilage and therefore, extend shelf-life. The total viable count (TVC) of chicken meat post-slaughter is approximately 4.3 $\log_{10}$ colony forming unit (CFU)/gram chicken and microbial growth increases as a function of storage time (Chouliara *et al.* 2007).

1.4.1.8 Sensorial properties

Antioxidant and antimicrobial agents are employed in chicken muscles to extend the shelf-life of chicken products. For centuries, plant-derived extracts have been used in poultry products to efficiently delay lipid oxidation and microbial growth (Botsoglou *et al.* 2010). However, their addition either into chicken diets and subsequently chicken meat or as active packaging components may affect the appearance, texture and flavour of chicken products which ultimately influences consumer acceptability. Therefore, the sensory attributes of chicken products perceived by human responses are very important to validate consumer acceptability. Common attributes or sensory descriptors being evaluated are; appearance, texture, flavour and overall acceptability parameters.

1.5 Strategies for enhancing the shelf-life quality of chicken meat using plant-derived extracts

1.5.1 Supplementation of plant-derived extracts into chicken diets

The shelf-life of chicken meat can be enhanced using plant-derived extracts by number of ways, supplementation of extracts into chicken diets, exogenous addition during post-mortem processing or incorporation as active agents into active packaging systems. Compounds present in plant-derived extracts ingested by chicken may enter the circulatory system and subsequently be distributed and retained in muscle tissues (Smet *et al.* 2008; Botsoglou *et al.* 2010). Direct addition of plant-derived extracts into processed poultry meat or into packaging efficiently improve the shelf-life of chicken meat throughout storage (Brannan 2007; Contini *et al.* 2014).

1.5.1.1 Fate of supplemental plant-derived extract compounds in chickens

There is an increasing interest in the effect of polyphenols to fully or partially substitute vitamin E in poultry diets due to their bioactive properties. Most polyphenols occurring in plants or extracts are in the form of esters, glycosides or polymers which cannot be absorbed in their native form (Manach *et al.* 2004). This complicates the absorption and digestion of polyphenols, because this process depends greatly on the molecular complexity of extracts and/or degree of polymerization and conjugation of polyphenols with other phenols present in the poultry feed (Brenes *et al.* 2016). To date, there is a lack of research on the digestibility of polyphenols and their effects on the digestibility of other nutrients in poultry. Although the scientific literature contains a limited number of studies examining the digestion and absorption of polyphenols in a monogastric digestion systems, Brenes *et al.* (2016) has reviewed the general digestion and absorption of polyphenols in monogastrics as illustrated in Figure 1.7.

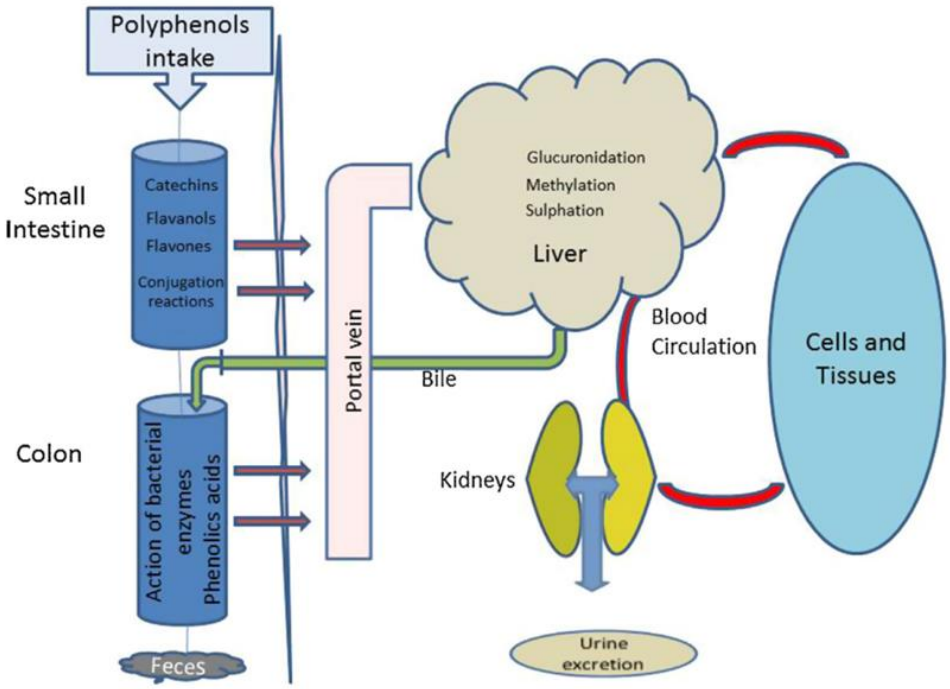


Figure 1.7 A schematic representation of the metabolic fate of dietary polyphenols in monogastric digestive system.
(Brenes *et al.* 2016).

Depending on the structural complexity and degree of polymerisation, low molecular weight polyphenols (such as monomeric and dimeric structures) may be readily absorbed in the small intestine while the oligomeric and polymeric polyphenols may reach the colon in unchanged form and some are bio-transformed by the colonic microbiota prior to absorption (Monagas *et al.* 2010). Microbial-derived phenolic metabolites may be absorbed or excreted in the faeces and in the case of absorption, compounds reach the liver through the portal vein, where they may be subjected to extensive metabolic reactions (including glucuronidation, methylation, sulphation) until they finally enter the systemic circulation and are distributed in tissues or eliminated in the urine (Brenes *et al.* 2016).

1.5.1.2 Antioxidant/antimicrobial activity of dietary plant-derived extracts in chickens

Previously, *in vivo* poultry studies have demonstrated that plant-derived extracts containing polyphenols influence growth performance and gut morphology, antioxidant, antimicrobial and meat quality indices (Table 1.9). These studies demonstrate that dietary polyphenols are being digested and absorbed by the poultry monogastric digestion system and distributed and retained in the organs and muscle tissues to elicit a response in poultry meat. The efficacy of *in vivo* supplementation depends greatly on many factors such as type of phenolic compounds included in the diet, concentration, duration of feeding and other ingredients present.

Table 1.9 Effect of dietary supplementation of plant-derived extracts on selected poultry parameters.

Extract	Duration and dose	Effect on growth performance, antioxidant status and meat quality	Reference
Grape by-products			
Grape seed (GS) extract	Chickens fed: low GS extract (25,900 mg/kg), low GS extract + methionine (1,500 mg/kg), high GS extract (51,800 mg/kg) and high GS extract + methionine (1,500 mg/kg) for 28 days.	- Reduce weight gain. - Impair growth. - Methionine: ineffective to counteract the effect of tannin on growth.	(Lau and King 2003)
Grape pomace (GP)	Broilers fed: 5,000, 15,000 and 30,000 mg/kg GP and α-tocopheryl acetate (200 mg/kg) for 42 days.	- No effect on feed efficiency. - No effect on growth performance and protein and amino acids digestibility. - Improve oxidative stability of raw chicken breast and thigh meats (7 days storage), less effective than vitamin E. - Further analyses show GP (30,000 mg/kg): 1) No effect on antioxidant activity of serum. 2) Increases antioxidant activity in diet and excreta.	(Goñi *et al.* 2007)
GS extract	Broilers fed: 100 and 200 mg/kg GS extract, 300 mg/kg synthetic antioxidant (BHT, EQ and BHA) and 200 mg/kg tocopherols for 42 days.	- GS extract (100 mg/kg): no inhibitory activity in raw and defrosted chicken breast patties. - GS extract (200 mg/kg): 1) Reduces lipid oxidation in raw (10 days storage) and defrosted chicken breast patties (frozen for 8 months) (11 days storage) similar effect to synthetic antioxidant. 2) The effect in defrosted chicken patties comparable to those fed with tocopherols.	(Smet *et al.* 2008)

Extract	Duration and dose	Effect on growth performance, antioxidant status, gut profile and meat quality	Reference
Grape pomace concentrate (GPC)	Broilers fed: 15,000, 30,000 and 60,000 mg/kg GPC and α-tocopheryl acetate (200 mg/kg) for 42 days.	- No impairment on growth performance, digestive organs size and protein digestibility. - Inhibition of lipid oxidation in raw chicken breast fillets (7 days storage). - Protective effect is equal to vitamin E.	(Brenes *et al.* 2008)
Grape seed proanthocyanidin extract (GSPE)	<u>Experiment 1</u> - 5, 10, 20, 40 and 80 mg/kg GSPE supplemented to broilers infected with *Eimeria tenella* for 15 days. <u>Experiment 2</u> - 12 mg/kg GSPE supplemented to broilers infected with *E. tenella* for 21 days.	<u>Experiment 1</u> - All levels decrease mortality and increase weight gain after infection. - Protective effect in dose-dependent manner. - 10 to 20 mg/kg: lowest mortalities and greatest growth gains. <u>Experiment 2 (GSPE (12 mg/kg)</u> - Reduce mortalities and lesion scores - Improve weight gains - Low concentration GSPE: restores the balance of antioxidant/oxidant system exerted by oxidative stress due to infection.	(Wang *et al.* 2008a)
GPC and GS extract	Broilers fed: 60,000 mg/kg GPC and 7,200 mg/kg GS extract for 21 days.	- In ileal: increase *Enterococcus* populations and decrease *Clostridium* populations - In cecal digesta: higher population of *E. coli*, *Lactobacillus*, *Enterococcus* and *Clostridium* spp. - Modify gut morphology and intestinal microflora and increase intestinal bacteria biodiversity.	(Viveros *et al.* 2011)

Extract	Duration and dose	Effect on growth performance, antioxidant and antimicrobial status and meat quality	Reference
Olive by-products			
Olive leaf (OL) extract	Turkeys supplemented: 10,000 mg/kg OL extract and 150 and 300 mg/kg α-tocopheryl acetate for 98 days.	- Improves oxidative and microbial stability of raw turkey breast fillets (12 days storage). - Better effect than control and 150 mg/kg α-tocopheryl acetate diets.	(Botsoglou *et al.* 2010)
Olive extract (contain polyphenols and hydroxytyrosol).	Chickens supplemented: 6 and 12 mg per bird per day for 42 days (drinking water).	- No enhancement on feed consumption, body weight and feed conversion. - No lipid oxidation inhibition in fresh turkey thigh patties, cooked thigh patties and cooked-salted (1%) thigh patties (3 days storage).	(King *et al.* 2014)
Olive oil mill waste water (OMWW) ~ tyrosol & hydroxytyrosol.	Broilers fed: 40,000 mg/kg OMWW permeate and 40,000 mg/kg OMWW retentate for 37 days.	Effect in plasma and tissue: - Reduce oxidative damage of biological molecules (protein and lipid oxidation). - Increase antioxidant mechanism (catalase activity, glutathione (GSH) and total antioxidant capacity level).	(Gerasopoulos *et al.* 2015)

Extract	Duration and dose	Effect on growth performance, antioxidant and antimicrobial status and gut profile	Reference
Sweet chestnut by-products			
Sweet chestnut wood (SCW) extract	Chickens fed: 1,500, 2,000 and 2,500 mg/kg SCW extract for 42 days.	- All levels: 1) No effect on proximate composition of raw chicken breast and thigh. 2) No inhibitory activity in raw breast homogenate system. - SCW extract (2,000 mg/kg) improves growth.	(Schiavone *et al.* 2008)
SCW extract	Chickens fed: 250, 500 and 1,000 mg/kg SCW extract for 42 days.	- 250 and 500 mg/kg SCW extract: 1) No effect body weight and feed conversion. 2) Well-developed tissue structure and functioning jejunal wall. - 1,000 mg/kg SCW extract: 1) Reduce body weight. 2) Reduce *E. coli* and coliform bacteria in small intestine (day 28). 3) Disturb intestinal wall morphology and function.	(Jamroz *et al.* 2009)
SCW extract	Broilers fed: 250, 500, 750 and 1,000 mg/kg SCW extract for 42 days.	- No effect on mortality. - Improve body weight gain and feed conversion. - Promote drier litters.	(Hooge *et al.* 2012)

Extract	Duration and dose	Effect on antioxidant status	Reference
SCW extract	Chickens fed: 3,000 mg/kg SCW extract and 3,000 mg/kg SCW extract + 68 IU of α-tocopherol/kg for 25 days.	- Both treatments did not affect antioxidant enzymes and total antioxidant status. - SCW extract reduced DNA damage. - Combination of SCWE and α-tocopherol: 1) Reduces plasma MDA. 2) Increases α-tocopherol in blood plasma, liver and breast muscle.	(Voljč *et al.* 2013)

1.5.2 Smart packaging technologies for quality and shelf-life extension of foods

Smart packaging systems improve and enhance packaged products by employing non-traditional packaging functions to provide safer and more secure, nutritious or attractive food products whilst being environmentally friendly (O' Callaghan and Kerry 2016). Recently, various smart packaging technologies are being developed to meet the requirements of the food supply chain and their adoption not only extends the shelf-life and improves the quality and safety of food products, but also provides important information pertaining the packaged products (Biji *et al.* 2015).

Intelligent and active packaging systems are the example of smart packaging technologies. Intelligent and active packaging materials were introduced in the Japan's market in the mid 70's and in the mid 90's, these technologies received considerable attention throughout Europe and in the USA (Dainelli *et al.* 2008). Intelligent systems provide information on the condition of the packaged food and the constituent should not be released into the food throughout the storage and transportation (European Commission 2009), for example, sensor (biosensor, gas sensor or chemical sensor) and indicator (freshness indicator, time temperature indicator, radiofrequency identification) constitutes (Biji *et al.* 2015).

Commission Regulation (EC) No 450/2009 defines active packaging as a package system designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food, intentionally to extend the shelf-life or to maintain the condition of packaged food (European Commission 2009). Dainelli *et al.* (2008) classified active packaging into two main types: 1) non-migratory active packaging functions without intentional migration and 2) active releasing packaging allowing controlled migration of non-volatile agents or an emission of volatile compounds in the atmosphere surrounding the packaged foods.

Examples of active packaging technologies are oxygen scavengers, carbon dioxide absorbers and emitters, antimicrobial packaging, moisture control, antioxidant release, ethylene scavengers, flavour or odour absorbers and releasers (Kerry *et al.* 2006; Biji *et al.* 2015). Developments in these technologies has led to advancement in many key areas encompassing controlled respiration rate in horticultural products, delaying or inhibiting lipid oxidation in muscle foods, reducing microbial growth and moisture migration in dried products (Biji *et al.* 2015).

1.5.2.1 Antioxidant active packaging

Lipid oxidation is a major quality deterioration process in cooked meat products. This resulting in the generation of toxic compounds which potentially affect the nutritional, shelf-life and sensory qualities of cooked muscle foods (Cortinas *et al.* 2005). The extent and severity of lipid oxidation in muscle foods can be controlled by direct addition of antioxidant compounds into raw meats. This requires disruption of meat muscles prior to antioxidant inclusion to ensure homogenous distribution of antioxidants within the muscle network for efficient control of the lipid oxidation process.

Alternatively, antioxidant agents can be incorporated into packaging materials (having direct contact with food products) to efficiently protect cooked meats against lipid oxidation without a need to disrupt meat muscle structure (Bolumar *et al.* 2011). Antioxidant active packaging is a promising technology whereby antioxidant agents are incorporated into the packaging system to maintain or prolong shelf-life and quality of oxidation sensitive foods (Jongjareonrak *et al.* 2008; Gómez-Estaca *et al.* 2014). These include moisture absorber, films and other mechanisms capable of delivering antioxidant activity.

Advantages of adding antioxidant agents into packaging, compared to direct addition in muscle foods are as follows:

1) Migration of antioxidant agents from the packaging matrix to the food surface.
2) Localisation of antioxidant activity on the food surface where high levels of lipid oxidation occur due to surface exposure to oxygen.
3) Elimination of processing steps required to incorporate antioxidants into processed meats (Bolumar *et al.* 2011).

There are two main mechanisms for antioxidant packaging: 1) the release of antioxidants onto food via the direct contact between packaging and food and 2) the scavenging of undesirable compounds (such as oxygen, reactive oxygen species or metal ions) from the packaging headspace or food surface. Food additives incorporated into primary food packaging must be generally recognised as safe (GRAS) and the concentration used must comply with current regulations (Gómez-Estaca *et al.* 2014).

1.5.2.1.1 Active pads and trays

Absorbent pads and trays are commercially available in various sizes and colours for many types of food products (Figure 1.8) such as meat, fish, fruits and cheese. Principally, food absorbent pads are placed beneath food products and function to absorb exudates, juices and condensation produced by the food products within the packaging (usually trays). Manufacturing companies, such as Sirane Ltd and McAirLaid's GmbH, produced many types of food absorbent pads employing various principals and mechanisms to efficiently absorb unsanitary exudates thus preventing contamination of food products as well as maintaining aesthetic appeal of the packaged products (Fernández *et al.* 2009).

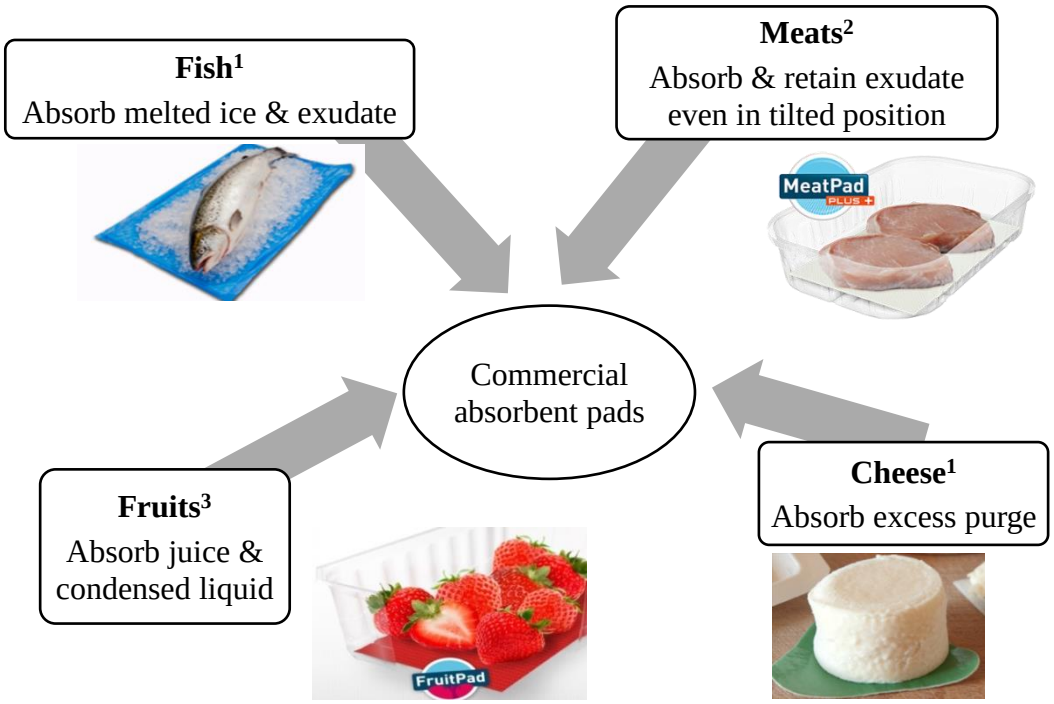


Figure 1.8 Commercial absorbent pads for food use.
Source: [1]http://www.sirane.com/dri-fresh.html, [2]http://www.meatpads.info/, [3]http://www.fruitpad.info/en/

The simplest form of absorbent pad comprised of an impervious upper surface (in-contact with food) and an absorption lower layer (in-contact with tray) (Figure 1.9a). This design allows the liquid exudate to drain to the sides of the tray, thereby being absorbed by the absorbing material/layer (usually cellulose). Alternatively, absorbent pads can also be composed of three layers which are upper, intermediate (absorbing material) and lower layer (b). The upper and lower layers can vary from impermeable thermoplastic material to coated cellulosic material to a perforated surface (Otoni *et al.* 2016). The perforated surface or thermoformed apertures (at least one layer or on both layers) facilitates the absorption of liquid by capillary action which transports the liquid into the absorbent layer (intermediate layer) (Pearlstein 1998; Otoni *et al.* 2016). The efficiency and success of absorption pads depend on the materials utilised and the construction of the pad matrix.

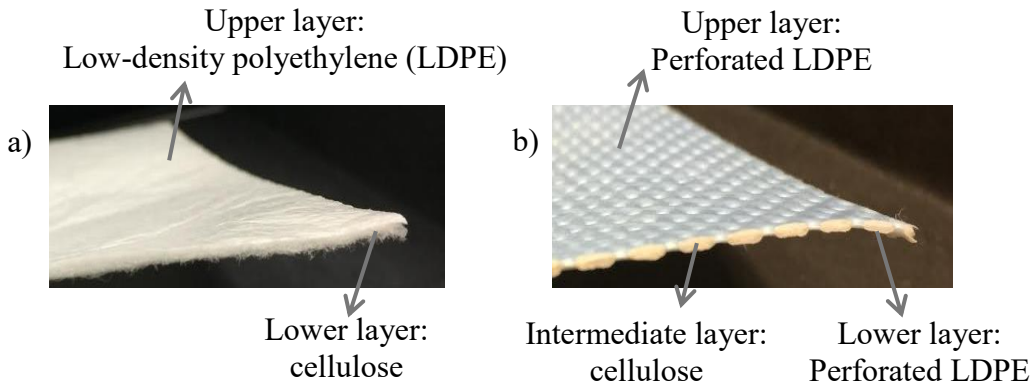


Figure 1.9 Composition of the absorbent pad matrix: (a) simple form of absorbent pad with low-density polyethylene (LDPE) upper layer with cellulose as lower layer and (b) perforated absorbent pad composed of perforated LDPE upper layer, intermediate cellulose layer and perforated LDPE lower layer.

Recent developments in pad manufacture has seen an evolution of the simple pad to a more advanced type which has excellent absorption capacity, ability to absorb undesirable odours from foods and deliver antimicrobial activity to the packaged food product. Sirane Ltd manufacture antimicrobial pads, known as the Dri-Fresh™ Fresh-Hold™ range, to improve microbial stability of meats, seafood and fruits.

Some research has been carried out to evaluate the efficacy and efficiency of absorbent pads containing antimicrobial agents to preserve and improve the shelf-life of perishable meat products. For example, absorbent pads (consist of perforated polyethylene, cellulose and polyethylene) containing oregano oil (0.015 ml/ml) extended the shelf-life of raw drumsticks for 2 days (Oral *et al.* 2009) and silver-loaded absorption pads (10 mg/ml) decreased the microbial load during the storage of fresh chicken breast and beef (Lloret *et al.* 2012). Lloret *et al.* (2012) proposed that the antimicrobial activity of the silver-loaded absorbent pads is due to the migration of silver ions from the absorbing material to food and the efficacy of the active pads depends greatly on the polymer of the absorbent pad and nature of the food matrix.

The key principle of active absorbent pads is the direct contact between one side of the pad with a surface of food to allow the migration of the active compounds to the food to elicit desirable responses. To date, scientific literature contains no information on the development of antioxidant pads containing plant extracts for increasing the oxidative stability of cooked muscle foods.

Contini *et al.* (2013) developed polyethylene terephthalate trays coated with citrus extract (100 mg/ml) which effectively reduced lipid oxidation in cooked turkey meat during refrigerated storage. Previous kinetic study demonstrated that citrus extract containing phenolic compounds and carboxylic acid has the affinity for water, suggesting their diffusion into the water phase of the meat to elicit antioxidative effect (Contini *et al.* 2014). The mechanism of action of antioxidant coated trays can be postulated as the mode of action for antioxidant absorption pads.

1.5.2.1.2 Active films

Petrochemical-based plastics (such as polystyrene, polyamides, polyethylene terephthalate, polyethylene) are widely used in food packaging applications due to their physical properties (gas or water barrier properties), aesthetic qualities, low cost and overall performance characteristics (Nur Hanani *et al.* 2014). The large consumption and utilisation of these materials by the food industry has resulted in serious environmental and ecological problems due to their non-biodegradable nature and negative implications on public health (Gómez-Estaca *et al.* 2009; Nur Hanani *et al.* 2014). These factors have led to numerous research studies which aim to develop biodegradable packaging to potentially reduce or replace traditional packaging materials, but must also be able to perform the basic functions of packaging such as containment, protection and preservation (Nur Hanani *et al.* 2014). Biodegradable materials are edible and derived from food ingredients such as

polysaccharides, proteins and lipids which can be obtained from agricultural, livestock rearing or fishing waste product sources (Gómez-Estaca *et al.* 2009; Nur Hanani *et al.* 2014).

This section of the literature review will focus on gelatin as an ingredient source as gelatin is used to make biodegradable films in the experimental component of this thesis. Gelatin is produced from the chemical denaturation of fibrous insoluble protein (known as collagen) obtained from the bones, skin and connective tissues of livestock generated during slaughtering and processing (Nur Hanani *et al.* 2014). Gelatin is widely used to manufacture biodegradable packaging films as such films possess good mechanical and gas barrier properties but have low water barrier properties (Gómez-Estaca *et al.* 2009; Nur Hanani *et al.* 2012).

The functional characteristics and physical properties of gelatin-based films can be improved by incorporating additives during film manufacturing (Nur Hanani *et al.* 2014). Addition of BHT (0.2 mg/g) improved the water vapour permeability and hydrophobicity of gelatin films compound dispersion within the matrix resulting in a denser film network (Jongjareonrak *et al.* 2008). The addition of BHT also improved the antioxidant properties of the gelatin films as a function of storage time. Currently, many research efforts are underway to substitute synthetic antioxidants with natural plant-derived extracts for safety and health reasons.

In Table 1.10, the development and characterisation of antioxidant active films containing plant extracts or polyphenols is summarised. In general, the incorporation of active compounds efficiently improved the antioxidant activities, water and UV light barrier properties of gelatin-based films but mechanical properties were only enhanced in green tea extract, ferulic and tyrosol containing films.

Table 1.10 Research on the development and characterisation of antioxidant gelatin-based films.

Film composition	Manufacturing technique	Film properties and characterisation	Reference
Fish gelatin films containing green tea extract (3.0 and 7.0 mg/ml)	- Continuous stirring and heating of extract solution with gelatin and other ingredients. - Casting and drying.	- Enhanced antioxidant activities (TPC, DPPH and reducing power). - Improved mechanical properties, water and UV light barrier properties. - Scanning electron microscope (SEM): smooth homogenous surface and compact structure (cross-section). - Fourier Transform Infra-Red (FTIR) spectra: evidence of protein-polyphenol interactions.	Wu *et al.* (2013)
Fish gelatin films containing green tea extract (1.0 mg/ml) and GS extract (1.0 mg/ml)	- Continuous stirring and heating of extract solution with gelatin and other ingredients. - Casting and drying.	- Improved antioxidant activities (DPPH scavenging and ferric ion reducing abilities). - Increased UV light and moisture barrier properties. - SEM: Heterogeneous surface and porous structure. - FTIR spectra: formation of hydrogen bonds with gelatin functional groups reducing the free hydrogen.	Li *et al.* (2014)

Film composition	Manufacturing technique	Film properties and characterisation	Reference
Pigskin gelatin films containing hydrolysable chestnut tannins (100 mg/ml)	- Continuous stirring and heating of extract solution with gelatin and other ingredients. - pH adjustment - Casting and drying.	- Improved DPPH radical scavenging ability. - Increased UV light and moisture barrier properties. - FTIR: hydrogen interactions between tannins and gelatin.	Peña-Rodriguez *et al.* (2015)
Chitosan-gelatin films containing ferulic acid or tyrosol (47.1 mg/g)	- Continuous stirring and heating of compounds with gelatin and other ingredients. - pH adjustment. - Casting and drying.	- Reduced water vapour permeability. - Increased tensile strength. - Kinetic release study: tyrosol and ferulic acid are released into the liquid medium (at different rate).	Benbettaïeb *et al.* (2016)

The proposed mechanism of action for antioxidant active films is based on close contact between the film and the food and the ability of the film to cover the entire food surface allowing optimal migration of compounds to provide desirable protective effects. Evidently, Benbettaïeb *et al.* (2016) demonstrated that the release of tyrosol and ferulic acid (47.1 mg/g) from chitosan-gelatin films into a water medium, where tyrosol was released at a higher rate than ferulic acid. Interaction between ferulic acid and the polymer network enhanced the cross-linking density, thereby reducing the ability of ferulic acid to diffuse into the liquid medium.

It can be postulated from a kinetic release study that the migration of active compounds from the film to packaged foods is dependent on cross-linking between active compounds within the polymer network and the surface nature (hydrophilic/hydrophobic) of the food to exert antioxidant activity (Benbettaïeb *et al.* 2016). To date, the scientific literature contains no information on the applicability/potential of gelatin films containing plant-derived extracts (particularly tea catechin, grape seed, olive leaf and sweet chestnut bark) to improve the oxidative stability of cooked chicken meat. However, previous studies have demonstrated that incorporation of green tea extract (200 mg/ml) into chitosan films improved the *in vitro* antioxidant activities (TPC and DPPH scavenging ability) of the films and overwrapping of cooked pork samples with such films successfully lowered levels of lipid oxidation during refrigerated storage, compared to the control (Siripatrawan and Harte 2010; Siripatrawan and Noipha 2012). It is important to highlight that the cooked pork meat contained a high level of moisture (~61%) (Siripatrawan and Noipha 2012) which may be a contributing factor in the release of hydrophilic antioxidant compounds from the film onto the packaged food in order to effectively inhibit lipid oxidation.

1.6 Conclusion

This review endeavours to present the state-of-the art in terms of use of natural plant-derived extracts as antioxidant agents in chicken diets and antioxidant agents in active packaging applications. The chemistry and chemical composition of grape seed, olive leaf, sweet chestnut bark and tea catechin extracts are reviewed and bioactive compounds present in each extract are highlighted. Methodologies for characterising polyphenolic compounds present in plant-derived extracts are presented.

In vitro antioxidant and antimicrobial assays and commonly used shelf-life indicators/assays are discussed in relation to muscle foods. Smart packaging technologies, in particular antioxidant active packaging (pads and films) are also examined.

1.7 Objectives

The studies in the thesis were undertaken with the following objectives:

- To characterise polyphenolic compounds present in grape seed (GS) extract and two mixtures of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) (GS/OL/CB_1 and GS/OL/CB_2) extracts and evaluate their potential antioxidant and antimicrobial activity for potential use in chicken meat.

- To investigate the effect of *in vivo* supplementation of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts on the shelf-life quality and oxidative stability of raw chicken meat.

- To evaluate the effect of *in vivo* supplementation of GS extract on the shelf-life quality of cooked chicken meat.

- To develop antioxidant active pads and investigate their potential use to improve the oxidative stability of cooked chicken breast meat.

- To develop and characterise antioxidant active films and evaluate their potential for use in enhancing the oxidative stability of cooked chicken meat.

CHAPTER 2

2 Characterisation of grape seed extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts for potential use as antioxidant and antimicrobial agents in poultry meat.

Abstract

Commercial grape seed (GS) extract and two mixtures of grape seed, olive leaf and sweet chestnut bark (GS/OL/CB_1 and GS/OL/CB_2) extracts were qualitatively characterised using liquid chromatography quadrapole-time-of-flight mass spectrometry (LC-Q-TOF-MS) and antioxidant activity was measured using *in vitro* antioxidant assays (TPC, FRAP and DPPH). The antioxidant potency (TBARS) of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts in 25% chicken breast and thigh homogenates (50 and 100 µg/ml) containing lipid pro-oxidants (45 µm $FeCl_3$:sodium ascorbate) was examined at pH 5.5 and 4°C. Potential antimicrobial activity of all extracts at a concentration of 50 mg/ml against Gram positive bacteria (*S. aureus* and *B. cereus*), Gram negative bacteria (*E. coli* and *P. fluorescens)* and chicken microflora was assessed using the disc diffusion method. The *in vitro* antioxidant activity of all extracts followed the order: GS/OL/CB_2 > GS/OL/CB_1 > GS ($p<0.05$). Similarly, after 24 hrs of storage, level of lipid oxidation were lower ($p<0.05$) in chicken breast and thigh homogenates containing GS/OL/CB_1 and GS/OL/CB_2 extracts (50 µg/ml) compared to GS and control homogenates containing lipid pro-oxidants. Level of lipid oxidation were not influenced ($p>0.05$) by extract concentration (50 and 100 µg/ml). Extract mixtures exhibited moderate antimicrobial activity against Gram positive bacteria. Overall results demonstrate the potential of natural extracts (and combination mixtures) for use as antioxidant ingredients in chicken.

2.1 Introduction

Plant-derived extracts are rich in phenolic compounds which possess one or more aromatic rings with one or more hydroxyl groups and are categorised as phenolic acids, flavonoids, stilbenes, coumarins and tannins (Liu 2004). Natural plant extracts containing phenolic compounds function as antioxidant and antimicrobial agents which render them useful to human and animal health, and as ingredients in muscle food products. For example, grape pomace can be used as a functional ingredient for incorporation into oxidation-sensitive muscle foods such as poultry, either by direct addition (processing) or dietary supplementation in order to improve the growth performance and shelf-life quality of the resulting meat (Goñi *et al.* 2007; Sáyago-Ayerdi, Brenes and Goñi 2009). The direct addition of rosemary into dehydrated chicken meat protected against oxidative deterioration similar to the effect provided by synthetic antioxidants (butylated hydroxyanisole and octyl gallate) (Nissen *et al.* 2000). Dietary supplementation with plant-derived extracts, such as grape pomace and tea catechin extract, improved lipid stability in chicken (Tang *et al.* 2001; Goñi *et al.* 2007).

The juice and winemaking industries produce large quantities of waste residue mainly consisting of grape pomace (seed, skin and stems). Grape (*Vitis vinifera*) seed represents 38 – 52% of grape pomace on dry matter basis and it can be separated, extracted, dried and purified to produce grape seed extract (Lau and King 2003; Brenes *et al.* 2016). The superior antioxidant properties of grape seed extract is due to the presence of monomeric and polymeric procyanidins (Yilmaz and Toledo 2004). From a meat quality perspective, grape seed extract reduced levels of lipid oxidation in chicken patties (Brannan 2007). The presence of phenolic compounds in grape seed extracts also exerted antimicrobial activity against the growth of *S. aureus*, *B. cereus* and *E. coli* on nutrient agar tested using the disc diffusion method (Baydar *et al.* 2004). In poultry diets, polyphenols

in grape seed extract are absorbed by the gut and modify gut morphology and increase biodiversity of intestinal microflora in broiler chicks (Viveros *et al.* 2011). The administration of grape seed extract in rat diets resulted in increased antioxidant enzyme activity thereby protecting the liver from lipid oxidation (Ahn *et al.* 2002).

Olive (*Olea europaea*) leaf extract contains oleuropeosides, flavones, flavonols, flavan-3-ols and substituted phenols (Benavente-García *et al.* 2000). Olive leaf extract has shown strong potential as an effective natural antioxidant and can be used to develop novel functional meat products (Hayes *et al.* 2009). The direct addition of olive leaf extract into meat systems reduced levels of lipid oxidation in beef and pork muscle model systems (Hayes *et al.* 2009) and in cooked beef and pork patties (DeJong and Lanari 2009). Dietary supplementation with olive leaf extract exerted an inhibitory effect against microbial growth in turkey breast meat (Botsoglou *et al.* 2010) and protected turkey breast meat and poultry eggs against lipid oxidation (Botsoglou *et al.* 2010, 2013).

Sweet chestnut (*Castanea sativa Mill*.) bark extract contains vescalin, castalin, gallic acid, vescalagin, 1-O-galloyl castalagin, castalagin and ellagic acid (Comandini *et al.* 2014). Sweet chestnut bark extract was reported to exert cardio-protective effects in rats (Chiarini *et al.* 2013) and neuro-protective effects in cell culture (Brizi *et al.* 2016). Dietary supplementation with sweet chestnut bark extract reduced DNA damage (Voljč *et al.* 2013) and improved growth performance in chickens (Schiavone *et al.* 2008). Information on the antimicrobial activity of sweet chestnut bark extract is not available in the scientific literature, however, it has been reported that the inner shell (pellicle) of sweet chestnut contains similar phenolic compounds to sweet chestnut bark extract, such as gallic acid, ellagic acid and ellagitannins (de Vasconcelos *et al.* 2010) and the disc diffusion assay demonstrated that the sweet chestnut inner shell extract exerted antimicrobial activity against the growth of *P. fluorescens* and *Bacillus* species (Hao *et al.* 2012).

While phenolic compounds can function as antioxidant and antimicrobial agents individually, various phenolic compounds in plants may interact to produce either synergistic, additive or antagonistic interactions. For example, Benavente-García *et al.* (2000) reported that a combination of phenolic compounds present in olive leaf extract extract demonstrated greater *in vitro* antioxidant activity compared to individual phenolic compounds alone. In support of this finding, a combination of dietary sweet chestnut bark extract and α-tocopherol reduced lipid oxidation in chicken (Voljč *et al.* 2013). Also, the inclusion of sweet chestnut bark extract and oregano essential oil in pig diets increased the antioxidant status of blood serum, reduced lipid oxidation in pork muscle and consequently improved the shelf-life of pork meat (Ranucci *et al.* 2015). Therefore, it is of interest to examine the suitability of grape seed extract alone, and in combination with olive leaf and sweet chestnut bark extracts as potential antioxidant and antimicrobial agents for stabilising chicken meat against quality deteriorative processes.

The aim of the study was to qualitatively characterise the compounds present in commercial grape seed (GS) extract and two mixtures of GS, olive leaf and sweet chestnut bark (GS/OL/CB_1 and GS/OL/CB_2) extracts using liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-Q-TOF-MS) and measure the antioxidant activity of extracts using *in vitro* assays (TPC, DPPH and FRAP). The antioxidant potency of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts on lipid oxidation in chicken breast and thigh muscle (25%) homogenates was examined. The antimicrobial properties of all extracts against Gram positive bacteria, Gram negative bacteria and chicken microflora were also investigated using the disc-diffusion technique.

2.2 Materials and Methods

2.2.1 Reagents and chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$), sodium acetate trihydrate ($C_2H_3O_2Na.3H_2O$), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) ($C_{18}H_{12}N_6$), L-ascorbic acid (sodium salt) ($C_6H_7O_6Na$), Folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), 2-thiobarbituric acid (TBA) ($C_4H_4N_2O_2S$), glacial acetic acid (CH_3CO_2H), trichloroacetic acid (TCA) ($C_2HCl_3O_2$), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) ($C_{18}H_{12}O_6$), L-histidine ($C_6H_9N_3O_2$) and methanol (CH_3OH) were supplied by Sigma-Aldrich Ireland, Ltd., Arklow, Co. Wicklow, Ireland. Potassium chloride (KCl) was manufactured by LP Chemical Ltd, Windsford, Cheshire and ferric chloride hexahydrate ($FeCl_3.6H_2O$) was supplied by BDH Limited Supplies, Poole, England. All chemicals used were of analytical grade. Maximum recovery diluent, Mueller Hinton Agar, Mueller Hinton Broth (MHB) and Streptomycin discs (10 µg) were purchased from Oxoid (Oxoid Ltd., Basingstoke, England). Tryptic soy agar (TSA), plate count agar and hydrochloric acid (HCl) were obtained from Merck, Darmstadt, Germany.

For chromatographic analysis, Ultrapure 18.2 MΩ water was used for sample preparation. Water with 0.1% formic acid (H_20) and acetonitrile (CH_3CN) were LC-MS grade and supplied by Sigma-Aldrich Ireland, Ltd., Arklow, Co. Wicklow, Ireland.
Commercial grape seed (GS) extracts and two mixtures of grape seed, olive leaf (OL) and sweet chestnut bark (CB) ($GS/OL/CB_1$ and $GS/OL/CB_2$) extracts were supplied by Envirotech Innovative Products Ltd (Dublin, Ireland). The chemical composition of all extracts was industrially confidential and required LC-MS analysis for identification of compound classes present.

2.2.2 Characterisation of natural-derived plant extracts

Individual compound classes present in GS, GS/OL/CB_1 and GS/OL/CB_2 extracts were identified using LC-Q-TOF-MS analysis. The antioxidant potency of the extracts was examined using *in vitro* antioxidant assays (TPC, FRAP and DPPH).

2.2.2.1 Liquid chromatography mass spectroscopy analysis of extracts

Mass spectrometry analysis was conducted using a Waters Acquity UPLC system coupled to a quadrupole time-of-flight mass spectrometer (Q-TOF-MS). The samples were eluted using a Waters Acquity UPLC HSS T3 column, 1.8 µm particle size, 2.1 x 100 mm preceded by an Acquity UPLC HSS T3 VanGuard, 1.8 µm particle size, 2.1 x 5 mm with the column set to 45°C. All samples were maintained at 4°C in the UPLC autosampler and a 10 µl injection volume (partial loop needle overfill) was used with a total flow rate of 0.65 ml/min over a total run time of 15 min. Gradient elution was carried out using Mobile phase A (water plus 0.1% formic acid) and B (100% acetonitrile) (Table 2.1).

Table 2.1 The mobile phase A and B gradient composition for LC-MS analysis.

Time (min)	**Flow rate (ml/min)**	**A (%)**	**B (%)**
Initial	0.65	99	1
1.0	0.65	99	1
10.0	0.65	70	30
12.0	0.65	5	95
12.1	0.65	99	1
15.0	0.65	99	1

*Mobile phase A: water + 0.1% formic acid; B: 100% acetonitrile

Mass spectrometry detection was conducted through electrospray ionization using an ms^e centroid experiment; negative mode was screened in the *m/z* scan range of 50 -1200

Da. Data was acquired in both positive and negative polarity with the analyser set to resolution mode. Scanning conditions were set to 1 scan every 0.7 s with the data format in centroid mode. Collision energy was set for two functions, function one at low energy with no collision energy applied and function two at high energy using a collision energy ramp from 20 volts to 65 volts. The following MS tune file settings were used in both positive and negative mode: capillary voltage 3.0 kV, sampling cone 40 V, extraction cone 4.0 V, source temperature 120°C, desolvation temperature 600°C, desolvation gas flow 1200 l/hr, cone gas flow 20 l/hr. The accurate mass of the instrument was initially calibrated through direct infusion of a sodium iodide calibrant solution prior to sample analysis. In addition, leucine enkephalin (Leuenk) lockmass solution (2 ng/µl) was infused at 5 µl/min in parallel to the mobile phase flow, scanned and automatically corrected to verify exact mass which ensured high mass accuracy (< 3 ppm) throughout the scan range over the course of the submitted sequence. Masslynx v4.1 software was used to control the instrument and also analyse the data.

2.2.2.2 *In vitro* antioxidant assays

2.2.2.2.1 Total phenol content (TPC)

The TPC of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts was measured using the Folin-Ciocalteu method described by Singleton & Rossi (1965) with slight modifications. Extracts (0.5 ml) (0.06 – 0.2 mg/ml stock concentration) were mixed with 2.5 ml Folin-Ciocalteu reagent (10% in distilled water) in test tubes and held at room temperature. After 5 min, 2 ml of Na_2CO_3 (7.5% in distilled water) was added and tubes were vortex mixed and incubated in the dark for 2 hr at room temperature. The absorbance of the coloured mixture was measured at 750 nm using a spectrophotometer (Cary 60 UV-Vis Agilent Technology, Australia) against a blank containing all reagents and distilled water. A

standard curve of aqueous gallic acid (0.004 – 0.08 mg/ml) was prepared and results were expressed as milligrams (mg) of gallic acid equivalents (GAE)/gram (g) extract.

2.2.2.2.2 Ferric reducing antioxidant power (FRAP)

The total antioxidant activity of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts was measured using the FRAP method of Benzie and Strain (1999) with slight modifications. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_20$ solution in the ratio 10:1:1, respectively. The reagent was incubated at 37°C for 10 min prior to use. The TPTZ and $FeCl_3.6H_20$ solutions were made fresh on each analysis day. FRAP reagent (2.85 ml) was added to test tubes containing 0.15 ml extracts (0.06 – 0.3 mg/ml), vortex mixed and incubated for 30 min in the dark at room temperature. Following incubation, absorbance measurements were recorded at 593 nm using a spectrophotometer (Cary 60). A standard curve of methanolic Trolox (0.013 – 0.19 mg/ml) was prepared and results were expressed as mg of Trolox equivalent (TE)/g extract.

2.2.2.2.3 DPPH free radical scavenging activity (DPPH)

The DPPH free radical scavenging activity of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts was determined as described by Yen and Wu (1999) with slight modifications. Extracts (0.06 – 0.2 mg/ml) were added to 2 ml 0.2 mM DPPH in methanol, vortexed and incubated in the dark for 30 min at room temperature. Absorbance measurements were recorded at 517 nm using a spectrophotometer (Cary 60). A standard curve of methanolic Trolox (0.01 – 0.05 mg/ml) was prepared and results were expressed as mg of TE/g extract.

2.2.3 Antioxidant potency of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts in chicken muscle homogenates

2.2.3.1 Preparation of muscle homogenates

Chicken samples were obtained from Shannon Vale Foods Ltd. Gullane, Clonakilty, Co. Cork, Ireland and stored at -20°C until required for analysis. Muscle homogenates (25%) were prepared by homogenising 50 g of chopped chicken breast (*pectoralis major*) and thigh (*gastrocnemius interna*) muscles in 150 ml of buffer (0.12 M KCl, 5 mM histidine, pH 5.5) using an Ultra Turrax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH & Co., Germany) at 24,000 rpm for 5 min. An ice bath was used to control the temperature during homogenisation. Stock solutions (2000 µg/ml) of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts were added to homogenate incubates (final volumes 20 ml) at concentrations of 50 and 100 µg/ml. Lipid oxidation was initiated in incubates by the addition of equimolar 45 µM ferric chloride/sodium ascorbate ($FeCl_3$/Asc) pro-oxidants. Sample beakers containing homogenates alone and lipid pro-oxidants were run simultaneously as controls. Lipid oxidation in muscle homogenates was measured after 1 and 24 hr of storage at 4°C.

2.2.3.2 Measurement of lipid oxidation

A modified version of the 2-thiobarbituric acid-reactive substances (TBARS) assay by Siu and Draper (1978) was used to measure lipid oxidation. 10% TCA (4 ml) was added to 4 ml of muscle homogenate and centrifuged (J2-21 Model centrifuge Beckman Inc., Palo Alto, CA, USA) at 6,160 g for 15 min at 4°C. The supernatant was filtered through Whatman No.1 filter paper. In screw-capped test tubes, 4 ml of the clear filtrate was added to 1 ml of 0.06 M TBA reagent. The assay blank contained 2 ml of buffer, 2 ml of 10% TCA and 1 ml of 0.06 M TBA reagent. Tubes were heated in water bath at 80°C for 90

min. Absorbance was measured at 532 nm against a blank using a spectrophotometer (Cary 60) and results were reported directly as absorbance (A_{532}).

2.2.4 Antimicrobial analysis

2.2.4.1 Microbial strains and growth conditions

Gram positive species (*Staphlycoccus aureus* (*S. aureus*, NCIMB 13062) and *Bacillus cereus* (*B. cereus*, NCIMB 9373)) and Gram negative species (*Escherichia coli* (*E. coli*, NCIMB 11943) and *Pseudomonas fluorescens* (*P. fluorescens*, NCIMB 9046)) microbial strains were examined. Microbial strains were initially cultivated on Tryptic Soy Agar (TSA) slants, maintained and stored at 4°C. Microbial cultures were generated twice from TSA slants into Mueller Hinton Broth (MHB) incubated for 18 hr at 30°C (*P. fluorescens* and *B. cereus*) and 37°C (*S. aureus* and *E. coli*).

Microflora isolated from locally sourced chicken was also examined. Chicken breast (10 g) meat was placed in Stomacher bags with 90 ml of MHB and stomached for 2 min (Colworth Stomacher 400, Seward Ltd., England). A 10 ml aliquot of the resulting homogenate was incubated for 18 hr at 37°C. Subsequently, a loopful of incubated homogenate was inoculated into a fresh MHB and further incubated for 18 hr at 37°C to produce chicken microflora to be used in disc diffusion analysis.

2.2.4.2 Antimicrobial activities of GS, GS/OL/CB_1 and GS/OL/CB_2: Disc diffusion

The extracts were dissolved in sterile distilled water at a concentration of 50 mg/ml. Extracts (20 µl) were pipetted onto 6 mm discs. Mueller Hinton agar plates were swabbed with targeted microorganisms (~10^6 colony forming unit (CFU)/ml). The discs-containing extracts were placed on the surface of swabbed agar plates and incubated at 30°C (*P. fluorescens* and *B. cereus*) and 37°C (*S. aureus, E. coli* and chicken microflora) for 24 hr.

A streptomycin (10 µg) disc was used as positive control. The inhibition zone around the disc indicated antimicrobial activity against the targeted microorganism and was measured using an electronic calliper device (Model ECA 015D Moore & Wright, Paintain tools, Ltd., Birmingham, UK) in millimetres (mm). The sensitivity of the microorganisms to the antimicrobial agents was classified by the diameter of the inhibition zone as follows: < 8 mm (not sensitive); 9 – 14 mm (sensitive); 15 – 19 mm (very sensitive) and > 20 mm (extremely sensitive) (Ponce *et al.* 2003).

2.2.5 Statistical analysis

Statistical analysis was carried out using SPSS 22.0 for Windows (SPSS Statistical Software, Inc., Armonk, NY, USA) software package. All analyses were performed in duplicate and three independent experimental trials were carried out. One way ANOVA was used to examine data from the *in vitro* antioxidant (TPC, FRAP and DPPH) and antimicrobial assays. A full repeated measure ANOVA was conducted to investigate the effects of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts concentration, time and their interactions for TBARS measurements. GS, GS/OL/CB_1 and GS/OL/CB_2 extracts concentration represented the 'between-subjects' factor and the effect of time measured using the 'within-subject' factor. Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means. All data is presented as mean values ± standard deviation.

2.3 Results and Discussion

2.3.1 Characterisation of grape seed (GS) extract and two mixtures of GS/OL/CB_1 and GS/OL/CB_2 extracts

2.3.2 Identification of compounds present in GS, GS/OL/CB_1 and GS/OL/CB_2 extracts

LC-MS analysis was used to identify compounds present in GS, GS/OL/CB_1 and GS/OL/CB_2 extract (Table 2.2). Phenolic compounds present in the GS extract were similar to previously reported studies i.e. gallic acid, monomer procyanidin ((+)-catechin, (-)-epicatechin and (-)-epicatechin gallate) and dimer procyanidin (B1 and B2) (Escribano-Bailon *et al.* 1992) and trans-resveratrol (Rockenbach *et al.* 2011). Typically, (+)-catechin and (-)-epicatechin are present in greater quantities than procyanidins in GS extract (Fuleki and Da Silva 1997).

In addition to compounds present in GS, plant extract mixtures (GS/OL/CB_1 and GS/OL/CB_2) contained compounds typically present in OL extract such as catechin, luteolin-7-glucoside, apigenin-7-glucoside, tyrosol, hydroxytyrosol and caffeic acid which commonly present in OL extract (Benavente-García *et al.* 2000). Hydroxytyrosol is the second major compound present in OL extract with the concentration of 2.3 g hydrosytyrosol/100 g fresh olive leaves (Benavente-García *et al.* 2000; Bouaziz and Sayadi 2005). In addition, extract mixtures also contained ellagic acid, vescalin, castalin and gallic acid, compounds typically present in CB extracts (Comandini *et al.* 2014) and it was reported ellagic acid was identified as one of the major components present in CB extracts (Chiarini *et al.* 2013) at the concentration of 0.43 – 0.93 g ellagic acid/100 g sweet chestnut bark sample (Comandini *et al.* 2014).

Table 2.2 LC-MS characterisation of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts.

Class	Sub class	Compounds	GS	GS/OL/CB_1	GS/OL/CB_2
Phenolic acids	Hydroxybenzoic acids	*p*-hydroxybenzoic acid	√	√	√
		Gallic acid	√	√	√
		Ellagic acid		√	√
	Hydroxycinnamic acids	Ferulic acid		√	√
		Sinapic acid		√	√
		Caffeic acid		√	√
		p-coumaric acid		√	√
		t-cinnamic acid			√
		trans-caftaric acid			√
Flavonoids	Flavanols	Catechin	√	√	√
		D-(+)-catechin	√	√	√
		(-)-epicatechin gallate	√	√	√
		Procyanidin B1	√	√	√
		Procyanidin B2	√	√	√
	Flavonols	Quercetin 3-β-galactoside	√	√	√
		Quercetin 3-β-glucuronide	√	√	√
		Quercetin 3-β-methylether	√	√	√
		Kaempferol 3-β-glucuronide	√	√	√
		Syringetin 3-O-β-D-glc		√	√
		Isorhamnetin 3-β-D-glc	√	√	√
		Myricetin 3-O-β-D-glc		√	√
		Kaempferol 3-O-β-rutinoside	√		
		Myricetin 3-O-β-D-glucuronide		√	
		Kaempferol 3-O-β-D-glc		√	√
		Laricitrin 3-β-D-glc		√	√
	Flavones	Apigenin-7-*O*-glucoside		√	√
		Luteolin-7-*O*-glucoside		√	√
	Dihydrochalcones	Phloridzin		√	√
Other polyphenols	Tyrosols	Hydroxytyrosol		√	√
		Tyrosol		√	
Stilbenes	Stilbenes	*trans*-resveratrol	√	√	√
		cis-resveratrol		√	√
		trans-polydatin		√	√
Tannins	Hydrolysable tannins	Vescalin		√	√
	(Ellagitannins)	Castalin		√	√
Others		5-hydroxymethyl-2-furaldehyde	√	√	√

Both extract mixtures were found to contain the same phenolic compounds with the exception of two compounds for GS/OL/CB_1 extract (tyrosol and myricetin 3-O-β-D-glucuronide) and GS/OL/CB_2 extract (t-cinnamic acid and trans-caftaric acid). Presumably due to the different percentage or amount of GS, OL and CB extracts incorporated into the extract mixtures formulation. For instance, it was reported that tyrosol (0.71% absolute content dry basis) present in OL extract at a lower concentration compared to hydroxytyrosol (1.46% absolute content dry basis) (Benavente-García *et al.* 2000). Tyrosol may be detected in GS/OL/CB_1 extract (Table 2.2) possibly because this extract contains greater amount of OL extract compared to GS/OL/CB_2 extract. However, the actual formulation of extract mixtures cannot be revealed due to confidentiality. Also, quantitative analysis of phenolic compounds present in the commercial extracts was not feasible in the present study, therefore *in vitro* antioxidant assays were used to characterise the antioxidant activities of the extracts.

2.3.3 *In vitro* antioxidant activity of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts

The antioxidant potency of the extracts for all *in vitro* antioxidant assays (TPC, FRAP, DPPH) followed the order: GS/OL/CB_2 > GS/OL/CB_1 > GS ($p<0.05$) (Table 2.3). The TPC of GS extract in present study was within the range of previously reported TPC values (TPC: 50.74 – 99.28 mg GAE/g) in the scientific literature (Xu *et al.* 2010). However, Xu *et al.* (2010) reported FRAP (0.08 – 0.15 mg TE/g) and DPPH (0.06 – 0.11 mg TE/g) activities in GS extract compared to the present study. Previous study reported the antioxidant activities (TPC, DPPH and FRAP) of GS extracts varies among 18 different grape cultivars, as it is believed that the genotype is a determining factor leading this variation (Xu *et al.* 2010). The antioxidant activity of GS extract was attributed to the presence of monomers and polymeric procyanidins (Yilmaz and Toledo 2004).

Table 2.3 *In-vitro* antioxidant activity of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts.

Extract	TPC (mg GAE/g)	FRAP (mg TE/g)	DPPH (mg TE/g)
GS	53.21 ± 12.63^{a}	185.72 ± 1.81^{a}	161.45 ± 32.16^{a}
GS/OL/CB_1	588.51 ± 7.94^{b}	1177.59 ± 13.87^{b}	2455.45 ± 31.83^{b}
GS/OL/CB_2	900.12 ± 15.77^{c}	1899.30 ± 37.72^{c}	4452.15 ± 29.87^{c}

[abc]Within each assay (TPC, FRAP, DPPH), mean values (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
GAE = Gallic acid equivalent.
TE = Trolox equivalent.

OL extract has been previously shown to possess TPC (66 mg GAE/g), FRAP (102 mg TE/g) and DPPH (0.21 mg TE/g) radical scavenging activities (Ahmad-Qasem *et al.* 2013; Botsoglou *et al.* 2013). CB extract had higher TPC (561 mg GAE/g) levels (Comandini *et al.* 2014) compared OL extract. Previous study reported sweet chestnut inner shell (pellicle) possessed DPPH scavenging ability (DPPH: 39.99 – 43.70 mg TE/g) (Ham *et al.* 2015). However, no FRAP data for CB extract is available in the scientific literature. The combination of GS extract with OL and CB extracts (GS/OL/CB_1 and GS/OL/CB_2 extracts) resulted in significantly higher TPC, FRAP and DPPH activities compared to the GS extract alone (Table 2.3).

The increased antioxidant activity is presumably due to the compounds present in OL and CB. For example, hydroxytyrosol has a good scavenging ability in comparison to tyrosol, apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside (Benavente-García *et al.* 2000). Also, ellagic acid was reported to have superior scavenging activity owing to the present of four phenolic hydroxyl (OH) groups on a fused benzofuran structure (Hayes *et al.* 2011).

The present study demonstrated that GS/OL/CB_1 extract was less potent than the GS/OL/CB_2 extract. This may be due to the fact that GS/OL/CB_1 extract contains tyrosol, a compound reported to be inefficient in inhibiting DPPH radical (Vlachogianni *et al.* 2015) and has less ability to scavenge ABTS radical as compared to hydroxytyrosol (Benavente-García *et al.* 2000). LCMS confirmed that GS/OL/CB_2 extracts contain caftaric acid, a compound found to possessed radical scavenging ability and react rapidly with DPPH radical as compared to (+)-catechin and (-)-epicatechin (Villaño *et al.* 2007).

The superior antioxidant activity of the extract mixtures may also be attributed to the interaction between phenolic compounds present in the extracts. Benavente-García *et al.* (2000) reported the OL extracts possessed greater antioxidant activity (ABTS) than individual phenolic compounds (tyrosol, apigenin 7-glucoside, luteolin 7-glucoside and caffeic acid) possibly due to the interaction between phenolic compounds in OL extracts implying potential synergistic activity between compound classes.

2.3.4 Antioxidant potency of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts in chicken muscle homogenates

Chicken breast and thigh homogenates containing GS, GS/OL/CB_1 and GS/OL/CB_2 extracts (50 and 100 µg/ml) were subjected to $FeCl_3$/Asc-induced lipid oxidation. After 24 hr storage at 4°C, lipid oxidation increased significantly ($p<0.05$) in breast and thigh homogenates containing lipid pro-oxidants (P), compared to controls (Table 2.4). After 24 hr storage at 4ºC, GS extract (50 and 100 µg/ml) did not significantly reduce lipid oxidation in breast homogenates containing lipid pro-oxidants. In thigh muscle homogenates, a significant reduction in lipid oxidation was observed in homogenate containing 100 µg/ml. GS/OL/CB_1 and GS/OL/CB_2 extracts (50 µg/ml) significantly ($p<0.05$) reduced lipid oxidation in both muscle homogenates compared to GS extract containing homogenates

(50 µg/ml) demonstrating superior antioxidant potency of the extract mixtures. No further reduction in lipid oxidation was observed at the higher level of extract addition (100 µg/ml) indicating that lower levels of extract addition (50 µg/ml) were sufficient in this test system.

Chicken muscle homogenates containing lipid pro-oxidants and extracts were used to screen the suitability and ability of the extracts to stabilise oxidation-sensitive muscle against lipid oxidation. Although GS/OL/CB_2 extract demonstrated the higher *in vitro* antioxidant activity compared to GS/OL/CB_1 extract (Table 2.3), both mixtures demonstrated similar antioxidant activity in chicken muscle homogenate system. Therefore, it is important to use *in vitro* antioxidant assays in conjunction with additional test systems when comparing the potency of antioxidants for use in muscle foods.

Table 2.4 Lipid oxidation in 25% chicken breast and thigh homogenates following the addition of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts.

Incubate	**Level (µg/ml)**	**Storage time at 4°C, hr**			
		Breast muscle		**Thigh muscle**	
		TBARS[1]		**TBARS**	
		1	24	1	24
H[2]		0.05 ± 0.01[a]	0.08 ± 0.02[a]	0.07 ± 0.02[a]	0.11 ± 0.04[a]
H + P[3]		0.08 ± 0.02[b]	0.57 ± 0.16[c]	0.11 ± 0.03[a]	0.48 ± 0.15[c]
H + P + GS	50	0.07 ± 0.01[ab]	0.43 ± 0.12[c]	0.09 ± 0.02[a]	0.35 ± 0.12[bc]
H + P + GS	100	0.07 ± 0.01[ab]	0.34 ± 0.10[bc]	0.08 ± 0.02[a]	0.26 ± 0.08[ab]
H + P + GS/OL/CB_1	50	0.07 ± 0.01[ab]	0.12 ± 0.05[ab]	0.08 ± 0.02[a]	0.12 ± 0.03[a]
H + P + GS/OL/CB_1	100	0.07 ± 0.01[ab]	0.10 ± 0.02[a]	0.08 ± 0.01[a]	0.10 ± 0.01[a]
H + P + GS/OL/CB_2	50	0.05 ± 0.01[ab]	0.09 ± 0.03[a]	0.06 ± 0.01[a]	0.08 ± 0.01[a]
H + P + GS/OL/CB_2	100	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.06 ± 0.00[a]	0.06 ± 0.01[a]

[abc]Mean values (± standard deviation) in the same column, bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
[1]Unit of absorbance at 532 nm (A_{532}), [2]25% chicken breast and thigh homogenate, [3]$FeCl_3$/sodium ascorbate (45µm).

2.3.5 Antimicrobial activity of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts

The antimicrobial activities of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts (50 mg/ml) were examined against Gram positive bacteria (*S. aureus* and *B. cereus*), Gram negative bacteria (*E. coli* and *P. fluorescens)* and chicken microflora using the disc diffusion method. This assay measures the antimicrobial activity of compounds which diffuse out from the disc and inhibit microbial growth on the agar surface, known as the inhibition zone (halo effect). The size of the inhibition zone is directly related to the potency of the extract (Table 2.5).

In general, the antimicrobial potencies of extracts against Gram positive bacteria were as follows: Streptomycin (positive control) > GS/OL/CB_1 ≈ GS/OL/CB_2 > GS (Table 5). GS extract shown to be insensitive to all tested bacteria and had no activity against chicken microflora. By contrast, it was previously reported that the minimum inhibitory concentration (MIC) required for GS extract to inhibit the growth of *S. aureus* using the disc diffusion method was > 40 mg/ml (Klančnik *et al.* 2010). Baydar *et al.* (2004) reported that GS extract (40 mg/ml) was capable of inhibiting the growth of *S. aureus* (0 - 10 mm), *B. cereus* (25 – 28 mm) and *E. coli* (22 – 27 mm) possibly due to the high total phenolic content (627.98 – 667.89 mg GAE/g) present. The lack of antimicrobial activity observed in present study presumably due to the low total phenolic content (Table 2.3) of GS extract used. It was also reported that a low concentration of GS extracts (1 and 2%) was ineffective to prevent microbial growth (Baydar *et al.* 2004).

Table 2.5 Antimicrobial activities of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts against Gram positive and negative bacteria and chicken microflora.

Extract	**Diffusion diameter (mm) and level of antimicrobial sensitivity[1]**				
	Gram positive		**Gram negative**		
	S. aureus	***B. cereus***	***E. coli***	***P. fluorescens***	**Chicken microflora**
GS	7.65 ± 0.09 (ns)	6.82 ± 0.62 (ns)	7.18 ± 0.48 (ns)	6.76 ± 0.34 (ns)	na[2]
GS/OL/CB_1	12.37 ± 1.15 (s)	10.28 ± 0.33 (s)	na[2]	6.97 ± 0.72 (ns)	7.75 ± 0.99 (ns)
GS/OL/CB_2	11.55 ± 0.31 (s)	11.66 ± 0.66 (s)	6.88 ± 0.33 (ns)	7.39 ± 0.42 (ns)	7.18 ± 1.02 (ns)
Streptomycin[3]	18.08 ± 0.98 (vs)	20.42 ± 1.13 (es)	17.64 ± 0.65 (vs)	14.54 ± 1.23 (s)	8.56 ± 2.55 (ns)

[1]Antimicrobial activity (sensitivity) of extracts interpreted depending on halo diameter: < 8mm not sensitive (ns); 9 – 14 mm sensitive (s); 15 – 19 mm very sensitive (vs); > 20 mm extremely sensitive (es).
[2]na = no activity
[3]Streptomycin 10 µg/disc
n = 3 independent experiments, measurements recorded in duplicate.

The extract mixtures, similar to GS, were insensitive to the growth of Gram negative bacteria, except for GS/OL/CB_1 extract which demonstrated no activity against the growth of *E. coli*. Antimicrobial activity of OL extract has been reported previously where the concentration of OL required to inhibit 25% growth of *B. cereus*, *E. coli* and *S. aureus* was 0.63, 1.81 and 2.68 mg/ml, respectively, probably due to high content of oleuropein (26.5 mg/g) and other phenolic compounds (caffeic acid, verbascoside, luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside and luteolin 4-*O*-glucoside) contributing to antimicrobial activity (Pereira *et al.* 2007). By contrast, Bisignano *et al.* (1999) reported hydroxytyrosol was more potent against *S. aureus* compared to oleuropein possibly because oleuropein has a glycosidic group which may prevent it from penetrating into cell membranes or reaching the target site. In addition, caffeic acid and p-coumaric acid were found to possess antimicrobial activity against *E. coli* and *B. cereus* (Lee and Lee 2010; Makwana *et al.* 2015).

The scientific literature contained no information on the antimicrobial activity of CB against the tested bacteria and chicken microflora. However, the chestnut inner shell extract was reported to effectively inhibit 50% (EC_{50}) of the growth of *P. fluorescens* and *Bacillus sp* at the concentration of 4.39 µg/ml and 5.02 µg/ml, respectively (Hao *et al.* 2012). The extract mixtures did not demonstrate antimicrobial activity possibly because CB extract may not contain similar level of the active compounds as the inner shell and the used of different medium in testing the antimicrobial activity in the previous (Lysogeny broth – Miller agar medium) and present study (plate count agar).

Individual phenolic (oleuropein, rutin and vanillin) containing discs (0.8 mg/disc) had no activity against *B. cereus*, *S. aureus* and *E. coli* possibly because the concentration of phenolic was too low to exert inhibitory activities against highly populated colonies (10^8 CFU/ml) (Lee and Lee 2010). Interestingly, combined phenolic compounds (0.8 mg/disc

containing oleuropein, rutin, vanillin and caffeic acid) greatly inhibited the growth of *B. cereus* suggesting synergistic activity of phenolic compound mixtures (Lee and Lee 2010). Therefore, the profound potency of the mixes against Gram positive bacteria is possibly due to the interaction among various compounds derived from OL and CB resulting in synergistic antimicrobial activity in present study.

In general, Gram negative bacteria showed great resistance against the antimicrobial activity of GS and both extract mixtures. Gram negative bacteria has an outer membrane surrounding the cell wall function to restrict diffusion of compounds through lipopolysaccharide layer (Vaara 1992) which may reduce antimicrobial effectiveness of natural extracts (Delgado Adámez *et al.* 2012).

It is important to note that chicken microflora demonstrated resistance against streptomycin as compared to individual bacterial strains because bacteria function less as individuals and more as coherent groups that are able to inhabit multiple ecological niches (Lazdunski *et al.* 2004). In addition, chicken microflora resistance may be attributed to the interactions (such as antagonism, metabiosis and quorum sensing (QS)) which occur among various microorganisms in food ecosystems in order to facilitate the growth and survival (Gram *et al.* 2002). QS (known as bacteria cell-to-cell communication) is an important tool in the battle for survival and relies on activation of a signal molecule (Cámara 2006). The concentration of the signal molecule reflects the number of bacterial cells in a particular niche and perception of a threshold concentration of that signal molecule indicates that the population is ready to make a behavioural decision or changes (Cámara 2006).

2.4 Conclusions

Grape seed, olive leaf and sweet chestnut bark extract were evaluated as antioxidant and antimicrobial ingredients for potential use in oxidation-sensitive muscle foods such as chicken. *In vitro* antioxidant activity (TPC, FRAP and DPPH) was greater in the two mixtures of grape seed, olive leaf and sweet chestnut bark extracts (GS/OL/CB_1 and GS/OL/CB_2) than grape seed (GS) extract alone. Extract mixtures exhibited greater antioxidant activity in chicken muscle homogenates compared to GS extract alone. GS extract did not exhibit antimicrobial activity against all tested strains and chicken microflora. Extract mixtures (GS/OL/CB_1 and GS/OL/CB_2) possessed antimicrobial activities against Gram positive bacteria but were not active against Gram negative bacteria or chicken microflora. Results from the present study highlight the potential for utilising grape seed, olive leaf and sweet chestnut bark extract mixtures as natural antioxidants in poultry processing.

CHAPTER 3

3 Supplementation of grape seed extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts in chicken diets: effects on quality and shelf-life characteristics of raw chicken meat.

Abstract

Chicken diets were supplemented with grape seeds (GS) extract at three levels (100, 200 and 300 mg/kg feed) (GS100, GS200 and GS300) and two mixes of GS, olive leaf (OL) and sweet chestnut bark (CB) extracts at the concentration of 50 mg/kg (GS/OL/$CB_1$50 and GS/OL/$CB_2$50) for 35 days pre-slaughter. The effect of supplementation on the total antioxidant status (TAS) of plasma, lipid stability of chicken muscles (breast and thigh) and organs (liver, heat and lung) and proximate composition of chicken breasts and thighs were examined. The effect of diets on drip loss after 48 hr aerobic storage and shelf-life quality (colour, pH, cook loss, texture, microbiology and lipid oxidation) of breast fillets stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) for up to 6 and 12 days, respectively were investigated. The TAS of plasma, lipid stability of chicken organs and proximate composition of chicken were not affected by the diets. In iron induced system, GS300 and GS100 significantly ($p<0.05$) reduced lipid oxidation of breast and thigh muscle homogenates, respectively. GS/OL/$CB_1$50 resulted to significantly ($p<0.05$) higher drip loss as compared to the controls. Most of the shelf-life qualities of chicken fillets stored in both types of packaging were not significantly affected by the diets, except the surface colour of aerobic and MAP breast samples was significantly ($p<0.05$) affected by the diets on certain storage days, however no clear trends were observed. Sensory evaluation demonstrated that GS300 had significant effect on chicken fillets attributes stored in aerobic and modified atmosphere packs. In conclusion, GS300 exerted great potencies against lipid oxidation in breast

homogenate muscles and improve the sensory attributes of the breasts while GS100 had protective effect on thigh muscles.

3.1 Introduction

Poultry meat (chicken, turkey and duck) is consumed and produced worldwide. The production of poultry, in particular chicken (broiler) meat, in Europe is expected to grow in 2016 and 2017 due to increased exports and domestic demand, low production cost, consumer preference and nutrient profile (Salmon and Audran 2016). From a nutritional perspective, chicken meat is a source of high-value animal protein in human diets. Chicken meat is low in fat and contains relatively high concentrations of polyunsaturated fatty acids (PUFAs) compared to beef or pork. The composition and nutrients present render chicken meat susceptible to many quality deteriorative processes, such as microbial growth and lipid oxidation. Such processes result in the loss of nutritional and organoleptic (sensory) characteristics, thus compromising chicken meat quality and shelf-life (Cortinas *et al.* 2005).

Quality deterioration in chicken meat can be controlled by the direct addition of natural/synthetic additives during post-mortem processing or by supplementing additives into chicken diets for a specific time period pre-slaughter. Rising concerns over the safety and toxicity of synthetic additives has prompted much research into the use of natural compounds from plant sources and their influence on muscle food quality and shelf-life (Botsoglou *et al.* 2010).

Vitamin E (as α-tocopheryl acetate) is included in commercial broiler diets in order to support bird growth, health, disease resistance and immune function. At cellular level, vitamin E also functions as an antioxidant capable of scavenging free radicals and reactive oxygen species which can initiate oxidation of unsaturated muscle lipids. Typical α-tocopheryl acetate inclusion levels are 80, 65 and 55 mg/kg feed in starter, grower and finisher broiler diets, respectively. When fed at elevated levels (200 mg/kg), vitamin E is deposited in broiler muscle tissues resulting in chicken meat with enhanced lipid stability

and shelf-life characteristics (De Winne and Dirinck 1996). Dietary supplementation of vitamin E is more effective in stabilising chicken meat against oxidation than post-mortem direct addition as supplementation allows for the incorporation of vitamin E into the membrane lipids, where lipid oxidation is initiated (Frankel 1998b).

Plant-derived extracts, manufactured from by-products or wastestreams from various food and beverage industries (for example grape seeds and olive leaves), contain a wide range of polyphenolic compounds with numerous multi-functional bioactivities including antioxidant and antibacterial activities. Potential exists for such extracts to act as a replacement for vitamin E in poultry diets. In addition to possibly conferring superior antioxidant properties to poultry meat, plant extracts may also reduce the need for use of antimicrobials in poultry diets and help alleviate associated issues with drug residues in poultry meat.

Grape seed (*Vitis vinifera*) contains gallic acid, monomer procyanidin ((+)-catechin, (-)-epicatechin and (-)-epicatechin gallate) and dimer procyanidin (B1 and B2) (Escribano-Bailon *et al.* 1992). The antioxidant capacity of grape seed extract is derived from their dimeric, trimeric, oligomeric and/or polymeric procyanidins (Yilmaz and Toledo 2004). Grape seed extract supplemented into chicken diets (100 and 200 mg/kg) was absorbed and deposited in the muscle, thus protecting minced chicken meat against lipid oxidation in a dose-response manner (Smet *et al.* 2008). Direct addition of grape seed extract (10,000 mg/kg) protected cooked turkey thigh muscles against lipid oxidation to a greater extent than the addition of 20,000 mg/kg. It was concluded that an optimum concentration for the direct addition of grape seed extract into poultry meat ranges between 1000 and 10,000 mg/kg (Lau and King 2003).

Olive (*Olea europaea*) leaf extract contains oleuropeosides, flavones, flavonols, flavan-3-ols and substituted phenols (Benavente-García *et al.* 2000). Phenolic compounds

present in olive leaf extract reduced lipid oxidation and lowered mesophilic, psychrotrophic and lactic acid bacterial growth on raw turkey breast meat from turkeys supplemented (10,000 mg/kg) with olive leaf extract (Botsoglou *et al.* 2010). In addition, olive leaf extract in hen diets (10,000 mg/kg) also exerted a significant antioxidant effect in *n*-3 enriched eggs (Botsoglou *et al.* 2013). Both studies indicated that active compounds present in olive leaf extract were absorbed in a monogastric digestive system and distributed in muscles tissue and eggs.

Sweet chestnut *(Castanea sativa* Mill.) bark and wood contain hydrolysable tannins and phenolic compounds, such as vescalagin, castalagin, vescalin, castalin, gallic acid and ellagic acid in varying concentrations (Viriot *et al.* 1994; Brizi *et al.* 2016). Sweet chestnut bark possesses *in vitro* antioxidant activity due to the presence of hydrolysable tannins (Brizi *et al.* 2016). To date, the scientific literature does not appear to contain any information regarding the supplementation of sweet chestnut bark in poultry or animal diets. Sweet chestnut wood supplemented into broiler diets (3,000 mg/kg) reduced lymphocyte DNA damage but exerted no protection against lipid oxidation in chicken breast muscle (Voljč *et al.* 2013).

The antioxidant potency and potential of grape seed extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts was demonstrated using *in vitro* antioxidant assays and in chicken (breast and thigh) homogenate model systems (Chapter 2). The behaviour and metabolic fate of these extracts (and mixtures) in poultry diets, including potential synergistic or additive effects of the compound classes present, or deposition in muscle tissue is unknown. Therefore, the effect of supplementing poultry diets with grape seed extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts on the quality and shelf-life parameters of chicken meat merits investigation.

The objective of this study was to assess the effect of dietary supplementation with commercial grape seed (GS) extract (100 mg/kg, GS100; 200 mg/kg, GS200; 300 mg/kg, GS300) and two mixtures of GS, olive leaf (OL) and sweet chestnut bark (CB) extracts (50 mg/kg, GS/OL/$CB_1$50 and GS/OL/$CB_2$50) on the metabolic fate of extracts in chicken tissues (plasma total antioxidant status and oxidative stability of organ (liver, heart and lung) and muscle (breast and thigh) homogenates). The effect of dietary GS, GS/OL/CB_1 and GS/OL/CB_2 plant-derived extracts on the quality and shelf-life parameters (proximate composition, colour, pH, drip loss, cook loss, texture, microbiology, lipid oxidation and sensory properties) of chicken meat stored in aerobic and modified atmosphere packaging (40% CO_2 : 60% N_2) conditions at 4°C was also investigated.

3.2 Materials and Methods

3.2.1 Reagents

Sulfuric acid (H_2SO_4), boric acid (H_3BO_3), hydrochloric acid (HCl), iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$), hydrogen peroxide (H_2O_2), L-ascorbic acid (sodium salt) ($C_6H_7O_6Na$), 2-thiobarbituric acid (TBA) ($C_4H_4N_2O_2S$), trichloroacetic acid (TCA) ($C_2HCl_3O_2$) and L-histidine ($C_6H_9N_3O_2$) were supplied by Sigma-Aldrich Ireland, Ltd., Arklow, Co. Wicklow, Ireland. Potassium chloride (KCl) was manufactured by LP Chemical Ltd, Windsford, Cheshire and ferric chloride hexahydrate ($FeCl_3.6H_2O$) was supplied by BDH Limited Supplies, Poole, England. The total antioxidant status (TAS) Randox-Trolox kit was obtained from Randox Laboratories Ltd., Co. Antrim, UK. All chemicals used were of analytical grade. Maximum recovery diluent was purchased from Oxoid (Oxoid Ltd., Basingstoke, England). Plate count agar was obtained from Merck, Darmstadt, Germany.

Commercial grape seed (GS) extracts and two mixes of grape seed, olive leaf (OL) and chestnut bark (CB) ($GS/OL/CB_1$ and $GS/OL/CB_2$) extracts were supplied by Envirotech Innovative Products Ltd (Dublin, Ireland). The chemical composition of GS, $GS/OL/CB_1$ and $GS/OL/CB_2$ extracts was reported in Chapter 2.

3.2.2 Animals and diets

Six hundred chickens (Ross 308 breed) were randomly assigned to one of six treatments (n = 100) and fed with experimental diets for 35 days pre-slaughter. The control group (control) were fed with a basal diet (Table 3.1). The second, third and fourth groups were fed with grape seed (GS) extract at inclusion rate of 100 (GS100), 200 (GS200) and 300 (GS300) mg/kg of feed, respectively. The fifth and sixth groups were fed with the basal diet plus commercial mixes of grape seed (GS), olive leaf (OL) and sweet chestnut bark

(CB) extracts, at inclusion rate of 50 mg/kg of feed, GS/OL/$CB_1$50 and GS/OL/$CB_2$50, respectively.

The chickens were housed together and divided into six different cages were grown in Shannon Vale Foods Ltd. Gallanes, Clonakilty, Co. Cork, Ireland. Cage was made from timber and 1-inch chicken mesh and cages were place along one wall of the house. Each cage was equipped with two drinkers and two feeders. The ambient environmental temperature within the house maintained at 20 – 30°C and 60 - 70% relative humidity. All chickens were fed in three stages: (1) starter diet was given from days 1 to 10, (2) grower diet (days 11 to 22) and (3) finisher diet (days 23 to 35). All birds had access to food all the times until 8 hrs before slaughter. The chickens were electrically-stunned prior to slaughter. A blood sample for plasma analysis was taken immediately following slaughtering. Then, the liver, heart, and lung were removed from the carcasses. The organs and blood were transported at 4°C to the School of Food and Nutritional Sciences at University College Cork (UCC), Ireland. The organ tissues were placed in vacuum pack bags (composed of polyamide and polyethylene sealing layer), vacuum packed and stored at -18°C prior to analysis. Blood samples were centrifuged to separate the plasma fractions and stored at -18°C prior to analysis. The carcasses were being held at 4°C for 24 hr. Then, the skins were removed and the breast and thigh muscles were removed from each carcass and transported to UCC at 4°C.

Table 3.1 Composition and chemical analysis of the experimental diet (%).

Composition	Basal diet at different stages of feeding		
	Starter (%)	Grower (%)	Finisher (%)
Barley (9% CP 12.5 MJ)	2.50	0.00	5.00
Maize (7.5% CP)	15.00	10.00	10.00
Wheat (10% CP 13 MJ)	39.67	44.18	41.65
Whole wheat (10% CP 13 MJ)	0.00	5.00	5.00
HiPro Soya - (GM/AO)	30.28	23.56	17.52
Rape - full fat (00) 38% Oil A + LPL	3.50	6.00	8.00
Golden distillers	3.00	5.00	7.50
Soya oil + LPL	1.64	1.72	1.24
Vegetable oil blend (Min 28% C18:2) + LPL	0.50	1.00	1.00
Xylanase 40000/Phyzyme XP 10000L	0.03	0.03	0.03
Sodium bicarbonate	0.18	0.14	0.11
Limestone	1.09	1.05	0.99
B-salt	0.10	0.06	0.04
Mono-calcium phosphate 22.7%	0.79	0.61	0.39
L-Lysine mono	0.27	0.21	0.20
DL-methionine	0.25	0.17	0.13
L-threonine	0.00	0.08	0.00
CR BR Premix	1.20	1.20	1.20
Total	100.00	100.00	100.00
Analysed composition			
Dry matters	86.44	86.58	86.68
Protein	21.85	20.02	18.40
A oil (acid hydrolysis method)	5.43	6.92	7.41
B oil (ether extract method)	6.30	7.94	8.48
Fibre	3.90	4.07	4.30
Ash	5.63	4.99	4.65
MEP (megajoule of energy for poultry)	12.61	13.11	13.31
Lysine	1.46	1.32	1.20
Methionine	0.71	0.64	0.56
Methionine and cysteine	1.09	1.00	0.92
Cysteine	0.37	0.36	0.35
THR	1.00	0.91	0.82
Trytophan	0.27	0.25	0.23
Calcium	0.85	0.80	0.73
Total phosphorus	0.59	0.55	0.51
Sodium	0.15	0.14	0.13
Salt	0.35	0.32	0.32

3.2.3 Poultry processing and packaging

Upon arrival, the fresh skinless chicken breast fillets were trimmed off visible fats and other non-muscular tissues and they were packed in aerobic and modified atmosphere packaging (MAP) stored at 4°C within 24 hours.

For aerobic packaging, chicken breast fillets were placed in individual trays and over-wrapped in oxygen permeable (6000 – 8000 cm^3 m^{-2} 24 h^{-1} at STP) polyvinylchloride (PVC) film (Musgrave Wholesale Partners, Dublin, Ireland). All aerobic samples were stored up to 6 days at 4°C.

For modified atmosphere packaging (MAP), chicken fillets were placed in low oxygen permeable (<1 cm^3 m^{-2} 24 hr^{-1} at STP) polystyrene/ethylvinylalcohol (EVOH)/polyethylene (PE) trays and flushed with 40% CO_2 : 60% N_2 using a vacuum-sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH & Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3 cm^3 m^{-2} 24 hr^{-1} at STP) laminated barrier film a polyolefin heat sealable layer. All MAP samples were stored up to 12 days at 4°C. The gas atmosphere in the MAP was checked using a CheckMate 9900 (PBI-DanSensor, Denmark). Immediately after gas flushing, MAP composition in MAP was 40.47 ± 0.30% CO_2 and 59.4% ± 0.30% N_2. The average gas composition in MAP at the end of the 12 days of storage period was 33.35 ± 0.78% CO_2 and 66.64 ± 0.78% N_2.

3.2.4 Plasma total antioxidant status

Blood samples (10 ml) were collected by jugular veni-puncture using vacutainers containing lithium/heparin as anti-coagulant (Becton Dickinson, Rutherford, NJ, USA) from all animal immediately after slaughter. The blood was centrifuged at 4,720 g for 20 min at 4°C using J2-21 Model centrifuge (Beckman Inc., Palo Alto, CA, USA). The plasma

layer was removed from the red blood cell layer and stored at -18°C for subsequent analysis. The total antioxidant status (TAS) of poultry blood plasma measured according to the manufacturer's instruction. Plasma (20 µl) was added to 1 ml chromogen (metmyoglobin/ABTS®), mixed thoroughly and incubated in a water bath at 37°C for 10 min. An initial absorbance of the coloured complex was recorded after 10 min of using a spectrophotometer (Cary 60 UV-Vis Agilent Technology, Australia) at 600 nm (A_{600nm}) against a blank containing all reagents and double deionised water. Tubes were returned to the water bath and 200 µl of substrate (H_2O_2 in stabilised form) was added. A second absorbance (A_{600nm}) measurement of the colour complex was recorded 3 min following substrate addition. The antioxidant activity was calculated using the following equation:

mmol/l = Factor x (ΔA Blank – ΔA Sample)

Factor = (2.04 mmol/l)/(ΔA Blank – ΔA Standard)

TAS was expressed as mmol of trolox equivalent antioxidant capacity (TEAC)/l plasma.

3.2.5 Iron-induced oxidation of organ tissues and chicken muscle homogenates

3.2.5.1 Preparation of organ tissue homogenates

Liver, heart and lung tissue homogenates (25%) were prepared by homogenising 7 g of tissues in 21 ml buffer (0.12M KCl, 5 mM histidine, pH 5.5) using a homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH & Co., Germany) at 24,000 rpm for 3 min. Lipid oxidation was initiated in individual plastic beakers by the addition of 1 mM $FeSO_4$ pro-oxidant. Lipid oxidation was measured after 4 and 24 hr of storage at 4 °C.

3.2.5.2 Preparation of chicken breast muscle homogenates

Muscle homogenates (25%) were prepared by homogenising 10 g of chopped chicken breast (*pectoralis major*) and thigh (*biceps femoris*) muscles in 30 ml of buffer

(0.12 M KCl, 5 mM histidine, pH 5.5) using an Ultra Turrax T25 homogeniser (Janke and Kunkel) at 24,000 rpm for 5 min. Lipid oxidation was initiated in individual plastic beakers by the addition of equimolar 45 µM ferric chloride/sodium ascorbate ($FeCl_3$/Asc) pro-oxidants. Lipid oxidation in muscle homogenates was measured after 1 and 24 hr of storage at 4°C.

3.2.5.3 Measurement of lipid oxidation in organ tissue and chicken muscle homogenates

A modified version of the 2-thiobarbituric acid-reactive substances (TBARS) assay by Siu and Draper (1978) was used to measure lipid oxidation. 10% TCA (4 ml) was added to 4 ml of organs and muscles homogenates and centrifuged at 6,160 g for 15 min at 4°C using J2-21 Model centrifuge (Beckman Model J2-21). The supernatant was filtered through Whatman No.1 filter paper. In screw-capped test tubes, 4 ml of the clear filtrate was added to 1 ml of 0.06 M TBA reagent. The assay blank contained 2 ml of buffer, 2 ml of 10% TCA and 1 ml of 0.06 M TBA reagent. Tubes were heated in water bath at 80°C for 90 min. Absorbance was measured at 532 nm against a blank using a spectrophotometer (Cary 60). The malondialdehyde (MDA) content was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg MDA/kg chicken

3.2.6 Proximate composition

The protein content of chicken breast was measured using the Kjeldahl method, with slight modifications (Suhre *et al.* 1982). The digestion block (Foss Tecator™ Digestor, Hillerød, Denmark) was pre-heated to 410°C. Well-homogenised of chicken breasts (~ 0.5 g) were weighed accurately into digestion tubes. Two “kjeltabs”, 15 ml of

H_2SO_4 (nitrogen free) and 10 ml H_2O_2 were added to each tube. Assay blanks tubes were prepared in the same way without samples. Tubes were placed in the heated digestion block until they become colourless (~ 1 hr). Tubes were removed from the digestion block and left to cool. Then, 50 ml of distilled water was added to each digested sample in the fume-hood. Tubes were placed in the Kjeldahl distillation unit (Foss Kjeltec 2100, Hillerød, Denmark) along with a receiver flask containing 50 ml of H_3BO_3 with indicator (Bromocresol Green and Methyl Red). After the distillation cycle was complete, the contents of the receiver flask were titrated with 0.1 N HCl until green colour reverted back to the original red colour. The protein was calculated using nitrogen conversion of 6.25. A SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany) was used to measure the moisture and fat content of the chicken breast samples. The ash content was determined using a muffle furnace (AOAC 1996) method 920.153. The muffle furnace was pre-heated to 550°C. Chicken samples (~5 g) were placed in the muffle furnace until a grey ash was produced. The samples were removed and placed in a desiccator to cool. Samples were then weighed and the ash content was calculated.

3.2.7 Colour measurement

The surface colour was measured using a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Japan). The chroma meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400) (Konica Minolta Sensing, Inc., Japan). The chroma meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79, a = -0.11, b = 2.69). The '*L' value represents lightness and 'a*' and 'b*' values represent redness and yellowness, respectively. Colour measurement of fillets were measured on days 0, 3 and 6 for aerobic samples and days 0, 3, 6, 9 and 12 for MAP samples, respectively.

3.2.8 pH measurement

The pH of breast fillet was determined using digital pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by direct insertion of the glass probe into the chicken breasts. The pH was measured on days 0, 3 and 6 for aerobic samples and days 0, 3, 6, 9 and 12 for MAP samples, respectively.

3.2.9 Drip loss measurement

The drip loss of chicken breast was determined by measuring the weight difference between 0 and 48 hr. The fillet was placed on ridged-trays, overwrapped with cling film and stored at 4°C for 48 hr. The drip loss was calculated using following equation:

$$\text{Drip loss (\%)} = \frac{\text{(initial weight, g)} - \text{(final weight, g)}}{\text{(initial weigh, g)}} \times 100\%$$

3.2.10 Cook loss measurement

The chicken breast was cooked at 90°C to an internal temperature of 75°C for 10 min in an electric steam-convection oven (Zanussi Professional, Model 10 GN1/1, Conegliano, Italy) (Rodríguez-Calleja *et al.* 2012). The cook loss of breast fillets was measured on days 0, 3 and 6 for aerobic samples and days 0, 3, 6, 9 and 12 for MAP samples, respectively.

Cook loss was calculated using following equation:

$$\text{Cook loss (\%)} = \frac{\text{(raw meat weight, g)} - \text{(cooked meat weight, g)}}{\text{(raw meat weigh, g)}} \times 100\%$$

3.2.11 Texture measurement

Warner-Bratzler shear force (WBSF) values were determined according to the method by Lyon and Lyon (1996) on 1.9 cm diameter meat cores (n = 2) cut parallel to the muscle fibre orientation. The peak shear force of each core was measured using a texture analyser (Stable Micro Systems, TA-XT2i, Surrey, UK) fitted with a Warner-Bratzler V-Blade shear cell, using a crosshead speed of 5 mm/s and a load cell of 25 kg. Maximum force measured to cut the meat was expressed in Newton (N). Texture measurement of fillets were measured on days 0, 3 and 6 for aerobic samples and days 0, 3, 6, 9 and 12 for MAP samples, respectively.

3.2.12 Microbiological analysis

Chicken breast samples (10 g) were transferred into filter stomacher bag, diluted with 90 ml of maximum recovery diluent and stomached for 2 min (Steward Stomacher 400 Lab Blender, London, UK) resulting in a 10^{-1} dilution used for analysis. Serial dilutions were prepared and 1 ml aliquots from each dilution were pipetted onto plate count agar. The plates were incubated at 30°C for 48 hr to determine mesophilic counts. Microbiological analysis of chicken fillets were carried out on days 0, 3 and 6 for aerobic samples and days 0, 3, 6, 9 and 12 for MAP samples. Results were expressed as $\log_{10}$ CFU (colony forming units)/g chicken.

3.2.13 Measurement of lipid oxidation

Lipid oxidation was measured using the 2-thiobarbituric acid assay (2-TBA) (Siu and Draper,1978). The malondialdehyde (MDA) content was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg MDA/kg chicken. Lipid oxidation in chicken breasts were

measured on days 0, 3 and 6 for aerobic samples and on days 0, 3, 6, 9 and 12 for MAP samples, respectively.

3.2.14 Sensory analysis

Chicken breasts were cooked for sensory analysis in an oven (Zanussi Professional) at 90°C to an internal temperature of 75°C for 10 min. Following cooking, chickens were cooled, sliced and identified with random three-digit codes. Sample presentation order was randomised to prevent any flavour carryover effects (MacFie *et al.* 1989). Prior to serving the panellists, chicken samples were re-heated in a microwave for 10 seconds to release the meat odour and flavour. Sensory analysis was undertaken in the panel booths at the university sensory laboratory in accordance with the ISO (1988) international standard regulations. Assessors were also provided with water to cleanse their pallets between samples. Hedonic sensory analysis descriptors were appearance, texture, flavour and overall acceptability. Intensity sensory analysis descriptors were juiciness, tenderness, chicken flavour, off-flavour and off-flavour oxidation. Assessors were asked to indicate their opinions on a 10 cm line scale ranging from 0 (extremely dislike/none) to 10 (extremely like/extreme). Results for sensory analysis scores were measured in centimetre (cm) and scores were statistically analysed using ANOVA-Partial Least Squares Regression (APLSR). Results were presented as a significance of regression coefficient, analysed by jack-knife testing. All samples were presented in duplicates. Sensory analysis of cooked chicken breasts (previously stored in aerobic and MAP) was performed by 10 naïve assessors on days 0 and 3 for aerobic samples and days 0, 3, and 6 for MAP samples, respetively.

3.2.15 Statistical analysis

Statistical analysis was carried out using SPSS 22.0 for Windows (SPSS Statistical Software, Inc., Armonk, NY, USA) software package. All analyses were performed in duplicate. A full repeated measures ANOVA was conducted to investigate the effect of treatment, time and their interactions. Treatment represented the 'between-subject' factor and the effect of time was measured using the 'within-subject' factor. One-way ANOVA was used to examine the data from proximate composition and drip loss analysis. Tukey's post-hoc test was used to adjust for multiple comparisons between means. All data is presented as mean values ± standard deviation.

Sensory data was analysed with ANOVA-Partial Least Square Regression (APLSR) to process the mean data accumulated from the 10 test subjects. The X-matrix was designated as 0/1 for treatment and days with the Y-matrix designated as sensory variables. To derive significance indications for the relationship determined in the quantitative APLSR, regression coefficients were analysed by jack-knifing which is based on cross-validation and stability plots (Martens and Martens, 2001). All analyses were performed using the Unscrambler Software, version 9.8 (CAMO ASA, Trondheim, Norway).

3.3 Results and Discussion

3.3.1 Antioxidant status of chicken plasma and tissue systems

3.3.2 Plasma total antioxidant status

Detection of antioxidant compounds in blood plasma is a useful indicator of the fate of the extracts, suggesting that the compounds or their metabolites are readily available for potential uptake by the muscles (Moroney *et al.* 2012). The average TAS was 1.38, 1.44, 1.43, 1.43, 1.66 and 1.26 mmol TEAC/L plasma for the control, GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 treatments, respectively. The inclusion of the different levels of GS and two mixtures (GS/OL/$CB_1$50 and GS/OL/$CB_2$50) extracts did not significantly ($p > 0.05$) increase the plasma TAS relative to the control. The plasma of chicken fed with GS/OL/CB_1 showed the highest increase (not significant) as the concentration of polyphenol in the plasma may vary depending on the nature of the polyphenol and the food source (Manach *et al.* 2004).

This is in agreement to previous studies reported that grape seed extract (100 and 200 mg/kg) and grape pomace (30,000 mg/kg) supplemented to chickens did not improve antioxidant capacities of the plasma as compared to chicken receiving control diets (Goñi *et al.* 2007; Vossen *et al.* 2011). Similarly, the same effect was observed in the plasma of chicken fed with sweet chestnut wood (3,000 mg/kg) (Voljč *et al.* 2013). There is no literature available on the effect of poultry supplementation with olive leaf extract, but Visioli *et al.* (2001) reported that when rats supplemented with hydroxytyrosol-rich diet (10 mg/kg) increases the antioxidant capacity of the plasma after 15 min ingestion. The increased in antioxidant capacity of the plasma can be observed during the postprandial period due to rapid excretion of polyphenols from the plasma (Alía *et al.* 2003). The fact that the chickens were fed 8 hr pre-slaughter may be the contributing factor to the insignificant differences across treatments in present study.

3.3.3 Oxidative stability of organ and muscle tissues

The insignificant level of TAS in the plasma did not suggest the availability of supplemental extracts for potential muscles uptake. Therefore, the organs (liver, heart and lung) and muscles (breast and thigh) were further subjected to iron induced lipid oxidation to investigate the antioxidant compounds behaviour under oxidative stress to confirm the fate of supplemental extracts distribution and functionality in chicken tissues.

3.3.4 Lipid oxidation in liver, heart and lung tissues

Liver, heart and lung tissue homogenates were subjected to iron ($FeSO_4$) induced lipid oxidation and following the addition of $FeSO_4$, lipid oxidation increased in all tissue homogenates over the 24 hr of storage period. The dietary supplementation of GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts did not significantly ($p > 0.05$) reduce lipid oxidation in the liver, heart and lung tissues relative to the organs of chickens fed with control diet (Table 3.2). In agreement to previous study, no antioxidative effect was observed in chicken liver when chickens were supplemented with high dose of sweet chestnut wood extract (3,000 mg/kg) for 25 days (Voljč *et al.* 2013). To date, the research on the effect of grape seed and olive leaf extracts supplementations on lipid oxidation of chicken organs are not currently available. However, rats fed with polymeric grape seed tannin (71 mg/kg diet) in high cholesterol-vitamin E deficient diet for longer duration (70 days) capable to inhibit the lipid oxidation of the tissues (aorta, heart, gastrocnemius, intestinal mucosa, kidney and liver) (Tebib *et al.* 1997) while rats drink hydroxytyrosol and its triacetylated derivatives containing water (3 mg/kg body weight) for 112 days exerted antioxidant activities on the organs (liver, heart, kidney, and aorta) (Jemai *et al.* 2008). The previous rat studies suggested the possible deposition of the extracts in the organs to

Table 3.2 Effect of dietary grape seed (GS) extract at three different levels (GS100, GS200 and GS300) and two mixes of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) (GS/OL/$CB_1$50 and GS/OL/$CB_2$50) on the iron-induced lipid oxidation (TBARS) in chicken organs (liver, heart and lung) and muscles (breast and thigh) homogenates after 24 hr of storage at 4°C.

Incubate	**Organ**						**Muscle**			
	Liver		**Heart**		**Lung**		**Breast**		**Thigh**	
	Storage time at 4°C, hours									
	4	**24**	**4**	**24**	**4**	**24**	**1**	**24**	**1**	**24**
Control	0.50 ± 0.01[a]	0.59 ± 0.03[a]	0.37 ± 0.05[a]	0.56 ± 0.20[a]	0.53 ± 0.18[a]	0.69 ± 0.32[a]	0.07 ± 0.02[a]	1.33 ± 0.39[a]	0.19 ± 0.11[a]	1.53 ± 0.53[a]
GS100	0.44 ± 0.03[a]	0.52 ± 0.03[a]	0.29 ± 0.07[a]	0.36 ± 0.06[a]	0.68 ± 0.26[a]	1.02 ± 0.45[a]	0.11 ± 0.07[a]	1.28 ± 0.53[ab]	0.09 ± 0.03[b]	0.80 ± 0.26[b]
GS200	0.47 ± 0.01[a]	0.54 ± 0.06[a]	0.27 ± 0.04[a]	0.29 ± 0.04[a]	0.75 ± 0.23[a]	1.16 ± 0.46[a]	0.08 ± 0.02[a]	1.51 ± 0.48[a]	0.09 ± 0.04[b]	1.03 ± 0.41[ab]
GS300	0.47 ± 0.02[a]	0.58 ± 0.03[a]	0.34 ± 0.05[a]	0.61 ± 0.24[a]	0.61 ± 0.03[a]	0.97 ± 0.09[a]	0.07 ± 0.02[a]	0.70 ± 0.15[b]	0.10 ± 0.02[b]	1.38 ± 0.46[ab]
GS/OL/$CB_1$50	0.47 ± 0.04[a]	0.55 ± 0.04[a]	0.39 ± 0.10[a]	0.58 ± 0.18[a]	0.45 ± 0.05[a]	0.58 ± 0.13[a]	0.10 ± 0.05[a]	1.20 ± 0.31[ab]	0.10 ± 0.03[b]	1.24 ± 0.54[ab]
GS/OL/$CB_2$50	0.43 ± 0.02[a]	0.52 ± 0.04[a]	0.31 ± 0.05[a]	0.38 ± 0.04[a]	0.63 ± 0.23[a]	0.88 ± 0.41[a]	0.08 ± 0.03[a]	1.23 ± 0.51[ab]	0.11 ± 0.03[b]	1.19 ± 0.46[ab]

[ab]Within each parameter and storage time, mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $p<0.05$.
n = 6 fillets/treatment, measurements recorded in duplicate.

elicit protective effect against oxidative stresses. However, this effect was not observed in the chicken study.

3.3.5 Lipid oxidation in chicken breast and thigh muscles

The chicken breast and thigh muscle homogenates were subjected to iron ($FeCl_3$/Asc) induced lipid oxidation and following the addition of $FeCl_3$/Asc, lipid oxidation increased in all breast and thigh muscle homogenates over the 24 hr of storage period (Table 3.2). Interestingly, *in vivo* supplementation of different concentrations of GS extract showed potencies in breast and thigh muscle homogenates against iron induced lipid oxidation. The inclusion of GS300 extract in the diet significantly ($p<0.05$) reduced lipid oxidation in the breast homogenate as compared to GS200 extract and the control. Meanwhile, GS100 diet significantly ($p<0.05$) exerted protection against lipid oxidation in thigh muscle homogenates relatives to the controls. The antioxidant capacity of GS extract derived from the phenolic content, free radical scavenging and ferric reducing antioxidant activities as reported in Chapter 2.

In agreement, grape pomace polyphenol diet (15,000 and 30,000 mg/kg) has shown to exert significant antioxidant activity of the raw chicken breast and thigh muscles against oxidative stress as compared to the control group, the concentration exhibited plateau effect (Goñi *et al.* 2007). The finding in present study reflects that antioxidant compounds in grape seed extract were absorbed, deposited and remained functional in both breast and thigh muscles. This lend the support to previous studies which reported that diets supplemented with grape seed (3,600 mg/kg) and grape pomace (30,000 mg/kg) extract result to significantly higher extractable polyphenols in chicken intestinal tract as compared to the control groups, suggesting that the extractable polyphenols were partially absorbed in the small intestine (Goñi *et al.* 2007; Brenes *et al.* 2010). In addition, Brenes *et al.* (2010)

also reported that grape seed extract diet result to significantly high antioxidant activity in the excreta which indicates the ability of chicken intestinal microflora to degrade parts of the extractable polyphenols.

The iron induced study showed that dietary supplementation with GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts did not improved the oxidative stability of raw breast and thigh muscles against lipid oxidation relative to the controls. Previous study showed that daily ingestion of olive extract drinking water (containing 2.5% hydroxytyrosol), at the concentration of 6 and 12 mg/chicken, for 42 days did not improve oxidative stability of post mortem thigh meat (King *et al.* 2014). The author suggested that the residual remaining in chicken tissues may be low as the by-product and hydroxytyrosol are highly water-soluble, thus make them unavailable to be deposited and functioned as antioxidant in the tissue and subsequently may end up in the urine and faeces. Similarly, high dose supplementation of sweet chestnut wood extract (1,500 – 2,500 mg/kg) for 42 days did not protect chicken breast muscle homogenates against iron induced oxidation as compared to the control (Schiavone *et al.* 2008).

Chapter 2 demonstrated the GS/OL/CB_1 and GS/OL/CB_2 extract mixtures exhibit excellent *in vitro* antioxidant activities greater than grape seed extract. The direct addition of mixes (50 and 100 µg/ml) in breast and thigh muscle homogenates, protected the muscles homogenates against lipid oxidation (plateau effect) which suggested the suitability of the mixes to be used in chicken muscle (Chapter 2). Despite these, the GS/OL/$CB_1$50 and GS/OL/$CB_2$50 diets exhibited lack of activities in the muscles probably due to the low concentration of the mixes (50 mg/kg) which may not allow sufficient absorption of the supplemental extracts. This is in agreement to Gladine *et al.* (2007), who reported that *in vitro* and *in vivo* antioxidant capacities of natural extracts rich in polyphenols are not

correlated. It may due the possibility of some polyphenols losing their antioxidant capacity *in vivo*, resulting to low availability for muscle uptakes (Manach *et al.* 2004).

3.3.6 Proximate composition of chicken breast and thigh muscles

Dietary supplementations with GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts did not significantly ($p > 0.05$) affect the composition of breast and thigh muscles as compared to the controls (Table 3.3). The composition of breast similar to the values reported in the literature i.e. 72.77 – 75.40% moisture and 22.02 – 25.19% protein (Choo *et al.* 2014), 3.80 – 5.25% fats (Fanatico *et al.* 2005) and 1.29% ash (Barbanti and Pasquini 2005). The proximate composition of thigh was similar to values reported by Jung *et al.* (2011) i.e. 73.89 – 75.34% moisture, 3.14 – 6.59% fats, 16.90 – 17.63% protein and 0.99 – 1.25% ash. In agreement, Schiavone *et al.* (2008) also reported that sweet chestnut wood diet did not affect the proximate composition of chicken breast and thigh muscles.

Table 3.3 Effect of dietary GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts on the proximate composition of chicken breast and thigh muscles.

Treatment	Muscle	% Moisture	% Fat	% Protein	% Ash
Control	Breast	72.63 ± 0.89[a]	2.97 ± 0.26[a]	22.44 ± 0.44[a]	1.10 ± 0.10[a]
GS100		71.67 ± 1.47	3.18 ± 0.16	23.57 ± 0.21	1.23 ± 0.07
GS200		71.89 ± 0.62	3.28 ± 0.21	23.18 ± 0.31	1.23 ± 0.02
GS300		72.45 ± 1.31	2.99 ± 0.11	22.99 ± 0.36	1.19 ± 0.02
GS/OL/$CB_1$50		71.86 ± 1.34	3.55 ± 0.73	22.64 ± 1.12	1.20 ± 0.04
GS/OL/$CB_2$50		72.01 ± 0.86	3.26 ± 0.20	23.20 ± 0.29	1.15 ± 0.12
Control	Thigh	73.00 ± 0.63[a]	3.68 ± 0.54[a]	19.83 ± 2.46	1.07 ± 0.07[a]
GS100		73.29 ± 1.00	3.75 ± 0.36	19.67 ± 0.47	1.07 ± 0.05
GS200		71.98 ± 1.08	3.81 ± 0.17	20.58 ± 0.42	1.07 ± 0.05
GS300		74.15 ± 0.83	3.37 ± 0.39	20.30 ± 0.70	1.13 ± 0.09
GS/OL/$CB_1$50		74.12 ± 0.55	3.49 ± 0.33	20.08 ± 1.41	1.07 ± 0.03
GS/OL/$CB_2$50		73.45 ± 1.23	3.84 ± 0.58	19.69 ± 1.35	1.05 ± 0.02

[a]Within each column, mean values (± standard deviation) are not significantly difference, $p > 0.05$.GS100: GS extract at 100 mg/kg, GS200: GS extract at 200 mg/kg, GS300: GS extract at 300 mg/kg GS/OL/CB1 and GS/OL/CB2: two mixtures of grape seed, olive leaf and sweet chestnut bark extract at 50 mg/kg.
n = 6 fillets/treatment, measurements recorded in duplicate.

3.3.7 Colour and pH of breasts muscles

The inclusion of GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts in the diets significantly ($p<0.05$) affect the lightness (L^*), redness (a^*) and yellowness (b*) of aerobic samples and L^* and a^* of MAP samples on certain days of analysis (Table 3.4). On day 0 of aerobic and modified atmosphere packaging, chicken fed with GS100 and GS200 extracts was significantly ($p<0.05$) darker than those fed with GS300. GS/OL/$CB_2$50 breasts had significantly ($p<0.05$) lighter surface than GS100 breasts on day 3 and 6 of aerobic storage. On day 0 of aerobic and modified atmosphere storage, control and GS200 breasts were significantly ($p<0.05$) redder than GS300 breasts. GS100 breasts were significantly ($p<0.05$) redder than GS200 and GS300 breasts on day 3 of aerobic

storage. Meanwhile, control breasts were significantly ($p<0.05$) redder than GS100 (day 3) and GS300 (day 6) breasts in MAP. In terms of yellowness, only G300 breasts were significantly ($p<0.05$) yellower than GS100 and GS/OL/$CB_1$50 breasts on day 6 aerobic storage but no effect was observed for MAP samples. The inclusion of extracts in the diets seem to affect the surface colour of raw breasts which may derived from the colour of the extracts used in chicken diets which were GS (light purplish), GS/OL/CB_1 (dark purple) and GS/OL/CB_2 (ochre). However, no trends were observed across treatments for breast fillets stored in aerobic and modified atmosphere packs. Therefore, the colour differences of raw breasts between treatments may be due to the variations of breast meats which exist at retail level (Fletcher 1999).

The supplementation of chicken diets with GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts did not significantly ($p > 0.05$) affect the pH of chicken breasts where measurements were recorded at 3 day intervals over 6 and 12 days of storage in aerobic and modified atmosphere packs, respectively (Table 3.5). Since there was no significant effect of the diet treatments, therefore the results were presented as overall data ranges from pH 5.4 – 6.3 (aerobic) and pH 5.9 – 6.5 (MAP). The pH values were similar to reported literature of breast pH stored in aerobic and modified atmosphere packs (pH 5.9 – 6.4) (Chouliara *et al.* 2007). Previous study showed that sweet chestnut wood diet did not affect the pH of chicken breast as compared to the control (Schiavone *et al.* 2008; Voljč *et al.* 2013).

Table 3.4 Effect of dietary GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts on colour of raw chicken breast stored in aerobic and modified atmosphere packs (MAP) (40% CO_2 : 60% N_2) at 4°C for up to 6 and 12 days, respectively.

Treatment/ Parameters	Storage time at 4°C, days						
	0	3		6		9	12
	Aerobic/MAP	Aerobic	MAP	Aerobic	MAP	MAP	MAP
Lightness (*L)**							
Control	54.8 ± 0.9^{ab}	54.9 ± 2.9^{ab}	53.6 ± 3.3^{a}	53.9 ± 2.3^{ab}	53.3 ± 2.3^{a}	55.4 ± 4.4^{a}	53.9 ± 2.3^{a}
GS100	53.3 ± 1.3^{a}	51.8 ± 0.7^{a}	54.8 ± 1.1^{a}	51.7 ± 0.5^{a}	57.6 ± 8.1^{a}	56.5 ± 2.0^{a}	51.6 ± 0.8^{a}
GS200	53.5 ± 1.6^{a}	56.5 ± 1.1^{ab}	54.5 ± 2.8^{a}	55.1 ± 2.4^{ab}	54.4 ± 1.5^{a}	56.1 ± 3.4^{a}	56.9 ± 1.8^{a}
GS300	57.3 ± 0.2^{b}	56.1 ± 2.3^{ab}	54.4 ± 1.1^{a}	56.6 ± 2.1^{ab}	53.8 ± 1.6^{a}	53.5 ± 0.9^{a}	55.1 ± 0.7^{a}
GS/OL/$CB_1$50	54.3 ± 2.0^{ab}	54.8 ± 1.8^{ab}	56.8 ± 2.2^{a}	56.6 ± 1.7^{ab}	54.0 ± 1.3^{a}	56.4 ± 3.3^{a}	56.5 ± 1.9^{a}
GS/OL/$CB_2$50	55.0 ± 0.5^{ab}	58.0 ± 1.4^{b}	56.6 ± 2.5^{a}	57.9 ± 1.7^{b}	55.6 ± 1.1^{a}	55.5 ± 1.2^{a}	58.0 ± 1.8^{a}
Redness (*a)**							
Control	2.5 ± 0.0^{b}	2.0 ± 0.5^{ab}	2.7 ± 0.5^{b}	2.2 ± 0.4^{a}	3.9 ± 1.3^{b}	2.6 ± 0.6^{a}	2.2 ± 0.4^{a}
GS100	1.7 ± 0.6^{ab}	3.1 ± 0.7^{b}	1.1 ± 0.3^{a}	2.1 ± 0.3^{a}	2.5 ± 0.4^{ab}	1.7 ± 0.2^{a}	2.7 ± 0.7^{a}
GS200	2.4 ± 0.8^{b}	1.7 ± 0.1^{a}	1.7 ± 0.3^{ab}	2.0 ± 0.6^{a}	1.7 ± 0.3^{ab}	1.7 ± 0.1^{a}	1.6 ± 0.3^{a}
GS300	1.0 ± 0.4^{a}	1.4 ± 0.6^{a}	2.1 ± 0.1^{ab}	1.4 ± 0.5^{a}	1.6 ± 0.4^{a}	2.1 ± 0.5^{a}	1.8 ± 0.3^{a}
GS/OL/$CB_1$50	2.3 ± 0.3^{ab}	1.9 ± 0.5^{ab}	1.9 ± 0.5^{ab}	2.2 ± 0.5^{a}	2.1 ± 0.2^{ab}	2.3 ± 0.4^{a}	2.3 ± 0.6^{a}
GS/OL/$CB_2$50	1.9 ± 0.6^{ab}	2.2 ± 0.4^{ab}	2.1 ± 0.5^{ab}	1.9 ± 0.3^{a}	2.1 ± 1.4^{ab}	1.8 ± 0.2^{a}	1.9 ± 0.4^{a}
Yellowness (*b)**							
Control	2.5 ± 1.0^{a}	3.1 ± 0.7^{a}	1.4 ± 2.0^{a}	2.9 ± 0.9^{ab}	3.0 ± 0.7^{a}	3.4 ± 0.8^{a}	3.0 ± 0.6^{a}
GS100	2.2 ± 0.4^{a}	2.8 ± 0.7^{a}	2.8 ± 0.1^{a}	2.5 ± 0.5^{a}	4.6 ± 1.0^{a}	3.5 ± 1.3^{a}	2.6 ± 0.7^{a}
GS200	2.8 ± 0.5^{a}	3.3 ± 0.5^{a}	2.6 ± 0.8^{a}	2.8 ± 0.7^{ab}	4.1 ± 0.8^{a}	3.0 ± 0.3^{a}	3.9 ± 1.3^{a}
GS300	3.3 ± 0.7^{a}	3.9 ± 1.9^{a}	2.4 ± 0.8^{a}	4.9 ± 0.8^{b}	3.5 ± 0.3^{a}	2.3 ± 0.9^{a}	4.7 ± 1.8^{a}
GS/OL/$CB_1$50	2.6 ± 0.3^{a}	2.4 ± 0.1^{a}	2.6 ± 0.6^{a}	2.5 ± 0.5^{a}	3.5 ± 0.1^{a}	4.5 ± 0.9^{a}	3.0 ± 0.4^{a}
GS/OL/$CB_2$50	2.8 ± 0.3^{a}	4.2 ± 0.8^{a}	2.1 ± 0.5^{a}	4.5 ± 1.2^{ab}	3.0 ± 0.7^{a}	3.9 ± 0.5^{a}	4.3 ± 0.5^{a}

^{ab}Within each parameter and storage day, mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $p<0.05$.
n = 6 fillets/treatment, measurements recorded in duplicate.

Table 3.5 Effect of dietary GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts on pH, cook loss, texture, microbiology and lipid oxidation of chicken breast stored in aerobic and modified atmosphere packs (40% CO_2: 60% N_2) at 4°C for up to 6 and 12 days, respectively.

Treatment	**Aerobic packaging**				
	pH	**Cook loss (%)**	**Texture (N)**	**Mesophilic count (log^{10} CFU/g)**	**Lipid oxidation (mg MDA/kg)**
Control	5.8 – 6.1	20.2 – 25.5	8.7 – 10.0	3.9 – 6.1	0.09 – 0.21
GS100	5.4 – 6.1	19.7 – 24.6	7.3 – 11.1	4.2 – 6.1	0.10 – 0.15
GS200	5.8 – 6.0	18.5 – 23.8	9.6 – 11.3	3.6 – 6.1	0.09 – 0.20
GS300	5.7 – 6.1	19.9 – 24.9	8.9 – 10.5	3.8 – 6.2	0.10 – 0.15
GS/OL/$CB_1$50	5.8 – 6.1	17.0 – 25.8	10.2 – 10.7	4.3 – 6.1	0.10 – 0.17
GS/OL/$CB_2$50	5.9 – 6.3	19.4 – 24.2	8.7 – 11.3	4.1 – 6.1	0.09 – 0.18
Treatment	**Modified atmosphere packaging**				
	pH	**Cook loss (%)**	**Texture (N)**	**Mesophilic count (log^{10} CFU/g)**	**Lipid oxidation (mg MDA/kg)**
Control	6.0 – 6.2	20.1 – 25.5	8.7 – 12.2	3.9 – 7.2	0.10 – 0.16
GS100	6.0 – 6.2	19.7 – 24.6	10.1 – 11.6	4.2 – 7.4	0.10 – 0.16
GS200	6.0 – 6.5	19.0 – 23.8	7.6 – 12.8	3.6 – 7.2	0.10 – 0.17
GS300	6.0 – 6.3	21.1 – 25.1	10.0 – 12.0	3.8 – 7.2	0.10 – 0.15
GS/OL/$CB_1$50	5.9 – 6.1	20.7 – 25.8	10.1 – 12.0	4.3 – 7.3	0.12 – 0.17
GS/OL/$CB_2$50	5.9 – 6.2	20.7 – 25.2	9.7 – 13.2	4.1 – 7.2	0.11 – 0.19

Data presented as overall ranges (minimum and maximum) of all values measured at 3 day intervals over 6 and 12 days in aerobic and modified atmosphere packs, respectively. n = 6 fillets/treatment, measurements recorded in duplicate.

3.3.8 Drip loss, cook loss and texture of chicken breasts

The drip losses of chickens were measured after 48 hr storage at 4°C. The drip losses of control, GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 chicken breasts were 0.34, 0.53, 0.47, 0.58, 0.70 and 0.52%, respectively. The drip loss of chicken fed with GS/OL/$CB_1$50 was significantly ($p<0.05$) higher as compared to the control. It means that the inclusion of GS/OL/$CB_1$50 extract reduced chicken muscle integrity in retaining water molecules during refrigerated storage. The drip loss of chicken supplemented with sweet chestnut wood extract (3,000 mg/kg) is similar to those received control diets which were in the range of 1.47 - 1.74% (Voljč *et al.* 2013). Although GS/OL/$CB_1$50 diet result to greater drip loss than the control group, but the value reported was lower than the literature.

Diet supplemented with GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts did not significantly ($p > 0.05$) reduce cook loss of chicken breasts stored in aerobic and modified atmosphere packaging relative to the controls (Table 3.5). The cook loss of breast samples was in the range of 17.0 – 25.8% (aerobic) and 19.0 – 25.8% (MAP). The cook loss values reported in present study was similar to reported literature which is 15.5 – 24.0% (Chae *et al.* 2007).

The GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 diets did not significantly ($p > 0.05$) affect the tenderness of chicken breasts as compared to the controls. The Warner-Bratzler shear force (WBSF) of aerobically- and MAP-cooked chicken breast was in the range of 7.3 – 11.3 N and 7.6 – 13.2 N, respectively (Table 3.5). The WBSF values was lower than the values reported by Lyon and Lyon (1996) ranging from 31.38 – 99.05 N.

3.3.9 Microbiological stability of raw chicken breasts

The inclusion of GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts into the diets did not improve microbial stability of the chicken breasts throughout the aerobic and modified atmosphere storage as compared to the controls (Table 3.5). The mesophilic plate counts of breasts increased gradually from day 0 up to day 6 and day 12 of aerobic and modified atmosphere storage, respectively. The mesophilic counts ranged from 3.6 – 6.2 log CFU/g (aerobic) and 3.6 – 7.4 log CFU/g (MAP). The microbial count was similar to previous study which was 4.3 – 7.2 log CFU/g for aerobic and MAP (Chouliara *et al.* 2007). The finding in present study is in agreement to Chapter 2, suggesting that GS, GS/OL/CB_1 and GS/OL/CB_2 extracts (50 mg/ml) exhibited no antimicrobial activities against chicken microflora in *in vitro* diffusion assay.

3.3.10 Lipid oxidation of raw chicken breasts

The lipid oxidation of raw chicken breasts increased over time in aerobic packaging (range: 0.09 – 0.21 mg MDA/kg) and MAP (range: 0.10 – 0.19 mg MDA/kg) (Table 3.5). The inclusion of GS100, GS200, GS300, GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts in the diet did not significantly ($p > 0.05$) reduced lipid oxidation of chickens relative to the control. Present study showed that all level of GS extract did not significantly ($p > 0.05$) improves oxidative stability of raw breasts as compared to the controls. Smet *et al.* (2008) reported that chickens fed with 100 and 200 mg/kg of grape seed extract reduced lipid oxidation of raw breast patties in a dose-dependent manner. The authors reported higher TBARS values (0.18 – 0.59 mg MDA/kg) as compared to the present study (0.10 – 0.21 mg MDA/kg) owing to meat processing which causes muscle structural disintegration, surface exposure to oxygen and subsequently increase the rate of lipid oxidation. In contrast, GS300 supplemented breasts were protected against oxidative stress induced by

iron ($FeCl_3$). This effect is not observed in raw breasts stored under refrigeration because the breast is raw and in intact form, which make it resistant towards lipid oxidation (Frankel 1998b).

The present study showed that the extract mixtures (GS/OL/$CB_1$50 and GS/OL/$CB_2$50) supplementation did not reduce lipid oxidation of raw breasts, in agreement to iron induced lipid oxidation of the breast of chicken fed with the mixes which demonstrate no protection against oxidative stress in the muscles. In contrast, broiler fed with sweet chestnut wood extract (3,000 mg/kg) for 25 days did not exert protective effect in raw breast muscle but capable to reduce lymphocyte DNA damage in chicken (Voljč *et al.* 2013). However, when turkeys were supplemented with high dose of olive leaf extract (10,000 mg/kg) for 42 days, the supplemental extracts successfully lower lipid oxidation of raw turkey breast when stored in refrigeration as compared to the control (Botsoglou *et al.* 2010). The protective effect is not observed in present study probably due to low concentration of extracts incorporated in the diets.

3.3.11 Sensory evaluation of chicken breast

Table 3.6 and Figure 3.1 summarised the APLSR of the treatments and sensory attributes of aerobically packed breasts as perceived by the panellists. GS100 significantly positively correlated to the juiciness and tenderness of chicken breasts on day 3. GS200 significantly positively correlated to tenderness on day 3 and significantly negatively correlated to tenderness (day 0) and juiciness (day 3). GS/OL/$CB_2$50 significantly negatively correlated to tenderness on day 0. GS300 exerted the greatest significantly positive effect on the attributes of fillets stored aerobically on day 0 which include the texture, flavour, overall acceptability, juiciness and tenderness. This is in agreement to the iron induced study which showed that the GS300 breasts exerted greatest activity indicating

Table 3.6 Significance coefficient (ANOVA results) for cooked chicken breast in aerobic packaging as derived by jack-knife uncertainty testing.

Treatment	**Sensory analysis descriptors, day 0 and 3**									
	Appearance		**Texture**		**Flavour**		**Overall acceptability**		**Juiciness**	
	0	**3**	**0**	**3**	**0**	**3**	**0**	**3**	**0**	**3**
Control	0.42	0.61	-0.57	-0.64	-0.98	0.19	-0.66	0.38	-0.53	0.23
GS100	0.71	-0.34	0.47	0.10	0.09	-0.67	0.99	-0.80	0.84	0.00**
GS200	0.83	0.36	-0.27	-0.10	-0.28	0.92	-0.79	0.82	-0.66	-0.00**
GS300	0.79	-0.68	0.00**	0.35	0.05*	-0.10	0.01**	-0.09	0.01**	0.25
GS/OL/$CB_1$50	0.52	0.41	-0.11	-0.69	-0.23	0.51	-0.09	0.25	-0.47	-0.97
GS/OL/$CB_2$50	0.62	0.93	-0.45	-0.44	-0.73	0.19	-0.56	0.21	-0.07	-0.84

Treatment	**Sensory analysis descriptors, day 0 and 3**							
	Tenderness		**Chicken flavour**		**Off flavour**		**Off flavour oxidation**	
	0	**3**	**0**	**3**	**0**	**3**	**0**	**3**
Control	-0.52	0.38	-0.95	0.80	-0.41	-0.53	-0.71	-0.37
GS100	0.25	0.01**	0.49	-0.31	0.25	0.39	0.77	0.93
GS200	-0.01**	0.03*	-0.77	0.37	-0.11	-0.13	-0.94	-0.56
GS300	0.00**	0.37	0.10	-0.39	0.54	0.28	0.47	0.21
GS/OL/$CB_1$50	-0.70	0.52	-0.33	0.59	-0.49	-0.46	-0.91	-0.63
GS/OL/$CB_2$50	-0.02*	0.52	-0.44	0.70	-0.38	-0.20	-0.49	-0.17

*95% significance, $p < 0.05$, **99% significance, $p < 0.01$. n = 10 fillets/treatment, measurements recorded in duplicate.

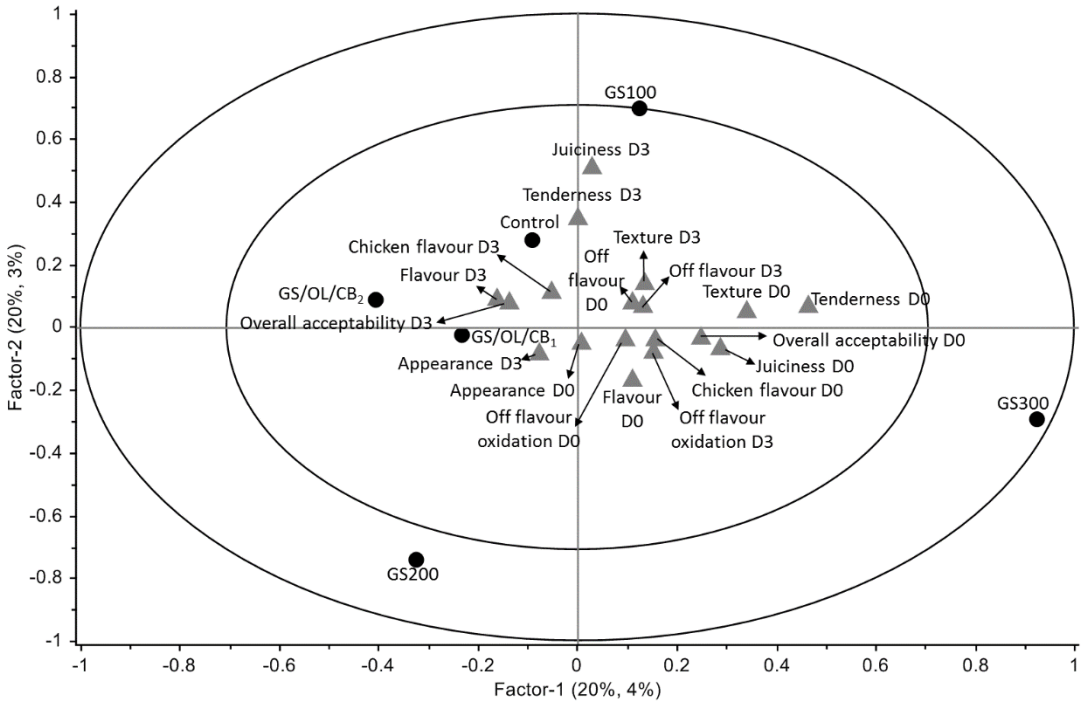


Figure 3.1 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 6 treatments stored in aerobic packaging. ●= treatments, ▲= sensory descriptors.

that high concentration of GS extract is optimum in improving the sensory quality of aerobically packed chicken fillets.

Table 3.7 and Figure 3.2 summarised the APLSR of the treatments and sensory attributes of MAP samples as perceived by the panellists. GS100 significantly negatively correlated to flavour on day 3. GS300 significantly positively correlated to texture, overall acceptability and tenderness (day 0), juiciness (day 0 and 3) and appearance (day 6) but significantly negatively correlated to flavour (day 3). GS/OL/$CB_1$50 significantly positively correlated to appearance on day 3. GS/OL/$CB_2$50 significantly positively correlated to flavour (day 3) but significantly negatively correlated to tenderness (day 0) and juiciness (day 3). The negative correlation of GS/OL/$CB_2$50 MAP samples to tenderness, in agreement to the WBSF values of 9.7 – 13.2 N (Table 5) which fall in the.

Table 3.7 Significance coefficient (ANOVA results) for cooked chicken breast in modified atmosphere packaging (40% CO_2; 60% N_2) as derived by jack-knife uncertainty testing.

Treatment	**Sensory analysis descriptors, day 0, 3 and 6**														
	Appearance			**Texture**			**Flavour**			**Overall acceptability**			**Juiciness**		
	0	3	6	0	3	6	0	3	6	0	3	6	0	3	6
Control	0.66	0.33	-0.40	-0.91	0.12	-0.38	-0.94	0.10	-0.77	-0.97	0.14	-0.44	-0.55	-0.50	-0.82
GS100	-0.47	-0.10	0.40	0.92	-0.08	0.16	0.83	-0.03*	0.74	0.86	-0.09	0.18	0.64	0.36	0.59
GS200	0.23	0.09	0.80	0.76	0.71	0.26	0.83	0.42	0.92	0.78	0.13	0.51	0.42	0.49	0.41
GS300	-0.76	-0.88	0.04*	0.00**	-0.13	0.58	0.22	-0.02*	0.87	0.00**	-0.67	0.77	0.03*	0.02*	0.94
GS/OL/$CB_1$50	0.49	0.02*	-0.66	-0.06	0.72	-0.78	-0.28	0.53	-0.84	-0.10	0.41	-0.83	-0.41	-0.81	-0.22
GS/OL/$CB_2$50	0.94	0.18	-0.16	-0.62	0.08	-0.39	-0.98	0.02*	-0.62	-0.90	0.13	-0.27	-0.13	-0.01*	-0.94

Treatment	**Sensory analysis descriptors, day 0, 3 and 6**											
	Tenderness			**Chicken flavour**			**Off flavour**			**Off flavour oxidation**		
	0	3	6	0	3	6	0	3	6	0	3	6
Control	-0.37	-0.73	0.77	-0.98	0.27	0.94	-0.85	-0.33	0.77	-0.86	-0.70	0.90
GS100	0.49	0.78	-0.59	0.92	-0.16	0.94	0.97	0.26	0.64	0.61	0.60	-0.89
GS200	-0.18	0.90	0.32	0.75	0.31	0.57	0.34	0.86	0.76	0.36	0.78	0.96
GS300	0.00**	0.76	-0.37	0.14	-0.90	1.00	0.48	0.19	0.57	0.46	0.46	-0.69
GS/OL/$CB_1$50	-0.92	-0.84	0.98	-0.40	0.59	0.31	-0.19	-0.76	0.71	-1.00	-0.31	0.82
GS/OL/$CB_2$50	-0.01*	-0.59	0.99	-0.79	0.48	0.69	-0.29	-0.14	-0.77	-0.77	-0.65	0.89

*95% significance, $p < 0.05$, **99% significance, $p < 0.01$. n = 10 fillets/treatment, measurements recorded in duplicate.

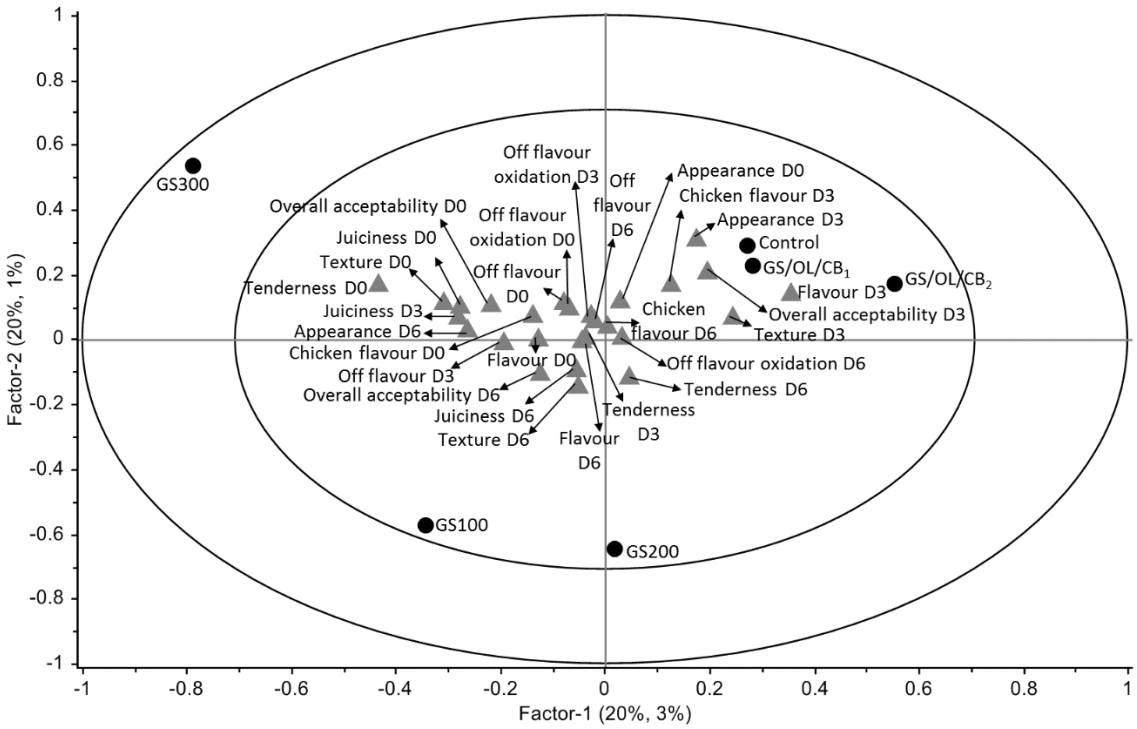


Figure 3.2 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 6 treatments stored in modified atmosphere packaging. ● = treatments, ▲= sensory descriptors.

category of neither tender nor tough (8.26 – 10.39 kg) to moderately tough (12.54 – 14.68 kg) as classified by Xiong *et al.* (2006)

Most importantly, the dietary supplementations did not contribute to the off flavour and off flavour oxidation of aerobic and MAP samples as compared to the control treatments. Overall, GS/OL/$CB_2$50 samples stored in both aerobic and modified atmosphere packs had negative effect on the tenderness of the fillets. Interestingly, GS300 diet produced the greatest positive effect on the sensory qualities of fillets stored in aerobic and modified atmosphere packs.

3.4 Conclusions

Grape seed (GS) extract and two mixture of grape seed, olive leaf and sweet chestnut bark (GS/OL/$CB_1$50 and GS/OL/$CB_2$50) extracts were incorporated into chicken diets in order to improve oxidative stability of chicken and chicken meat quality. The diets with GS (all levels), GS/OL/$CB_1$50 and GS/OL/$CB_2$50 extracts did not improve plasma total antioxidant status, proximate composition of chicken breast and thigh muscles and most of shelf-life quality (pH, cook loss, texture, microbiological and lipid oxidation) of raw breast fillets, except the surface colour which probably due to the variation among breast fillets. The GS/OL/$CB_1$50 diet resulted to greater drip loss of the fillets. Diet supplementation containing GS extract improved oxidative stability of breast and thigh muscle homogenates. Low concentration is required to reduce lipid oxidation of thigh muscle while high concentration is needed to protect breast muscle homogenate against oxidation. In addition, high concentration of GS extract diet improved the sensory qualities of the fillets stored both in aerobic and modified atmosphere packaging. The positive effects on oxidative stability and sensory qualities of chicken fed with high concentration of GS extract suggesting that supplemental extracts were absorbed by chicken digestive system and well distributed on breast muscles. Overall, the suitability of GS extract to be used in chicken diets in order to improve the meat quality has been demonstrated.

CHAPTER 4

4 Effect of dietary supplementation of grape seed extract on the quality and shelf-life of cooked chicken patties stored in aerobic and modified atmosphere packaging conditions.

Abstract

The shelf-life quality of cooked chicken breast and thigh patties, receiving chicken diets supplemented with grape seed (GS) extract at three different concentrations (GS100:100 mg/kg, GS200: 200 mg/kg and GS300:300 mg/kg) were evaluated against control and salt control (SC) patties. Salt (0.5% w/w) was added to SC, GS100, GS200 and GS300 patty formulations. Control and SC patties were made of chicken fed with a basal diet. The patties were packed in two types of packaging (aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) and stored under refrigeration for 14 (aerobic) and 28 days (MAP) respectively. Generally, supplementation did not affect the lightness and yellowness of cooked breast and thigh patties throughout aerobic and modified atmosphere packs. GS supplementations significantly ($p < 0.05$) affect the redness of cooked breast and thigh patties on certain days of aerobic and MAP storage. GS supplementation reduced lipid oxidation of cooked chicken breast and thigh patties stored in aerobic packaging, but only GS300 and GS200 supplementation significantly ($p < 0.05$) improve oxidative stability of aerobically stored cooked breast and thigh patties, respectively. No concentration effect was observed in the study. In MAP storage, all level of GS supplementation reduced lipid oxidation of cooked breast and thigh patties but the effect was not significant. No antimicrobial effect of dietary GS was observed in the present study. In addition, the supplementation of GS enhanced the sensory qualities of cooked chicken breast and thigh patties stored in both type of packaging indicated the acceptability of the supplemented cooked patties to panellists. In conclusion, GS extract demonstrated

its ability to improve oxidative and storage stability and enhance the sensory attributes of cooked breast and thigh patties through chicken diets.

4.1 Introduction

Poultry meat and meat products are widely consumed all over the world mainly due to their low price, consumer preference and nutrition (Salmon and Audran 2016). Other than protein, lipids, carbohydrate and mineral content, poultry meat also contains high level polyunsaturated fatty acids (PUFAs) which renders poultry meat susceptibility to lipid oxidation (Higgins *et al.* 1998). Lipid oxidation is believe to be initiated in the membrane-bound phospholipids due to the concentration of long chain PUFAs (Higgins *et al.* 1998). It can occur in both raw and cooked meat but the severity of lipid oxidation is greater in processed or cooked meat as compared to raw meat (Sheehy *et al.* 2014).

This is because processing of meat, such as grinding, chopping or heating, disrupt meat structure and expose the phospholipids to oxygen, enzymes, heme pigments and metal ions which bring the PUFAs in close contact to the catalysts, thus catalyse lipid oxidation (Frankel 1998a). Recently, consumers prefer to purchase processed chicken products as they are more convenient (Roenigk 1999), but these kind of products need to be stored under refrigeration. The practice of processing and refrigerated storage of chicken product has promoted deterioration of meat lipid fraction (Jensen *et al.* 1997; Cortinas *et al.* 2005). Therefore, lipid oxidation is a major threat to the quality of processed products (Estevez 2015) because it affects nutritional and sensory values as well as produces potential toxic compounds (Cortinas *et al.* 2005).

Nevertheless, the extent of lipid oxidation in processed chicken meat products can be controlled by the use of antioxidant agents. Antioxidant agents can be either directly added into chicken meat systems or supplemented in chicken diets in order to maintain nutritional quality and prolong shelf-life of meat products. Synthetic antioxidants, such as BHT, BHA, ethoxyquin and propyl gallate, have been widely incorporated in pre-mortem to post-mortem application to inhibit oxidative stress and reduce lipid oxidation thus

improve chicken meat quality (Nissen *et al.* 2000; Smet *et al.* 2008; Tavárez *et al.* 2011). Despite their effectiveness, consumers are aware regarding the health effect of consuming products containing synthetic additives (Błaszczyk *et al.* 2013). Therefore, numerous on-going research are conducted to replace the utilisation of synthetic additives with natural extracts to be added directly in chicken meats or diets, in order to improve chicken meat quality.

Many studies reported the ability of natural plant-derived extracts to improve the meat quality upon post-mortem addition of the extracts in chicken muscle (Tang *et al.* 2001a; Lau and King 2003). Post-mortem addition of natural antioxidants requires disintegration of meat muscle prior to incorporation and they may not be positioned closely to cellular membrane PUFAs to perform optimum antioxidative effect (Higgins *et al.* 1998). In contrast, diet inclusion of natural antioxidants can improve the health and stability of raw as well as processed chicken meat (Tang *et al.* 2001b; Goñi *et al.* 2007; Brenes *et al.* 2010). For example, chicken supplemented with α-tocopherol allows it to be incorporated into the membrane lipids (Frankel 1998a) and reported to improve the oxidative and storage stability of raw and cooked chicken meat (Sheehy *et al.* 2014).

Other than α-tocopherol supplementation, recent research is carried out to incorporate plant-derived extracts (such as green tea and grape-by products) due to their low price, abundance availability and the presence of polyphenolic compounds which contribute to radical scavenger and proton donating capabilities to reduce formation of free radical, delay lipid oxidation and improve chicken meat quality (Tang *et al.* 2001b; Sáyago-Ayerdi, Brenes, Viveros, *et al.* 2009). Chickens supplemented with grape pomace concentrate (30 and 60 mg/kg) protected aerobically stored cooked chicken breast patties against lipid oxidation and the protective effect derived from the polyphenolic compounds

which may act in a similar way to vitamin E on the lipid bilayers in the meat (Sáyago-Ayerdi, Brenes, Viveros, *et al.* 2009).

Chapter 2 demonstrated that grape seed extract contains polyphenols compounds, such as phenolic acids, flavanols, flavonols and stilbenes, contributing to the DPPH radical scavenging activity and ferric reducing antioxidant power. Smet *et al.* (2008) reported chicken diets supplemented at 100 and 200 mg/kg of grape seed extract protected raw breast patties in dose dependent manner and the protection effect was similar to those received 300 mg/kg synthetic antioxidant (mix of butylated hydroxytoluene, ethoxyquin and butylated hydroxyanisole). On the other hand, Chapter 3 reported that diet supplementation of grape seed extract (100 – 300 mg/kg) do not improve oxidative stability of raw chicken breasts stored in aerobic and modified atmosphere packs but the inclusion of grape seed extract at the concentration of 100 and 300 mg/kg in the diets had efficiently protected thigh and breast muscle homogenates against iron induced lipid oxidation, respectively.

Both studies suggested that grape seed extracts supplemented to chicken were well deposited and retained in chicken muscles to elicit positive responses against oxidative stresses. Therefore, the potential of deposited extracts to remain functional in cooked muscle foods after processing in order to improve lipid stability and enhance sensory quality of cooked meat merits investigation. The objective of this study was to examine the effects of supplementation of chicken diets with grape seed (GS) extract at three levels 100, 200 and 300 mg/kg feed on the colour, lipid oxidation, microbiology and sensory properties of cooked breast or thigh patties stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) stored at 4°C for up to 14 and 28 days, respectively.

4.2 Materials and Methods

4.2.1 Reagents

2-thiobarbituric acid (TBA) ($C_4H_4N_2O_2S$) and trichloroacetic acid (TCA) ($C_2HCl_3O_2$) were supplied by Sigma-Aldrich Ireland, Ltd., Arklow, Co. Wicklow, Ireland. All chemicals used were of analytical grade. Maximum recovery diluent was purchased from Oxoid Ltd., Basingstoke, England and plate count agar was obtained from Merck, Darmstadt, Germany. Commercial grape seed (GS) extracts was supplied by Envirotech Innovative Products Ltd., Dublin, Ireland. The chemical composition of GS extract was reported in Chapter 2.

4.2.2 Chicken patty processing and packaging

The chickens received experimental diets in Shannon Vale Foods Ltd. Gallanes, Clonakilty, Co. Cork, Ireland as described in Chapter 3. Only chickens fed with control diet and diet containing three levels of GS extract at the inclusion rate of 100, 200 and 300 mg/kg (GS100, GS200 and GS300) were used in this study because these treatments showed positive effect *in vivo*. The chicken breast and thigh muscles were separated from each carcass and transported (4°C) to the School of Food and Nutritional Sciences at University College Cork (UCC), Ireland. The samples were vacuum packed and stored in freezer (-20°C) until further analysis.

The chicken breast and thigh muscles were trimmed off visible fat and connective tissue and minced through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain). The control chicken breast and thigh muscles was assigned to two treatments i.e. control and salt control (SC). Another three treatments of breast and thigh meat patties were made from chicken muscles fed with GS100, GS200 and GS300. Following mincing, sodium chloride salt (0.5% w/w) was dissolved in water and added to minced breast or thigh at 5%

v/w to SC, GS100, GS200 and GS300 treatments. Only control treatment patties were not added with salt. Minced breast or thigh from each treatment was formed into 100 g patties using meat former (Ministeak burger maker, O.L. Smith Co. Ltd., Italy), placed on aluminium foil lined trays and cooked at 180°C in a fan-assisted convection oven (Zanussi Professional, Model 10 GN1/1, Conegliano, Italy) until an internal temperature of 72°C was reached and held for 8 min. Following cooling, cooked patties were placed in individual trays and over-wrapped in an oxygen permeable (6000 – 8000 cm^3 m^{-2} 24 h^{-1} at STP) polyvinylchloride cling film (Musgrave Wholesale Partners, Dublin, Ireland) without seal as to allow exposure to atmospheric air. All aerobic samples were stored up to 14 days at 4°C.

For modified atmosphere samples, cooked patties were placed in low oxygen permeable (<1 cm^3 m^{-2} 24 h^{-1} at STP) polystyrene/ethyl vinyl alcohol/polyethylene trays and using modified atmosphere packaging (MAP) technology, flushed with 40% CO_2:60% N_2 using a vacuum-sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH & Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3 cm^3 m^{-2} 24 h^{-1} at STP) laminated barrier film a polyolefin heat sealable layer. All MAP samples were stored up to 28 days at 4°C. The gas atmosphere (CO_2 and N_2) in the MAP was checked using a CheckMate 9900 (PBI-Dansensor, Denmark). Immediately after flushing, the MAP trays contained 60.59 ± 0.25% N_2 and 39.37± 0.25% CO_2 and after 28 days, the gases composition was 60.14 ± 0.60% N_2 and 39.81 ± 0.59% CO_2. The O_2 content was in the range of 0.04 – 0.05% throughout the storage.

4.2.3 Colour measurement

The surface colour was measured using a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Japan). The chroma meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400) (Konica Minolta Sensing, Inc., Japan). The chroma meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79, a = -0.11, b = 2.69). The '*L*' value represents lightness and 'a*' and 'b*' values represent redness and yellowness, respectively. Colour measurement of fillets were measured on days 0, 4, 7, 11 and 14 and days 0, 7, 14, 21 and 28 for aerobic and MAP samples, respectively.

4.2.4 Measurement of lipid oxidation

Lipid oxidation was measured using the 2-thiobarbituric acid assay (2-TBA) (Siu and Draper,1978). The malondialdehyde (MDA) content was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg MDA/kg chicken. Lipid oxidation in cooked patties were measured on days 0, 4, 7, 11 and 14 and days 0, 7, 14, 21 and 28 for aerobic and MAP samples, respectively.

4.2.5 Microbiological analysis

Cooked patties (10 g) were transferred into filter stomacher bag, diluted with 90 ml of maximum recovery diluent and stomached for 2 min (Steward Stomacher 400 Lab Blender, London, UK) resulting in a 10^{-1} dilution used for analysis. Serial dilutions were prepared and 1 ml aliquots from each dilution were pipetted onto plate count agar. The plates were incubated at 30°C for 48 hr to determine mesophilic counts. Microbiological analysis was carried out on days 0, 7 and 14 and days 0, 14 and 28 for aerobic and MAP

samples, respectively. Results were expressed as $\log_{10}$ CFU (colony forming units)/g chicken.

4.2.6 Sensory evaluation

The chicken patties were sliced and identified with random three-digit codes. Sample presentation order was randomised to prevent any flavour carryover effects (MacFie *et al.* 1989). Prior to serving the panellists, chicken samples were re-heated in a microwave for 10 seconds to release the meat odour and flavour. Sensory analysis was undertaken in the panel booths at the university sensory laboratory in accordance with the ISO (1988) international standard regulations. Assessors were also provided with water to cleanse their pallets between samples. Hedonic sensory analysis descriptors were appearance, texture, flavour and overall acceptability. Intensity sensory analysis descriptors were whiteness, tenderness, chicken flavour and oxidation flavour. Assessors were asked to indicate their opinions on a 10 cm line scale ranging from 0 (extremely dislike/none) to 10 (extremely like/extreme). Results for sensory analysis scores were measured in centimetre (cm) and scores were statistically analysed using ANOVA-Partial Least Squares Regression (APLSR). Results were presented as a significance of regression coefficient, analysed by jack-knife testing. All samples were presented in duplicates. Sensory analysis of cooked patties was performed by 10 naïve assessors on days 1 and 8 (aerobic samples) and days 1, 8, 15 and 22 (MAP samples).

4.2.7 Statistical analysis

Statistical analysis was carried out using SPSS 22.0 for Windows (SPSS Statistical Software, Inc., Armonk, NY, USA) software package. All analyses were performed in duplicate and three independent experimental trials were carried out. A full repeated

measures ANOVA was conducted to investigate the effect of treatment, time and their interactions. Treatment represented the 'between-subject' factor and the effect of time was measured using the 'within-subject' factor. Tukey's post-hoc test was used to adjust for multiple comparisons between means. All data is presented as mean values ± standard deviation.

Sensory data was analysed with ANOVA-Partial Least Square Regression (APLSR) to process the mean data accumulated from the 10 test subjects. The X-matrix was designated as 0/1 for treatment and days with the Y-matrix designated as sensory variables. To derive significance indications for the relationship determined in the quantitative APLSR, regression coefficients were analysed by jack-knifing which is based on cross-validation and stability plots (Martens and Martens, 2001). All analyses were performed using the Unscrambler Software, version 9.8 (CAMO ASA, Trondheim, Norway).

4.3 Results and Discussion

4.3.1 Colour of cooked patties

Generally, dietary supplementation of GS did not significantly affect the lightness (L^*) and yellowness (b^*) of cooked chicken breast patties in aerobic packaging and MAP (Table 4.1). On day 0 of aerobic and modified atmosphere packs, control breast patties were significantly ($p < 0.05$) redder than other treatments. The redness (a*) of control breast patties remained significant ($p < 0.05$) throughout aerobic storage. In MAP storage, control breast patties were significantly ($p < 0.05$) redder than other treatments on day 7. On day 14 and 21, control and SC breast patties were significantly ($p < 0.05$) redder than all GS-supplemented breast patties. At the end of MAP storage, control breast patties were significantly ($p < 0.05$) redder than other treatments while SC breast patties were significantly ($p < 0.05$) redder than control and GS200 breast patties.

Overall, GS inclusion in chicken diet did not significantly affect the lightness (L^*) and yellowness (b^*) of cooked chicken thigh patties in aerobic and MAP storage (Table 4.2). On day 4 of aerobic packaging, control and GS200 thigh patties were significantly ($p < 0.05$) redder than GS100 thigh patties. Control thigh patties were significantly ($p < 0.05$) redder than GS100 and GS300 thigh patties while GS200 thigh patties were significantly redder than GS100 thigh patties on day 7 and 11 of aerobic storage. However, the redness of thigh patties was less affected when stored in MAP.

The redness of breast patties seems to be affected by the addition of salt in both aerobic packaging and MAP. However, the significant differences in redness between control thigh patties and salt-containing thigh patties in both types of packaging were less as compared to observation in breast patties. It is possibly because thigh meat is darker in nature (lower L^* values), thus changes in redness were less obvious.

Table 4.1 Effect of *in vivo* supplementation of grape seed (GS) extract at three levels (GS100, GS200 and GS300) on the surface colour of cooked chicken breast patties stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) for up to 14 and 28 days, respectively at 4°C.

Treatment/	**Storage time at 4°C, days**								
Parameters	**0**	**4**	**7**		**11**	**14**		**21**	**28**
	Aerobic/MAP	**Aerobic**	**Aerobic**	**MAP**	**Aerobic**	**Aerobic**	**MAP**	**MAP**	**MAP**
Lightness, *L*									
Control	77.1 ± 1.3[a]	77.2 ± 2.4[a]	77.4 ± 3.3[a]	76.6 ± 2.6[a]	77.4 ± 3.4[a]	77.6 ± 2.1[a]	77.8 ± 1.4[a]	77.3 ± 0.3[a]	78.5 ± 1.0[a]
SC	74.6 ± 1.4[a]	74.1 ± 1.8[a]	74.2 ± 0.9[a]	76.5 ± 1.7[a]	74.7 ± 0.3[a]	75.4 ± 1.5[a]	75.6 ± 1.6[a]	75.2 ± 2.0[a]	76.6 ± 1.4[a]
GS100	76.6 ± 0.2[a]	75.2 ± 3.0[a]	74.5 ± 2.2[a]	77.1 ± 0.9[a]	74.9 ± 2.5[a]	75.2 ± 2.4[a]	76.8 ± 1.3[a]	76.6 ± 0.6[a]	79.2 ± 1.2[a]
GS200	76.4 ± 1.8[a]	77.8 ± 1.1[a]	76.4 ± 2.6[a]	76.8 ± 0.7[a]	76.6 ± 2.4[a]	77.2 ± 0.7[a]	77.6 ± 1.4[a]	77.0 ± 2.97[a]	77.5 ± 0.6[a]
GS300	76.1 ± 1.8[a]	76.5 ± 0.3[a]	76.1 ± 1.6[a]	75.8 ± 1.0[a]	75.7 ± 2.4[a]	74.1 ± 1.8[a]	78.0 ± 0.5[a]	77.7 ± 2.1[a]	77.1 ± 1.1[a]
Redness, *a**									
Control	2.5 ± 0.3[a]	2.4 ± 0.3[a]	2.3 ± 0.4[a]	2.3 ± 0.9[a]	2.4 ± 0.6[a]	2.3 ± 0.3[a]	1.7 ± 0.3[a]	1.9 ± 0.2[a]	1.2 ± 0.1[a]
SC	1.1 ± 0.6[b]	1.1 ± 0.6[b]	0.2 ± 0.1[b]	1.00 ± 0.2[b]	-0.3 ± 0.2[b]	-0.7 ± 0.4[b]	1.3 ± 0.1[a]	1.3 ± 0.2[a]	0.7 ± 0.1[b]
GS100	0.5 ± 0.3[b]	0.6 ± 0.2[b]	0.3 ± 0.1[b]	0.6 ± 0.3[b]	-0.4 ± 0.2[b]	-0.4 ± 0.3[b]	0.4 ± 0.1[b]	0.5 ± 0.1[b]	0.4 ± 0.2[bc]
GS200	0.5 ± 0.2[b]	0.3 ± 0.1[b]	0.1 ± 0.3[b]	0.6 ± 0.2[b]	-0.3 ± 0.3[b]	-0.7 ± 0.0[b]	0.3 ± 0.1[b]	0.4 ± 0.1[b]	0.2 ± 0.2[c]
GS300	0.2 ± 0.4[b]	0.3 ± 0.3[b]	-0.1 ± 0.0[b]	0.5 ± 0.4[b]	-0.7 ± 0.2[b]	-1.1 ± 0.3[b]	0.2 ± 0.1[b]	0.4 ± 0.5[b]	0.3 ± 0.3[bc]
Yellowness, *b**									
Control	20.9 ± 1.7[a]	21.8 ± 3.5[a]	20.9 ± 4.9[a]	21.6 ± 3.4[a]	21.2 ± 5.6[a]	19.6 ± 4.4[a]	20.7 ± 2.3[a]	21.0 ± 1.8[a]	21.4 ± 0.8[a]
SC	23.6 ± 1.0[a]	24.1 ± 0.8[a]	23.6 ± 0.6[a]	23.3 ± 1.6[a]	23.3 ± 2.8[a]	22.7 ± 0.9[a]	23.3 ± 3.1[a]	22.4 ± 1.7[a]	22.2 ± 0.7[a]
GS100	23.0 ± 0.6[a]	23.0 ± 1.0[a]	22.5 ± 1.8[a]	23.0 ± 1.6[a]	21.9 ± 1.8[a]	21.6 ± 1.1[a]	23.1 ± 0.1[a]	23.9 ± 1.1[a]	20.5 ± 1.6[a]
GS200	22.1 ± 0.6[a]	23.5 ± 0.5[a]	22.5 ± 0.4[a]	22.6 ± 0.7[a]	22.3 ± 0.7[a]	22.5 ± 0.6[a]	22.6 ± 0.8[a]	22.6 ± 1.6[a]	23.4 ± 0.6[a]
GS300	23.9 ± 1.9[a]	22.9 ± 1.2[a]	22.6 ± 0.8[a]	24.2 ± 1.1[a]	22.9 ±1.2[a]	23.1 ± 1.1[a]	22.3 ± 1.5[a]	23.3 ± 2.9[a]	23.2 ± 0.7[a]

[ab]Within each parameter and storage day, mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $p < 0.05$.
n = 3 independent experiments, measurements recorded in duplicate.

Table 4.2 Effect of *in vivo* supplementation of GS100, GS200 and GS300 on the surface colour of cooked chicken thigh patties stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) for up to 14 and 28 days, respectively at 4°C.

Treatment	**Storage time at 4°C, days**								
	0	**4**	**7**		**11**	**14**		**21**	**28**
	Aerobic/MAP	**Aerobic**	**Aerobic**	**MAP**	**Aerobic**	**Aerobic**	**MAP**	**MAP**	**MAP**
Lightness, *L*									
Control	63.6 ± 0.8^{a}	63.4 ± 1.3^{a}	62.0 ± 1.8^{a}	62.2 ± 2.1^{a}	62.9 ± 1.4^{a}	62.3 ± 1.9^{a}	63.5 ± 1.3^{a}	63.3 ± 1.4^{a}	65.2 ± 1.6^{a}
SC	61.1 ± 1.3^{a}	60.7 ± 0.9^{a}	61.1 ± 1.3^{a}	58.1 ± 6.4^{a}	61.8 ± 1.2^{a}	61.8 ± 1.0^{a}	62.9 ± 1.4^{a}	62.3 ± 1.9^{a}	64.3 ± 1.3^{a}
GS100	60.2 ± 2.7^{a}	63.9 ± 1.6^{a}	62.7 ± 2.3^{a}	63.1 ± 1.3^{a}	63.5 ± 2.3^{a}	62.9 ± 2.0^{a}	64.3 ± 2.2^{a}	64.3 ± 1.9^{a}	62.4 ± 1.0^{a}
GS200	62.0 ± 3.5^{a}	62.0 ± 1.5^{a}	62.6 ± 1.9^{a}	61.6 ± 1.7^{a}	63.5 ± 2.1^{a}	63.2 ± 1.4^{a}	64.6 ± 1.5^{a}	61.0 ± 2.3^{a}	61.5 ± 0.5^{a}
GS300	63.0 ± 1.5^{a}	63.1 ± 1.1^{a}	63.5 ± 1.8^{a}	63.4 ± 0.7^{a}	64.0 ± 1.1^{a}	63.6 ± 1.2^{a}	62.5 ± 3.4^{a}	63.2 ± 0.9^{a}	63.9 ± 1.5^{a}
Redness, *a**									
Control	3.9 ± 0.5^{a}	3.4 ± 0.3^{a}	3.2 ± 0.6^{a}	3.9 ± 0.4^{a}	2.6 ± 0.5^{a}	2.4 ± 0.6^{a}	3.8 ± 0.3^{a}	3.4 ± 0.3^{a}	4.2 ± 0.6^{a}
SC	4.0 ± 0.5^{a}	3.0 ± 0.1^{ab}	2.7 ± 0.4^{abc}	3.3 ± 1.0^{a}	2.0 ± 0.4^{abc}	1.5 ± 0.2^{a}	3.8 ± 0.2^{a}	3.0 ± 0.3^{a}	3.2 ± 0.4^{ab}
GS100	4.0 ± 1.1^{a}	2.2 ± 0.4^{b}	1.9 ± 0.2^{c}	3.1 ± 0.2^{a}	1.2 ± 0.5^{c}	1.4 ± 0.5^{a}	3.2 ± 0.5^{a}	3.2 ± 0.4^{a}	3.2 ± 0.1^{ab}
GS200	3.6 ± 0.4^{a}	3.7 ± 0.4^{a}	3.1 ± 0.5^{ab}	3.8 ± 0.3^{a}	2.3 ± 0.3^{ab}	2.1 ± 0.8^{a}	2.9 ± 0.2^{a}	3.1 ± 1.1^{a}	3.2 ± 0.2^{ab}
GS300	3.3 ± 0.4^{a}	2.9 ± 0.4^{ab}	2.2 ± 0.1^{bc}	3.7 ± 0.2^{a}	1.5 ± 0.3^{bc}	1.5 ± 0.1^{a}	3.1 ± 0.5^{a}	3.6 ± 0.7^{a}	2.8 ± 0.5^{b}
Yellowness, *b**									
Control	22.3 ± 0.2^{a}	22.8 ± 1.1^{a}	23.5 ± 0.1^{a}	23.8 ± 0.7^{a}	23.8 ± 0.5^{a}	24.2 ± 0.6^{a}	23.9 ± 0.4^{a}	23.0 ± 1.20^{a}	22.6 ± 1.3^{a}
SC	23.2 ± 0.6^{a}	22.4 ± 0.9^{a}	22.5 ± 1.0^{a}	22.5 ± 1.0^{a}	23.1 ± 0.8^{a}	23.4 ± 0.3^{a}	23.6 ± 0.6^{a}	21.9 ± 0.5^{a}	23.3 ± 0.6^{a}
GS100	22.7 ± 0.5^{a}	23.1 ± 0.7^{a}	23.2 ± 0.8^{a}	21.5 ± 2.6^{a}	23.5 ± 0.1^{a}	23.8 ± 0.7^{a}	22.5 ± 0.7^{a}	22.6 ± 1.5^{a}	24.3 ± 0.4^{a}
GS200	22.6 ± 1.2^{a}	22.8 ± 0.8^{a}	22.4 ± 0.8^{a}	21.9 ± 0.7^{a}	22.3 ± 0.9^{a}	22.5 ± 0.4^{a}	21.8 ± 0.9^{a}	21.8 ± 1.3^{a}	22.9 ± 0.8^{a}
GS300	23.6 ± 0.5^{a}	21.7 ± 1.00^{a}	21.2 ± 1.5^{a}	22.6 ± 1.1^{a}	21.9 ± 1.4^{a}	22.3 ± 0.9^{a}	23.2 ± 0.5^{a}	21.9 ± 2.3^{a}	23.2 ± 1.2^{a}

^{abc}Within each parameter and storage day, mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $p < 0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
SC: salt control, GS100: GS extract at 100 mg/kg, GS200: GS extract at 200 mg/kg, GS300: GS extract at 300 mg/kg.

4.3.2 Lipid oxidation of cooked chicken patties

The lipid oxidation of all treatments of cooked chicken breast and thigh patties increased throughout both aerobic and modified atmosphere storage (Table 4.3). Generally, the lipid oxidation of SC breast patties was higher than the control breast patties but the significant increase was only observed on day 4 and 7 of aerobic storage. Sodium chloride salt is a pro-oxidant that promotes lipid oxidation in cooked muscle patties (Tang *et al.* 2001a) because it can displace ionic iron from binding macromolecules such as myoglobin, thus provide free iron ions to catalyse lipid oxidation (Kanner *et al.* 1991).

The supplementation of all level of GS extract reduced lipid oxidation of cooked breast patties as compared to SC breast patties on day 4, 7 and 11. However, TBARS reduction was not significant. Interestingly, GS extract protected cooked breast patties against lipid oxidation until the end of aerobic storage. GS supplementation did not function in dose-dependent manner. But only GS300 supplementation significantly ($p < 0.05$) lowered the TBARS level in cooked breast patties in aerobic packaging on day 14 and in fact the value was even lower than control breast patties (not significant). It showed that GS extract not only protect cooked muscle foods against lipid oxidation catalysed by cooking but also capable to negate the pro-oxidant effect of salt.

The potency of GS300 supplementation in cooked breast patties corroborated to breast homogenates study reported in Chapter 3, which showed that breast muscle homogenates of chicken fed with GS300 demonstrated excellent potency against lipid oxidation. Both finding indicated that 300 mg/kg GS supplementation was optimum for absorption, distribution and retention in the breast muscles to elicits positive response against lipid oxidation after processing, which insinuate the thermal stability of the extract in cooked muscle foods.

Table 4.3 Effect of *in vivo* supplementation of GS100, GS200 and GS300 on the lipid oxidation (TBARS) of cooked chicken thigh patties stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) for up to 14 and 28 days, respectively at 4°C.

Treatment	Packaging	Storage time at 4°C, days									
		Breast					Thigh				
		0	4	7	11	14	0	4	7	11	14
Control	Aerobic	0.28 ± 0.05^{a}	0.68 ± 0.14^{a}	1.10 ± 0.23^{a}	1.76 ± 0.10^{a}	2.01 ± 0.19^{ab}	0.50 ± 0.06^{a}	2.97 ± 0.34^{a}	3.86 ± 0.07^{a}	4.53 ± 0.48^{a}	4.77 ± 0.34^{a}
SC		0.33 ± 0.04^{a}	1.18 ± 0.18^{b}	2.01 ± 0.52^{b}	2.07 ± 0.13^{a}	3.10 ± 0.80^{b}	0.45 ± 0.08^{a}	2.99 ± 0.18^{a}	3.85 ± 0.36^{a}	4.71 ± 0.54^{a}	4.82 ± 0.13^{a}
GS100		0.30 ± 0.02^{a}	1.07 ± 0.15^{b}	1.46 ± 0.20^{ab}	1.94 ± 0.07^{a}	2.52 ± 0.11^{ab}	0.49 ± 0.03^{a}	2.30 ± 0.17^{b}	3.25 ± 0.37^{ab}	3.99 ± 0.50^{ab}	4.07 ± 0.82^{a}
GS200		0.30 ± 0.03^{a}	1.02 ± 0.07^{ab}	1.46 ± 0.14^{ab}	1.63 ± 0.14^{a}	2.22 ± 0.40^{ab}	0.54 ± 0.10^{a}	2.09 ± 0.22^{b}	2.78 ± 0.14^{b}	3.33 ± 0.21^{b}	3.90 ± 0.67^{a}
GS300		0.34 ± 0.14^{a}	0.97 ± 0.05^{ab}	1.34 ± 0.31^{ab}	1.65 ± 0.29^{a}	1.67 ± 0.25^{a}	0.45 ± 0.06^{a}	2.33 ± 0.13^{b}	3.31 ± 0.18^{ab}	4.03 ± 0.40^{ab}	4.37 ± 0.02^{a}
		0	7	14	21	28	0	7	14	21	28
Control	MAP	0.28 ± 0.05^{a}	0.38 ± 0.11^{a}	0.51 ± 0.11^{a}	0.59 ± 0.02^{a}	1.23 ± 0.10^{a}	0.50 ± 0.06^{a}	0.72 ± 0.31^{a}	1.13 ± 0.44^{a}	1.45 ± 0.36^{a}	2.25 ± 0.42^{a}
SC		0.33 ± 0.04^{a}	0.41 ± 0.11^{a}	0.66 ± 0.12^{a}	1.02 ± 0.19^{a}	1.12 ± 0.08^{a}	0.45 ± 0.08^{a}	0.78 ± 0.25^{a}	1.26 ± 0.46^{a}	1.77 ± 0.24^{a}	2.23 ± 0.15^{a}
GS100		0.30 ± 0.02^{a}	0.46 ± 0.21^{a}	0.53 ± 0.10^{a}	1.01 ± 0.16^{a}	1.01 ± 0.12^{a}	0.49 ± 0.03^{a}	0.78 ± 0.06^{a}	1.14 ± 0.16^{a}	1.77 ± 0.03^{a}	1.83 ± 0.14^{a}
GS200		0.30 ± 0.03^{a}	0.40 ± 0.04^{a}	0.49 ± 0.06^{a}	0.78 ± 0.10^{a}	1.14 ± 0.10^{a}	0.54 ± 0.10^{a}	0.82 ± 0.15^{a}	1.40 ± 0.20^{a}	1.70 ± 0.15^{a}	2.08 ± 0.62^{a}
GS300		0.34 ± 0.14^{a}	0.30 ± 0.02^{a}	0.51 ± 0.16^{a}	0.65 ± 0.04^{a}	1.12 ± 0.15^{a}	0.45 ± 0.06^{a}	0.74 ± 0.22^{a}	1.54 ± 0.33^{a}	1.83 ± 0.23^{a}	2.69 ± 0.33^{a}

^{ab}Within each parameter and storage day, mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $p < 0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
SC: salt control, GS100: GS extract at 100 mg/kg, GS200: GS extract at 200 mg/kg, GS300: GS extract at 300 mg/kg.

The efficacy of grape seed extract in improving and enhancing raw and processed chicken meat via supplementation was also observed in study reported by Smet *et al.* (2008). The authors reported that chickens supplemented with grape seed extract (100 and 200 mg/kg) improve oxidative stability of raw and thawed breast patties (after 8 month freezing) and the protective effect was comparable to those received with 300 mg/kg synthetic antioxidant due to high polyphenol content. Sáyago-Ayerdi *et al.* (2009) also reported that grape pomace (30 and 60 mg/kg) incorporated in chicken diets exerted excellent protection against lipid oxidation catalysed by cooking and salt (1%) activity due to its polyphenolic content which may act similarly to vitamin E to the bilayer to inhibit lipid oxidation.

However, the addition of salt in thigh muscle did not significantly accelerate lipid oxidation of aerobically stored cooked thigh patties as compared to control thigh patties. In present study, no significant differences were observed across the treatments on day 0. Generally, the GS supplementation (at all concentration) reduced lipid oxidation in cooked thigh patties from day 4 to 14 of aerobic storage as compared to control and SC patties. All the GS treatments significantly ($p<0.05$) reduced TBARS level relative to the control and SC thigh patties on day 4, but only GS200 thigh patties remained to be significantly ($p<0.05$) protected against lipid oxidation up to day 11. No concentration effect of GS supplementation was observed in inhibiting lipid oxidation of cooked thigh patties. GS inclusion in chicken diets resulted to reduction of TBARS level in thigh patties until the end of the aerobic storage, although the effects were not significant.

In contrast to Chapter 3, thigh muscle homogenates of chickens supplemented with GS100 significantly reduced lipid oxidation as compared to chicken fed with control diet. Probably because GS extract may behave differently in protecting thigh muscle under different oxidative stresses. It can be deduced from this study and Chapter 3 finding, thigh

muscle requires low and intermediate concentrations (100 and 200 mg/kg) in inhibiting lipid oxidation as compared to breast muscle which requires high concentration of GS (300 mg/kg). This in agreement to Lau and King (2003), who reported that addition of grape seed at low concentration of grape seed (10 g/kg) protected cooked thigh turkey patties better than high concentration of the extract (20 g/kg).

The present study showed that TBARS values of cooked thigh patties were significantly ($p < 0.05$) higher than cooked breast patties between each muscle of the same treatment (for example between control breast and control thigh patties). Similarly, Goñi *et al.* 2007 reported that grape pomace supplementation (30 g/kg) effectively delayed lipid oxidation of aerobically stored raw breast and thigh meat but it was found that grape pomace efficiently prevent lipid oxidation in breast meat as compared to thigh meat, probably because thigh meat is more susceptible to lipid oxidation. It is because it contains higher fat content, PUFAs with more than two double bonds (PUFAs > 18:2), molar ratio of PUFAs > 18:2 to α-tocopherol and content of iron-containing proteins as compared to breast meat (Jensen *et al.* 1997). Evidently, the total lipids of thigh meats used in this study was higher (not significant) than total lipids of breast meats (Chapter 3) which may be the contributing factor to greater lipid oxidation in thigh cooked meat. The presence of α-tocopherol in meat muscle exert protection of thigh meat against lipid oxidation (Frankel 1998a) but thigh meat also contains high molar ratio of PUFAs > 18:2 to α-tocopherol render thigh meat susceptibility to lipid oxidation and this ratio is more important indicator of oxidative stability than α-tocopherol content alone (Jensen *et al.* 1997).

The dietary supplementation of GS (all levels) reduced lipid oxidation of cooked breast and thigh meat patties stored in MAP, but the reduction was not significant. It is likely that the removal of oxygen suppresses and inhibits lipid oxidation thus efficiently delayed lipid oxidation in cooked muscle foods (Smiddy, Papkovsky, *et al.* 2002). Despite

the insignificant effect of the dietary supplementation of cooked chicken patties stored in modified atmosphere packaging, it is important to highlight that the TBARS level of GS300 breast patties were lower than SC breast patties from day 7 to 21. This is consistent to the potency of GS300 supplementation in improving oxidative and storage stability of cooked breast patties stored in aerobic packaging.

Overall, the low TBARS values of cooked breast and thigh muscle foods indicated that supplemental GS extract were distributed and retained in the muscles and remained functional in cooked muscles food. This potency may be due to the ability of polyphenolic compounds to act on the membrane bilayer (Sáyago-Ayerdi, Brenes, Viveros, *et al.* 2009) and possibility that flavonoids can scavenge free radicals near the membrane surface (Terao *et al.* 1994). For example, tea catechin potency to inhibit lipid oxidation in cooked muscles food is due to their high affinity towards muscles lipid bilayer (Tang *et al.* 2001a). This study highlighted the thermal stability and efficacy of supplemented GS extract in cooked muscle foods to negate oxidation catalysed by salt pro-oxidant, heating and present of oxygen in the system.

4.3.3 Microbiological

The mesophilic plate counts of cooked breast and thigh patties stored in aerobic packaging ranged from 2.3 – 2.7 log CFU/g on day 0 to 4.1 – 4.3 log CFU/g on day 14. In MAP storage, initial mesophilic counts was 2.3 – 2.7 log CFU/g to 4.3 – 4.5 log CFU/g at the end of the storage. The dietary supplementation of GS100, GS200 and GS300 did not significantly affect ($p > 0.05$) microbial status of both cooked breast and thigh patties stored in both type of packaging. This is not surprising as GS extract (50 mg/ml) exhibited no *in vitro* antimicrobial activity against chicken microflora agar diffusion test (Chapter 2) and

the GS100, GS200 and GS300 supplementation did not improve the microbial stability of raw breasts fillets stored in aerobic and modified atmosphere packs (Chapter 3).

4.3.4 Sensory evaluation of cooked chicken patties

In aerobic storage, the control breast patties significantly negatively correlated to appearance, texture, flavour, overall acceptability and chicken flavour but significantly positively correlated to tenderness on day 2 and 8 (Table 4.4). Control breast patties significantly negatively correlated to whiteness on day 1. Visually, control was on the most left of APLSR plot (Figure 4.1) as control the negative association with most sensory attributes of cooked patties. The appearance of aerobically control breast patties maybe perceived less attractive corroborate to the colour measurement (Section 3.1) which showed that the patties were significantly redder than other treatments throughout aerobic storage. GS300 breast patties significantly positively correlated to chicken flavour, appearance (day 1) and flavour (day 8) but significantly negatively correlated to tenderness (day 8). This is in agreement to the level of lipid oxidation of cooked breast patties stored in aerobic packaging (Section 3.2), indicated that GS300 is optimum concentration for chicken supplementation in order to exert functional effects, thus improving the oxidative stabilities and sensory qualities of aerobically stored cooked breast patties. Evidently, Chapter 3 demonstrated GS300 supplemented breast improved most sensory attributes of raw chicken fillets stored in aerobic packaging.

Table 4.4 Significance coefficient (ANOVA results) for cooked chicken breast and thigh patties in aerobic packaging as derived by jack-knife uncertainty testing.

Treatment	**Muscle**	**Sensory analysis descriptors, day 1 and 8**							
		Appearance		**Texture**		**Flavour**		**Overall acceptability**	
		1	**8**	**1**	**8**	**1**	**8**	**1**	**8**
Control	Breast	**-0.00****	**-0.01***	**-0.04***	**-0.05***	**-0.00****	**-0.00****	**-0.00****	**-0.00****
SC		0.11	0.97	0.95	0.20	0.19	0.58	0.30	0.32
GS100		0.07	0.76	0.72	0.14	0.14	0.27	0.29	0.18
GS200		0.32	0.23	0.34	0.89	0.30	0.41	0.06	0.24
GS300		0.31	**0.04***	0.22	0.91	0.07	**0.03***	0.13	0.09
Control	Thigh	-0.45	-0.46	**-0.00****	**-0.00****	**-0.00****	**-0.00****	**-0.00****	**-0.00****
SC		0.61	0.58	0.29	**0.05***	**0.02***	0.12	0.07	**0.01***
GS100		0.70	0.63	0.12	0.17	0.14	0.26	0.10	0.10
GS200		0.27	0.92	0.84	0.48	0.93	**0.01***	0.86	0.23
GS300		0.40	0.97	0.23	0.94	0.47	0.36	0.31	0.52

Treatment	**Muscle**	**Sensory analysis descriptors, day 1 and 8**							
		Whiteness		**Tenderness**		**Chicken flavour**		**Oxidation flavour**	
		1	**8**	**1**	**8**	**1**	**8**	**1**	**8**
Control	Breast	**-0.00****	-0.20	**0.00****	**0.00****	**-0.00****	**-0.00****	0.84	-0.42
SC		0.07	0.54	-0.89	-0.26	0.56	0.27	0.75	0.45
GS100		0.12	0.45	-0.89	-0.08	0.27	0.15	0.61	0.58
GS200		0.29	0.76	-0.21	-0.51	0.38	0.37	0.56	0.73
GS300		0.37	0.69	-0.35	**-0.00****	**0.02***	0.30	-0.83	0.34
Control	Thigh	0.73	0.75	-0.85	-0.07	**-0.03***	**-0.00****	0.52	0.70
SC		-0.77	-0.54	0.63	0.72	0.32	0.24	-0.60	-0.67
GS100		-0.89	-0.85	0.88	0.33	0.17	0.24	-0.45	-0.89
GS200		-0.85	0.66	0.30	0.18	0.90	**0.04***	-0.42	-0.70
GS300		-0.66	-0.43	**0.04***	0.08	0.22	0.12	-0.60	-0.63

*95% significance, $p < 0.05$, **99% significance, $p < 0.01$. n = 3 independent experiments, measurements recorded in duplicate.

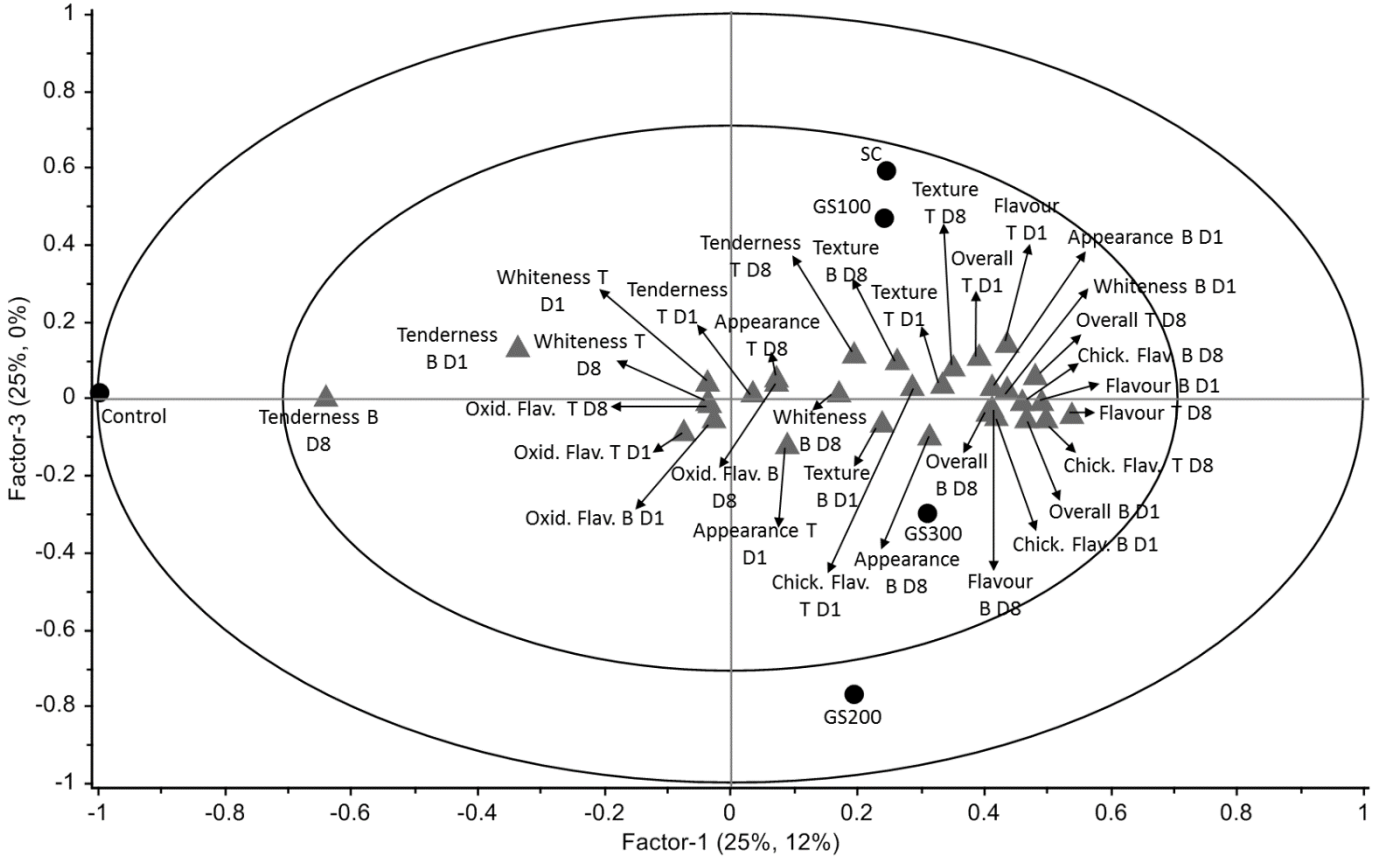


Figure 4.1 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 6 treatments stored in aerobic packaging. ● = treatments, ▲ = sensory descriptors.

The control thigh patties significantly negatively correlated to texture, flavour, overall acceptability and chicken flavour on day 1 and 8 of aerobic storage (Table 4.4). SC thigh patties significantly positively correlated to texture (day 8), flavour (day 1) and overall acceptability (day 8). The addition of salt enhance the flavour and texture of meat products (Desmond 2006), thus improve the overall acceptability of SC thigh patties. GS200 thigh patties significantly positively correlated to flavour and chicken flavour (day 8) and GS300 thigh patties significantly positively correlated to tenderness (day 1). GS200 supplementation improved the flavour and chicken flavour of cooked thigh patties in agreement to the efficient protective effect of GS200 supplementation in delaying lipid oxidation in cooked thigh patties (Section 4.3.2).

The control breast patties significantly negatively correlated to appearance, texture, flavour, overall acceptability, whiteness and chicken flavour on certain days of MAP storage (Table 4.5) as seen in Figure 4.2, control was at the most right of APLSR plot which clearly distant from most sensory attributes. Similarly, the redness of breast patties (Section 4.3.1) in MAP storage may contribute to negative perception to the appearance. However, control breast patties significantly positively correlated to tenderness on day 1 and 15 but significantly negatively correlated to the same attribute on day 22. On day 8 of MAP storage, SC breast patties significantly positively correlated to texture, flavour, overall acceptability and tenderness due to the addition of salt. Salt solubilises the functional myofibrillar proteins, activating the proteins to increase hydration and water binding capacity which subsequently improve the texture and tenderness of processed meat (Desmond 2006). GS100 breast patties were significantly positively correlated to appearance (day 1), flavour (day 1, 8, 15 and 22), overall acceptability (day 1 and 15), whiteness (day 1) and chicken flavour (day 1, 8 and 22). GS200 breast patties were significantly positively correlated to flavour and tenderness (day 22). GS300 breast patties

were significantly positively correlated to appearance (day 15 and 22) and chicken flavour (day 1 and 22). Overall, the supplementation of GS improved the sensory qualities of MAP stored cooked breast patties especially GS100 diet.

In MAP storage, control thigh patties significantly negatively correlated to texture, flavour, overall acceptability and chicken flavour on day 1, 8 and 15 (Table 4.5). The perceived attributes of control thigh patties stored in MAP was similar to those stored in aerobic packaging. On day 1 of MAP storage, SC and GS100 treatments significantly positively correlated to flavour and overall acceptability. GS200 significantly positively correlated to tenderness on day 15. GS300 of MAP thigh patties significantly positively correlated to appearance, flavour (day 8), tenderness (day 1 and 8) and chicken flavour (day 15).

In addition, it is important to highlight that the oxidation flavour of cooked chicken breast and thigh patties were not affected by any the treatments. Although the level of oxidation was high, but no panellists associated any treatment to negative sensory attribute to oxidation flavour.

Table 4.5 Significance coefficient (ANOVA results) for cooked chicken breast and thigh patties in modified atmosphere packaging (40% CO_2; 60% N_2) as derived by jack-knife uncertainty testing.

Treatment	Muscle	Sensory analysis descriptors, day 1, 8, 15 and 22															
		Appearance				Texture				Flavour				Overall acceptability			
		1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22
Control	Breast	**-0.00****	-0.31	**-0.00****	-0.12	**-0.04***	-0.10	**-0.04***	**-0.00****	**-0.00****	**-0.00****	**-0.00****	**-0.00****	**-0.00****	**-0.00****	**-0.00****	**-0.00****
SC		0.19	0.06	0.52	0.74	0.83	**0.02***	0.94	0.39	0.33	**0.03***	0.97	0.29	0.54	**0.02***	0.98	0.22
GS100		**0.02***	0.82	0.27	0.82	0.25	0.28	0.30	0.24	**0.02***	**0.04***	**0.05***	**0.01***	**0.01***	0.06	**0.05***	**0.01***
GS200		0.62	0.22	0.30	0.33	0.46	0.94	0.71	0.12	0.74	0.42	0.22	**0.05***	0.36	0.26	0.10	0.35
GS300		0.34	0.72	**0.00****	**0.05***	0.23	0.29	0.15	0.14	0.07	0.38	0.06	0.86	0.21	0.33	0.10	0.67
Control	Thigh	-0.46	-0.12	-0.09	0.96	**-0.00****	**-0.03***	**-0.05***	0.41	**-0.00****	**-0.00****	**-0.00****	0.19	**-0.00****	**-0.01***	**-0.03***	0.44
SC		0.27	0.84	0.68	0.52	0.29	0.99	0.17	-0.31	**0.03***	0.14	0.14	-0.15	**0.05***	0.59	0.23	-0.10
GS100		0.75	0.85	0.52	0.84	0.09	0.27	0.59	-0.97	**0.03***	0.14	0.43	-0.90	**0.05***	0.15	0.54	-0.97
GS200		0.33	0.45	0.68	0.81	0.98	0.66	0.23	-0.20	0.91	0.58	0.23	-0.18	0.99	0.97	0.25	-0.10
GS300		0.48	**0.04***	0.17	0.66	0.22	0.16	0.28	-0.32	0.46	**0.01***	0.10	-0.18	0.32	0.14	0.09	-0.34

Treatment	Muscle	Sensory analysis descriptors, day 1, 8, 15 and 22															
		Whiteness				Tenderness				Chicken flavour				Oxidation flavour			
		1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22
Control	Breast	**-0.00****	0.66	**-0.03***	0.99	**0.00****	-0.26	**0.02***	**-0.00****	**-0.00****	**-0.00****	-0.16	**-0.00****	0.80	0.47	0.28	0.38
SC		0.28	-0.75	0.75	0.79	-0.94	**0.01***	-0.30	0.91	0.85	0.12	0.59	0.47	-0.68	-0.45	-0.42	-0.07
GS100		**0.00****	-0.66	0.28	1.00	-0.13	0.48	-0.07	0.13	**0.04***	**0.04***	0.34	**0.01***	-0.96	-0.82	-0.22	-0.43
GS200		0.33	0.96	0.06	0.83	-0.64	0.71	-0.81	**0.03***	0.25	0.23	0.97	0.38	-0.67	-0.59	-0.75	-0.81
GS300		0.42	-0.94	0.85	0.95	-0.27	0.17	-0.88	0.74	**0.03***	0.76	0.53	**0.01***	-0.81	-0.59	-0.63	-0.15
Control	Thigh	0.75	0.83	-0.13	-0.14	-0.78	-0.10	-0.98	0.56	**-0.03***	**-0.05***	**-0.01***	0.24	0.49	0.87	0.52	-0.34
SC		-0.78	0.14	0.54	0.80	0.70	0.55	0.26	-0.85	0.43	0.96	0.06	-0.25	-0.37	0.46	-0.17	0.58
GS100		-0.97	0.91	0.68	0.36	0.62	0.77	0.78	-0.63	0.14	0.28	0.64	-0.79	-0.87	-0.77	-0.96	0.52
GS200		-0.92	0.29	0.50	0.39	0.51	0.87	**0.02***	0.90	0.81	0.81	0.09	-0.17	-0.45	0.85	-0.14	0.92
GS300		-0.67	0.84	0.07	0.68	**0.05***	**0.02***	0.23	-0.92	0.23	0.29	**0.01***	-0.34	-0.56	0.23	-0.33	0.93

*95% significance, $p < 0.05$, **99% significance, $p < 0.01$, ***99.9% significance, $p < 0.001$. n = 3 independent experiments, measurements recorded in duplicate.

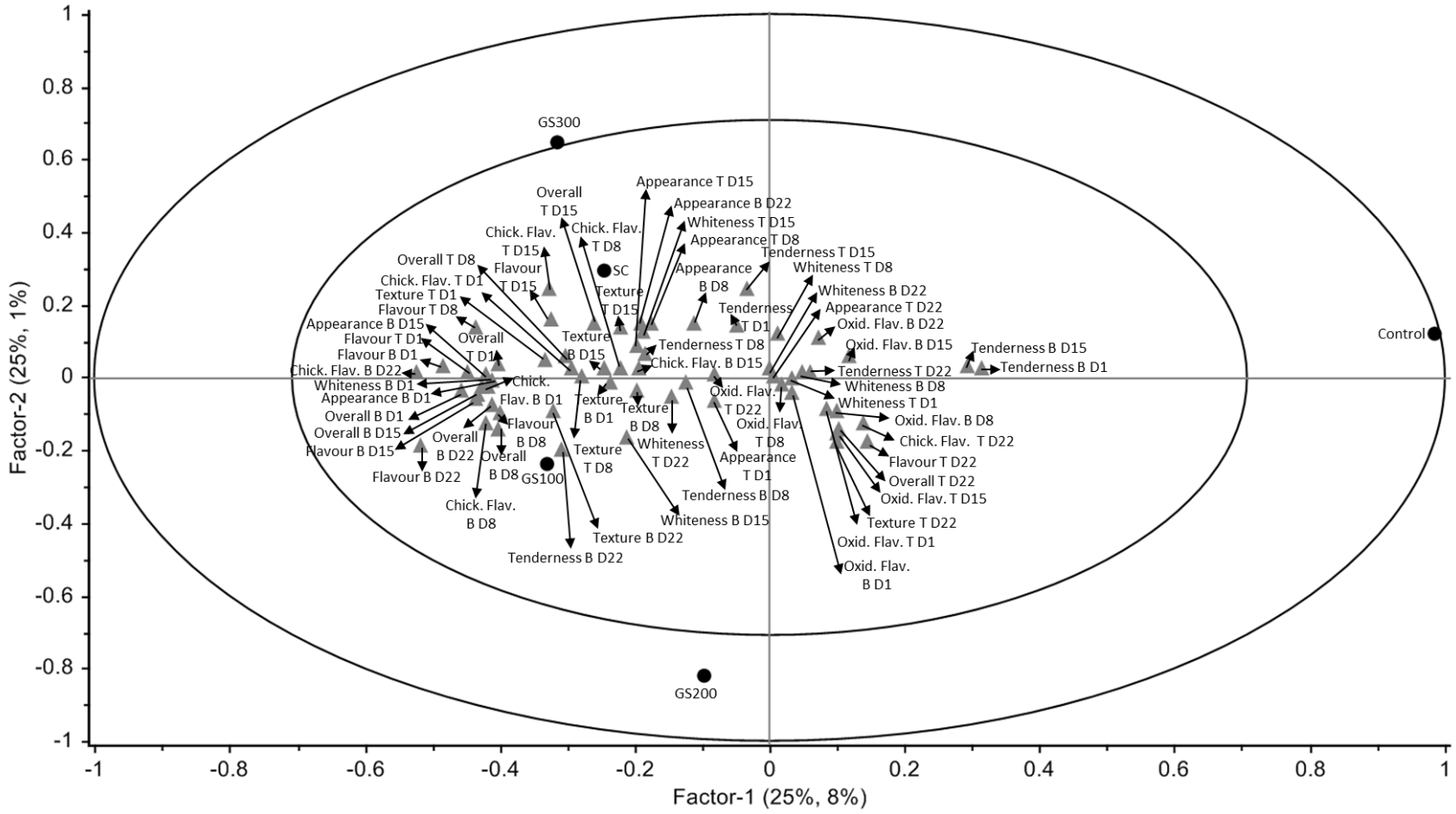


Figure 4.2 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 6 treatments stored in modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2). ● = treatments, ▲ = sensory descriptors.

4.4 Conclusions

The dietary supplementation of GS at three levels did not affect the surface lightness and yellowness but reduced redness of cooked breast and thigh patties stored in aerobic and modified atmosphere packs. The quality enhancing of supplementing chicken diets with GS was mediated through decreased level of lipid oxidation in cooked muscle foods. This is due to the deposition and retention of thermally stable supplemental extracts that remained functional during processing and throughout chill storage. In addition, the supplementation of GS enhanced the selected sensory qualities of cooked chicken breast and thigh patties stored in both aerobic and modified atmosphere packs indicated the acceptability of the supplemented cooked patties to panellists. Grape seed extract may be a good substitute to synthetic antioxidant in improving oxidative stability and enhancing the sensory qualities of cooked breast and thigh muscle foods when supplemented in chicken diets.

CHAPTER 5

5 The effect of plant extract-enriched 'active pads' on the oxidative stability of cooked chicken breast meat stored aerobically and in modified atmosphere packages.

Abstract

Two mixtures of grape seed, olive leaf and sweet chestnut bark (GS/OL/CB_1 and GS/OL/CB_2) and tea catechin (TC) extract (positive control) were incorporated into active pads and their influence on the oxidative stability of cooked chicken breast meat was examined. The diffusion ability of pads was determined by measuring the diffusion diameter of dye-impregnated pads on agar (~98% H_2O) stored at 4°C for 4 days. Dye diffusion increased as a function of storage time. The antioxidant activity of plant-derived extracts (TC, GS/OL/CB_1 and GS/OL/CB_2) was measured using *in vitro* antioxidant assays (TPC, FRAP and DPPH). Commercial absorbent pads, composed of micro-perforated (1429.88 µm diameter) low density polyethylene (LDPE) and cellulose fibres layers, were impregnated with TC, GS/OL/CB_1 and GS/OL/CB_2 extracts (1.9 and 3.8 mg/cm^2) and dried at 25°C (50% RH) to constant weight (~4 days) (antioxidant active pads). Chicken breast meat was cooked (85°C for 45 min), cooled, sliced (6 mm thickness), cored (3.5 cm diameter) and placed on active pads which were stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) at 4°C for up to 7 and 28 days, respectively. The influence of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts enriched pads on the quality (pH, colour and lipid oxidation) of cooked chicken breast meat was investigated. *In vitro* antioxidant activity followed the order: TC > GS/OL/CB_1 > GS/OL/CB_2 ($p<0.05$). Antioxidant active pads did not affect the pH of chicken stored aerobically and in MAP. The surface lightness of chicken was affected ($p<0.05$) by TC and GS/OL/CB_2 pads in both packaging conditions on certain days of storage. On day 7 of aerobic storage, all active

pads (1.9 mg/cm^2) and TC and GS/OL/CB$_1$ pads (3.8 mg/cm^2) reduced ($p<0.05$) lipid oxidation in cooked chicken meat relative to the control. TC pads reduced ($p<0.05$) lipid oxidation of chicken in a dose-dependent manner. In MAP storage, TC (3.8 mg/cm^2) and GS/OL/CB$_1$ (both concentrations) pads reduced ($p<0.05$) levels of lipid oxidation in chicken towards the end of storage. The mechanism of pad antioxidant activity was attributed to moisture activated release and diffusion of antioxidant compounds from the pad matrix onto the chicken surface. Overall results demonstrated the potential of plant-derived extracts for use as antioxidant agents in active packaging (pads) application for cooked chicken meat.

5.1 Introduction

Synthetic antioxidants, such as BHA, can be directly added into meat systems to reduce levels of lipid oxidation in processed chicken meat (Nissen *et al.* 2000). Health concern regarding the use of synthetic antioxidants has prompted much research into the use of natural alternatives from plant-derived sources. Many plant-derived extracts contain phenolic compounds, which possess excellent antioxidant activity, for example green tea and grape seed extract. Tea catechins are a major group of polyphenolic flavonoids found in green tea (O'Grady *et al.* 2006). The direct addition of tea catechins (0.3 mg/g) into cooked chicken patties reduced levels of lipid oxidation due to high affinity of tea catechins for the lipid bilayer (Tang *et al.* 2001a). Commercial mixtures of grape seed, olive leaf and sweet chestnut bark extracts contain diverse phenolic compounds classes such as hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, flavones, tyrosols and stilbenes. Extract mixtures possess good *in vitro* antioxidant activities and exhibited antioxidant potency in inhibiting lipid oxidation in chicken breast and thigh muscle homogenates (Chapter 2).

Active packaging is a technology which involves the inclusion of specific compounds into packaging systems to maintain or prolong product shelf-life and quality (Arvanitoyannis and Stratakos 2012). Examples include moisture absorbers and other mechanisms capable of delivering flavour or conferring antioxidant and antimicrobial properties to food products (Otoni *et al.* 2016). The advantages of adding antioxidant agents into packaging, compared to direct addition into muscle foods include: migration of antioxidant agents from the packaging onto food surface, localisation of antioxidant activity on the food surface where high levels of lipid oxidation occur due to surface exposure to oxygen and elimination of processing step required to introduce the agents into processed meats (Bolumar *et al.* 2011).

Antioxidant-releasing packaging is a promising technology used to protect muscle foods against lipid oxidation (Jongjareonrak *et al.* 2008; Gómez-Estaca *et al.* 2014). Absorbent pads have applications in the development of active packaging (Otoni *et al.* 2016). Pads are composed of an absorbent layer underneath a layer of polyethylene film having thermoformed apertures which functions to absorb moisture and exudate in packaged food efficiently by transporting liquid into the absorbent layer (Pearlstein 1998). Pads are placed underneath food products to prevent unsanitary exudate from contaminating packaged food and to maintain aesthetic appeal of food products during storage (Fernández *et al.* 2009).

Recent developments in active packaging have created novel dual functionality for absorbent pads i.e. to absorb moisture and confer antimicrobial properties for the preservation of foods (Otoni *et al.* 2016). Previous studies have reported that the incorporation of oregano essential oil (1.5%) in 3 layer absorbent pads (consisting of perforated polyethylene, cellulose and polyethylene) reduced microbial populations and extended the shelf-life of chicken drumstick for 2 days (Oral *et al.* 2009). The scientific literature contains no information regarding the mode of action of antioxidant absorbent pads in delivering antioxidant potency to cooked muscle foods. Recently, Contini *et al.* (2013) developed polyethylene terephthalate (PET) trays coated with citrus extract (100 mg/ml) which effectively reduced lipid oxidation in cooked turkey meat during refrigerated storage. Previously, reported kinetic studies demonstrated that citrus extract, containing a mixture of phenolic compounds and carboxylic acids, had an affinity for water, suggesting that their diffusion into the water phase of the meat facilitated their antioxidative effect (Contini *et al.* 2014). The mechanism of action of antioxidant coated trays can be postulated as a mode of action for antioxidant-containing absorbent pads. Therefore, the mechanism

and suitability of absorbent pads containing plant-derived antioxidant extracts to improve the oxidative stability of cooked muscle foods merits investigation.

The objective of the study was to assess the *in vitro* antioxidant activity (total phenol content (TPC), ferric reducing antioxidant power (FRAP) and DPPH free radical scavenging activity (DPPH)) of plant-derived extracts (tea catechins (TC) and two mixtures of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) (GS/OL/CB_1 and GS/OL/CB_2)). The development of antioxidant active pads involved microscopic examination and diffusion ability of dye-impregnated pads on agar. The colour and impregnation level of the TC, GS/OL/CB_1 and GS/OL/CB_2 pads were determined. The ability of antioxidant active pads containing natural extracts (TC, GS/OL/CB_1 and GS/OL/CB_2) to improve the shelf-life quality (pH, colour and lipid oxidation) of cooked chicken breast meat stored at 4°C in aerobic and modified atmosphere packaging (MAP: 40% CO_2 : 60% N_2) was investigated.

5.2 Materials and Methods

5.2.1 Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$), sodium acetate trihydrate ($C_2H_3O_2Na.3H_2O$), glacial acetic acid (CH_3CO_2H), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) ($C_{18}H_{12}N_6$), Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), trichloroacetic acid (TCA) ($C_2HCl_3O_2$), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) ($C_{18}H_{12}O_6$), 2-thiobarbituric acid (TBA) ($C_4H_4N_2O_2S$) and methanol were supplied by Sigma-Aldrich Ireland Ltd., Arklow, Co. Wicklow, Ireland. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was manufactured by BDH Laboratory Supplies, Poole, England. Plate count agar and hydrochloric acid (HCl) were obtained from Merck, Darmstadt, Germany. Tea catechins (TC) (81.43%) extract containing epigallocatechin gallate (37.62%), epicatechin gallate (16.22%), epicatechin (11.51%), epigallocatechin (9.64%), catechin (4.82%) and gallocatechin gallate (1.62%) was supplied by New Kinglong Natural Products Co. Ltd, Hunan, China. Two commercial mixtures of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) extracts ($GS/OL/CB_1$ and $GS/OL/CB_2$) were supplied by Envirotech Innovative Products Ltd., Dublin, Ireland. The classes of polyphenolic compounds present in $GS/OL/CB_1$ and $GS/OL/CB_2$ were reported in Chapter 2.

5.2.2 *In vitro* antioxidant activity of plant-derived extracts

The antioxidant potency of the plant-derived extracts was examined using *in vitro* antioxidant assays (TPC, FRAP and DPPH).

5.2.2.1 Total phenol content (TPC)

The TPC of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts was measured using the Folin-Ciocalteu method described by Singleton & Rossi (1965) with slight modifications. Extracts (0.5 ml) were mixed with 2.5 ml Folin-Ciocalteu reagent (10% in distilled water) in test tubes and held at room temperature. After 5 min, 2 ml of Na_2CO_3 (7.5% in distilled water) was added and tubes were vortex mixed and incubated in the dark for 2 hr at room temperature. The absorbance of the coloured mixture was measured at 750 nm using a spectrophotometer (Cary 60 UV-Vis Agilent Technology, Australia) against a blank containing all reagents and distilled water. A standard curve of aqueous gallic acid (0.004 – 0.10 mg/ml) was prepared and results were expressed as milligrams (mg) of gallic acid equivalents (GAE)/gram (g) extract.

5.2.2.2 Ferric reducing antioxidant power (FRAP)

The total antioxidant activity of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts was measured using the FRAP method of Benzie and Strain (1999) with slight modification. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_20$ solutions in the ratio 10:1:1, respectively. The reagent was incubated at 37°C for 10 min prior to use. The TPTZ and $FeCl_3.6H_2O$ solutions were made fresh on each analysis day. FRAP reagent (2.85 ml) was added to test tubes containing 0.15 ml extracts, vortex mixed and incubated for 30 min in the dark at room temperature. Following incubation, absorbance measurements were recorded at 593 nm

using a spectrophotometer (Cary 60). A standard curve of methanolic Trolox (0.006 – 0.19 mg/ml) was prepared and results were expressed as mg of Trolox equivalent (TE)/g extract.

5.2.2.3 DPPH scavenging activity (DPPH)

The DPPH free radical scavenging activity of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts was determined as described by Yen and Wu (1999) with slight modifications. Extracts (0.25 – 2.00 ml) were added to with 2 ml 0.2 mM DPPH in methanol, vortexed and incubated in the dark for 30 min at room temperature. Absorbance measurements were recorded at 517 nm using a spectrophotometer (Cary 60). A standard curve of methanolic Trolox (0.005 – 0.05 mg/ml) was prepared and results were expressed as mg of TE/g extract.

5.2.3 Development of plant extract-enriched active pads

5.2.3.1 Microscopy examination

Micro-perforated low-density polyethylene (LDPE) pad perforations were measured using a light and fluorescence microscope under reflected light observation (Olympus BX61 – Mason Technology, Dublin, Ireland).

5.2.3.2 Diffusion ability measurement

The micro-perforated LDPE absorbent pads (MeatPad MP 2500, McAirlaid's GmbH, Steinfurt, Germany) were cut into 4 cm^2 pieces and pad edges were sealed with Loctite® 431 instant adhesive (Henkel Ireland Operation and Research Ltd, Dublin, Ireland). Red dye (600 µl) was dispensed onto the sealed absorbent pads and pads were subsequently dried at 25°C and 50% relative humidity (RH) in a humidity chamber (Binder GmbH, Model KBF, Germany) until constant weight was achieved. Dye-impregnated dry

pads were then placed on the surface of plate count agar and stored in inverted position at 4°C to mimic the normal storage condition of food on pads. Diffusion of the red dye (diffusion diameter) on agar was measured using digital calliper gauge (Globaltronics GmbH & Co. KG, Hamburg, Germany) for up to 4 days. The diffusion diameter was measured daily.

5.2.3.3 Moisture analysis of agar and chicken

A SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany) was used to measure the moisture content of the plate count agar and cooked chicken breast meat samples.

5.2.4 Manufacture of plant extract-enriched active pads

5.2.4.1 Impregnation with plant-derived extracts

The TC, GS/OL/CB_1 and GS/OL/CB_2 active pads at the concentration of 1.9 and 3.8 mg/cm^2 were manufactured by impregnating 14.82 cm^2 absorbent pads (3.9 cm x 3.8 cm) with 2.22 ml of extract solutions at 12.5 and 25.0 mg/ml of TC, GS/OL/CB_1 and GS/OL/CB_2, respectively. The amount of solution used was sufficient to ensure uniform distribution of the extract solution on the pad. Control pads were impregnated with distilled water. Pads were subsequently dried at 25°C and 50% RH in a humidity chamber (Binder GmbH) until constant weight was achieved (~4 days). Extract impregnation of the pad can be calculated as follows:

$$\text{Impregnation (\%)} = \frac{\text{(final pad weight, g – initial pad weight), g}}{\text{initial pad weight, g}} \times 100\%$$

5.2.4.2 pH of plant-derived extract solutions

The pH of TC, GS/OL/CB_1 and GS/OL/CB_2 extract solutions at 12.5 and 25.0 mg/ml were measured at 20°C using a pH meter (Seven Easy portable, Mettler-Toledo GmbH, Schweizenbach, Switzerland).

5.2.4.3 Surface colour of TC, GS/OL/CB_1 and GS/OL/CB_2 impregnated active pads

The surface colour of active impregnated pads was measured using a portable Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Japan). The chroma meter consisted of a measuring head (CR-400) with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400) (Konica Minolta). The chroma meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79, a = - 0.11, b = 2.69). The 'L*' value represented lightness and 'a*' and 'b*' values represented redness and yellowness, respectively. The total colour difference (ΔE*) between the active pads and control pads was calculated using following formula (Stancil and Jordan 1985):

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

A ΔE* value of 3.0 - 6.0 for very distinct, 6.0 - 12.0 for great and a value of > 12 reflected that the colour of active pads was very different from the control pads (Drlange 1994).

5.2.5 Shelf-life of cooked chicken breast meat placed on plant extract impregnated active pads

5.2.5.1 Preparation and packaging of cooked chicken breast

Raw chicken breast meat was supplied by Shannon Vale Foods, Clonakilty, Co. Cork, Ireland. Chicken samples were vacuum packed individually and cooked in a circulating water bath (Julabo SW23-GB, Julabo Labortechnik GmBH, Seelbach, Germany) at 85°C for 45 min (internal temperature of 80°C). Following cooking, chicken

samples were immediately chilled in iced water (± 0.2°C) for 30 min. Cooked chicken breast samples were subsequently sliced into 6 mm thickness using a conventional meat slicer (Scharfen G330F, Witten, Germany) and cored to produce uniform size cooked chicken breast meat samples (3.5 mm diameter and weight ~6.0 g). Cooked chicken samples (surface area of 9.62 mm^2) was then pressed onto pads to ensure optimal contact between the cooked chicken.

5.2.5.2 Aerobic packaging

Cooked chicken breast samples on active pads were placed in individual trays and over-wrapped with an oxygen permeable (6000 – 8000 cm^3 m^{-2} 24 h^{-1} at STP) polyvinylchloride (PVC) film (Musgrave Wholesale Partners, Dublin, Ireland) and stored up to 7 days at 4°C.

5.2.5.3 Modified atmosphere packaging (MAP)

Cooked chicken breast samples on active pads were placed in low oxygen permeable (<1 cm^3 m^{-2} 24 h^{-1} at STP) polystyrene/ethylvinylalcohol (EVOH)/polyethylene (PE) trays and flushed with 40% CO_2 : 60% N_2 (modified atmosphere packaging, MAP) using a vacuum-sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH & Co. KG, Witten, Germany). Trays were covered and heat-sealed using low oxygen permeable (3 cm^3 m^{-2} 24 h^{-1} at STP) laminated barrier film with a polyolefin heat sealable layer. All packages were stored for up to 28 days at 4°C. The gas atmosphere in the MAP was checked using a CheckMate 9900 (PBI-Dansensor, Denmark). Immediately after flushing, the MAP tray contained 60.18 ± 0.19% N_2, 39.79 ± 0.19% CO_2 and 0.03 ± 0.00% O_2 and after 28 days, the gas composition was 61.48 ± 0.09% N_2, 38.37 ± 0.13% CO_2 and 0.15 ± 0.01% O_2.

5.2.5.4 Surface colour of cooked chicken samples

The surface colour of cooked chicken was measured using a Minolta CR-400 Chroma Meter (Konica Minolta). The chroma meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400) (Konica Minolta). The chroma meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79, a = -0.11, b = 2.69) where the 'L*' value represented lightness. Colour measurements of cooked chicken sample were recorded on days 1, 4 and 7 and days 1, 7, 14, 21 and 28 for aerobic and MAP, respectively.

5.2.5.5 pH of cooked chicken samples

Cooked chicken samples (5 g) were homogenised for 1 min at 24,000 rpm in 45 ml of distilled water using an Ultra Turrax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). The pH of the chicken homogenates was measured at 20°C using pH meter (Seven Easy portable). The pH of chicken was recorded on days 1 and 7 and days 1 and 28 for aerobic and MAP samples, respectively.

5.2.5.6 Lipid oxidation in cooked chicken samples

Lipid oxidation was measured using the 2-thiobarbituric acid assay (Siu and Draper, 1978). The malondialdehyde (MDA) content was calculated using extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1}. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg MDA/kg cooked chicken breast. Lipid oxidation in cooked chicken samples was measured on days 1, 4 and 7 and days 1, 7, 14, 21 and 28 for aerobic and MAP, respectively.

5.2.6 Statistical analysis

Statistical analysis was carried out using SPSS 22.0 for Windows (SPSS Statistical Software, Inc., Armonk, NY, USA) software package. All analyses were performed in duplicate and three independent experimental trials were carried out. One-way ANOVA was used to examine data from the *in vitro* antioxidant assays (TPC, FRAP and DPPH) and pad characteristics (colour and extract impregnation). One-way ANOVA was used to examine data from the shelf-life study and a full repeated measure ANOVA was conducted to investigate the effects of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts pads concentration (1.9 and 3.8 mg/cm^2), time and their interactions on the surface colour, pH and lipid oxidation of cooked chicken samples. TC, GS/OL/CB_1 and GS/OL/CB_2 active pads concentrations represented the 'between-subjects' factor and the effect of time measured using the 'within-subject' factor. Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means. All data is presented as mean values ± standard deviation.

5.3 Results and Discussion

5.3.1 *In vitro* antioxidant activity of plant-derived extracts

Antioxidant activity for all *in vitro* assays (TPC, FRAP and DPPH) followed the order: TC > GS/OL/CB_2 > GS/OL/CB_1 ($p<0.05$) (Table 5.1). The total phenol content for TC extract was higher than the reported literature i.e. 93 – 128 mg GAE/g (Ramiréz-Aristizabal *et al.* 2015). The high value of TPC reflected that tea catechin contains large amount of polyphenols compounds. Polyphenols is responsible for most of the antioxidant activity in plant products (Lee *et al.* 2014). However, scientific literature reported higher FRAP (389,200 mg TE/g) and DPPH (356162 mg TE/g) values than the present study (Lee *et al.* 2014). The antioxidant power of tea catechin strongly correlated with the TPC, indicating that the number of phenolic hydroxyl groups is a major determinant of antioxidant power (Veljković *et al.* 2013). High DPPH value of TC extract shows its ability to scavenge free radicals, may be attributed to the presence of epicatechin gallate, epigallocatechin and epicatechins which possessed good scavenging activites (Tang *et al.* 2002).

Table 5.1 *In vitro* antioxidant activity of tea catechin (TC) extract and two mixtures of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) extracts (GS/OL/CB_1 and GS/OL/CB_2) extracts.

Treatment	TPC (mg GAE/g)	FRAP (mg TE/g)	DPPH (mg TE/g)
TC	957.98 ± 3.40^{a}	2212.53 ± 1.65^{a}	5461.92 ± 24.85^{a}
GS/OL/CB_1	620.54 ± 63.31^{c}	1181.76 ± 18.59^{c}	2743.19 ± 1.88^{c}
GS/OL/CB_2	832.63 ± 13.64^{b}	1901.33 ± 41.06^{b}	4391.65 ± 80.92^{b}

^{abc}Within each column, mean values (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.

The antioxidant activity of GS/OL/CB_1 and GS/OL/CB_2 extracts may be attributed to the present of procyanidin B1, procyanidin B2, epicatechin gallate, epicatechin, gallic

acid, catechin, resveratrol, caffeic acid, ferulic acid, hydroxytyrosol and ellagic acid (Chapter 2). Generally, GS/OL/CB_1 and GS/OL/CB_2 extracts contain similar phenolic compounds with the exception of two additional compounds present in the GS/OL/CB_1 extract (tyrosol and myricetin 3-O-β-D-glucuronide) and GS/OL/CB_2 extract (t-cinnamic acid and trans-caftaric acid) (referring to Chapter 2). GS/OL/CB_2 extract demonstrated greater *in vitro* antioxidant activity possibly because the phenolic compounds present in the extract are more potent than those present in GS/OL/CB_1 extract. For instance, GS/OL/CB_2 extract contains caftaric acid, a compound found to possess radical scavenging ability and reacts rapidly with DPPH radical as compared to (+)-catechin and (-)-epicatechin (Villaño *et al.* 2007). By contrast, GS/OL/CB_1 extract contains tyrosol, a compound reported to be inefficient in inhibiting the DPPH radical (Vlachogianni *et al.* 2015).

5.3.2 Microscopy and diffusion properties of absorbent pads

Pads consisted of micro-perforated LDPE outer layer on each side with a patented matrix core of cellulose and super absorbent fibres with perforation diameters of 1429.88 ± 25.43 µm (Figure 5.1a). Micro-perforations on both the upper and lower pad surfaces facilitate liquid absorption by cellulose within the pad matrix through capillary action (Otoni *et al.* 2016). Prior to diffusion measurement, the pad edges were sealed with adhesive to ensure that any red colour which diffused out from the pad, solely diffused through perforated surface upon contact with the high moisture surface of the agar (97.66 ± 0.14%). The red colour diffusion diameter increased as a function of storage time. The initial diameter was 19.82 ± 0.31 mm which increased to 62.34 ± 2.14 mm on day 4 of refrigerated storage (Figure 5.1b). The proposed mechanism of action of the active pads is the absorption of water/drip by cellulose core through pad perforation upon contact with

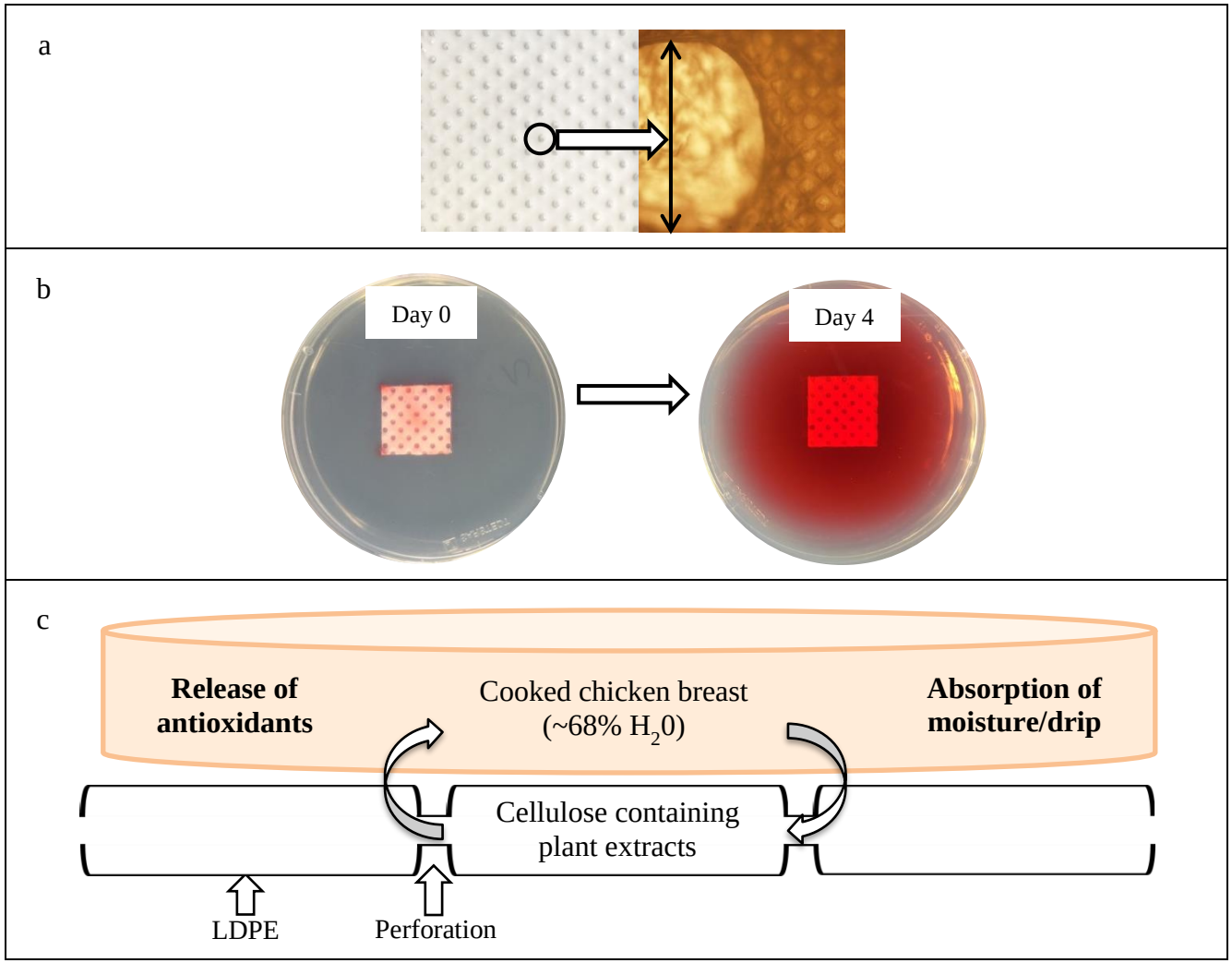


Figure 5.1 (a) Microscopic examination illustrating the perforation diameter of micro-perforated LDPE cellulose pads, (b) diffusion of dye-impregnated pad on agar after 4 days of storage at 4°C and (c) illustration of the proposed mechanism of action of impregnated active pads.

cooked chicken (67.77 ± 1.30% moisture) which subsequently released antioxidant impregnated compounds from the pad onto the chicken breast surface (Figure 5.1c).

5.3.3 Impregnation of antioxidant active pads with plant-derived extracts

The cellulose matrix of the pads absorbs large amount of plant extract solutions through the pad perforations by capillary action. The drying process fixed and retained the plant extracts containing phenolic compounds within the pad matrix. The low concentration of TC, GS/OL/CB_1 and GS/OL/CB_2 pads (1.9 mg/cm^2) was impregnated with 12.5 mg/ml TC, GS/OL/CB_1 and GS/OL/CB_2 extract solutions, respectively. Meanwhile, the high concentration active pads (3.8 mg/cm^2) were prepared from 25.0 mg/ml extract solutions. The low concentration (1.9 mg/cm^2) active pads had significantly ($p<0.05$) greater impregnation range (1.92 – 2.03%) compared to the high concentration active pads (3.86 – 3.88%) as summarised in Table 5.2. At the low concentration, GS/OL/CB_1 pads had significantly ($p<0.05$) higher impregnation than TC pads. However, the impregnation percentage of the higher concentration (3.8 mg/cm^2) TC, GS/OL/CB_1 and GS/OL/CB_2 pads were similar between treatments.

Table 5.2 Physical characteristic of TC, GS/OL/CB_1 and GS/OL/CB_2 antioxidant active pads at two concentrations (1.9 and 3.8 mg/cm^2)

Treatment	Extract concentration (mg/ml)	Pad concentration (mg/cm^2)	Impregnation (%)	Total colour difference (ΔE)
TC	12.5	1.9	1.92 ± 0.19^{a}	18.56 ± 0.56^{a}
TC	25.0	3.8	3.88 ± 0.14^{c}	20.13 ± 0.69^{a}
GS/OL/CB_1	12.5	1.9	2.03 ± 0.17^{b}	24.72 ± 1.98^{b}
GS/OL/CB_1	25.0	3.8	3.86 ± 0.12^{c}	27.64 ± 1.76^{c}
GS/OL/CB_2	12.5	1.9	1.95 ± 0.14^{ab}	19.96 ± 0.98^{a}
GS/OL/CB_2	25.0	3.8	3.86 ± 0.19^{c}	22.53 ± 1.23^{b}

[abc]Within each column, mean values (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
TC: tea catechin extract, GS/OL/CB_1 and GS/OL/CB_2: two mixtures of grape seed, olive leaf and sweet chestnut bark extracts.

5.3.4 Surface colour of impregnated active pads

The total colour differences (ΔE^*) of the TC, GS/OL/CB_1 and GS/OL/CB_2 active pads (1.9 and 3.8 mg/cm^2) was greater than 12 (Table 5.2) and are thus classified as greatly different from the control pads (Drlange 1994). The ΔE^* of the active pads in increasing order were as follows: (TC 1.9 mg/cm^2) ≈ GS/OL/CB_2 (1.9 mg/cm^2) ≈ TC (3.8 mg/cm^2) < GS/OL/CB_2 (3.8 mg/cm^2) ≈ GS/OL/CB_1 (1.9 mg/cm^2) < GS/OL/CB_1 (3.8 mg/cm^2). The ΔE^* of the TC pads was not affected by the concentration of TC extract solutions. The ΔE^* of GS/OL/CB_1 and GS/OL/CB_2 pads increased in a dose-dependent manner ($p<0.05$). Generally, the colour of the active pads was dependent on the natural colour of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts which was light brown, dark purple and ochra, respectively. However, coloured pads are not a limitation in the development of active pads since a number of commercial food pads are available in different colours such as black (Sirane Ltd.), pink, red, green and blue (Dri-Loc® Cryovac).

5.3.5 Effect of active pads on the shelf-life quality of cooked chicken breast stored aerobically and in modified atmosphere packages

5.3.5.1 Surface colour of cooked chicken samples

Table 5.3 summarised the effect of TC, GS/OL/CB_1 and GS/OL/CB_2 pads (1.9 and 3.8 mg/cm^2) on the surface lightness (L^*) of cooked chicken during aerobic and MAP (40% CO_2 : 60% N_2) storage. The TC (1.9 mg/cm^2) pads did not affect the colour of chicken samples throughout the storage, except on day 14 when the L^* of MAP cooked chicken samples were significantly ($p<0.05$) darker than control samples. The higher concentration TC (3.8 mg/cm^2) pads significantly ($p<0.05$) reduced the surface L^* of cooked chicken on day 4 and 7 (aerobic) and on day 7 and 14 (MAP), relative to the controls. A concentration-dependent effect was observed on day 7 (aerobic) and on day 7 and 14 (MAP) ($p<0.05$).

Low concentration GS/OL/CB_1 pads did not affect the L^* of cooked chicken samples throughout aerobic and MAP storage, except on day 7 of MAP storage where lower L^* values for cooked chicken samples compared to the controls were observed. The high concentration GS/OL/CB_1 pads resulted in significant ($p<0.05$) darker surface of cooked chicken samples on day 7 (aerobic) and on day 7 and 14 (MAP) in comparison to samples stored on control pads. The GS/OL/CB_1 pads reduced the L^* of MAP chicken samples stored on day 7 and 14 in concentration-dependent manner ($p<0.05$).

In contrast, both concentrations of GS/OL/CB_2 pads did not affect the surface lightness of cooked chicken samples stored in aerobic and modified atmosphere packs throughout the refrigerated storage. It was observed that high concentration of TC and GS/OL/CB_1 pads reduced the L^* of aerobically packed chicken towards the end of storage (day 4 and 7). Interestingly, both concentration of TC and GS/OL/CB_1 pads lowered the

Table 5.3 Effect of TC, GS/OL/CB_1 and GS/OL/CB_2 active pads on lightness (L^*) of cooked chicken breast stored in aerobic packaging and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) at 4°C for up to 7 and 28 days, respectively.

Treatment	**Concentration (mg/cm^2)**	**Storage time at 4°C, days**							
		1		**4**	**7**		**14**	**21**	**28**
		Aerobic	**MAP**	**Aerobic**	**Aerobic**	**MAP**	**MAP**	**MAP**	**MAP**
Control		85.1 ± 0.5^{a}	84.2 ± 1.0^{a}	83.8 ± 1.7^{abc}	83.3 ± 0.2^{ab}	84.8 ± 0.1^{a}	84.8 ± 0.8^{a}	83.6 ± 1.0^{a}	83.5 ± 1.0^{a}
TC	1.9	85.3 ± 0.5^{a}	84.2 ± 1.1^{a}	81.4 ± 1.3^{cd}	82.5 ± 1.1^{bc}	84.8 ± 0.3^{a}	82.9 ± 0.3^{bc}	83.8 ± 2.0^{a}	83.9 ± 2.0^{a}
TC	3.8	85.0 ± 0.7^{a}	85.7 ± 0.5^{a}	79.7 ± 0.9^{d}	79.6 ± 0.5^{d}	78.3 ± 0.5^{d}	80.6 ± 0.5^{d}	81.8 ± 1.3^{a}	81.1 ± 0.4^{a}
GS/OL/CB_1	1.9	84.9 ± 0.1^{a}	85.0 ± 1.0^{a}	82.5 ± 0.3^{bc}	82.4 ± 0.1^{bc}	83.3 ± 0.3^{b}	84.3 ± 1.1^{ab}	84.4 ± 0.6^{a}	83.9 ± 1.4^{a}
GS/OL/CB_1	3.8	84.9 ± 0.7^{a}	85.2 ± 1.1^{a}	81.4 ± 0.7^{cd}	80.9 ± 1.0^{cd}	80.5 ± 0.5^{c}	81.2 ± 0.5^{cd}	78.1 ± 5.1^{a}	81.7 ± 2.6^{a}
GS/OL/CB_2	1.9	84.9 ± 0.2^{a}	85.4 ± 1.6^{a}	84.8 ± 0.3^{ab}	84.5 ± 0.2^{a}	84.6 ± 0.4^{a}	85.4 ± 0.2^{a}	84.1 ±1.4^{a}	84.4 ± 0.8^{a}
GS/OL/CB_2	3.8	84.6 ± 0.5^{a}	85.8 ± 1.0^{a}	85.4 ± 0.2^{a}	84.7 ± 0.2^{a}	84.8 ± 0.5^{a}	83.6 ± 0.7^{ab}	83.3 ± 2.0^{a}	83.7 ± 1.7^{a}

^{abcd}Within each column and storage day, mean value (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
TC: tea catechin extract, GS/OL/CB_1 and GS/OL/CB_2: two mixtures of grape seed, olive leaf and sweet chestnut bark extracts.

*L** values of chicken samples in the middle of MAP storage (day 7 and 14). It can be deduced that the colour tainting process is time consuming, possibly due to the activated release and diffusion mechanism depicted in Figure 5.1 (b and c).

Chicken meat discolouration may be due to the natural colour of the plant-derived extracts, the level of pad impregnation or a reaction between the compounds present in the extracts with the chicken matrix. TC, GS/OL/CB_1 and GS/OL/CB_2 extracts possessed different hues but the pigments of GS/OL/CB_2 extract did not affect the surface colour of cooked chicken samples. In contrast, GS/OL/CB_1 extract is purplish in nature, most likely derived from the GS extract. Similarly, the addition of 10 and 20 mg/g grape dietary fibre in chicken hamburger significantly reduced the lightness of the cooked samples throughout 5 days of storage (Sáyago-Ayerdi, Brenes and Goñi 2009). The TC extract may cause discolouration in chicken due to possible binding of tea catechin with the iron component of myoglobin (Mitsumoto *et al.* 2005) which may be demonstrated by the TC pads.

5.3.5.2 pH of chicken samples

The pH of cooked chicken breast samples stored on control and active pads (TC, GS/OL/CB_1 and GS/OL/CB_2) in both types of packaging fell within the range 6.13 – 6.33 (Table 5.4), similar to the pH of cooked chicken breast stored in air and MAP (30% CO_2 : 70% N_2) which ranged from 6.25–6.42 (Patsias *et al.* 2006). The pH of the TC, GS/OL/CB_1 and GS/OL/CB_2 extracts was ranged from 4.18 – 4.24, 4.72 – 4.77 and 4.65 – 4.71, respectively. Despite their acidic nature, the TC, GS/OL/CB_1 and GS/OL/CB_2 pads did not significantly affect the pH of cooked chicken compared to those stored on control pads.

Table 5.4 Effect of TC, GS/OL/CB_1 and GS/OL/CB_2 active pads on pH of cooked chicken breast samples stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) at 4°C for up to 7 and 28 days, respectively.

Treatment	Concentration (mg/cm^2)	Storage time at 4°C, days			
		1		7	28
		Aerobic	MAP	Aerobic	MAP
Control		6.17 ± 0.13[a]	6.30 ± 0.03[a]	6.27 ± 0.01[a]	6.16 ± 0.13[a]
TC	1.9	6.25 ± 0.12[a]	6.33 ± 0.03[a]	6.27 ± 0.03[a]	6.13 ± 0.07[a]
TC	3.8	6.16 ± 0.09[a]	6.31 ± 0.02[a]	6.30 ± 0.05[a]	6.15 ± 0.05[a]
GS/OL/CB_1	1.9	6.22 ± 0.13[a]	6.31 ± 0.02[a]	6.21 ± 0.14[a]	6.22 ± 0.07[a]
GS/OL/CB_1	3.8	6.18 ± 0.14[a]	6.27 ± 0.05[a]	6.27 ± 0.03[a]	6.18 ± 0.07[a]
GS/OL/CB_2	1.9	6.26 ± 0.08[a]	6.25 ± 0.07[a]	6.24 ± 0.05[a]	6.20 ± 0.10[a]
GS/OL/CB_2	3.8	6.24 ± 0.11[a]	6.27 ± 0.03[a]	6.18 ± 0.03[a]	6.14 ± 0.05[a]

[a]Within each storage day, mean values (± standard deviation) in the same column are not significantly difference, $p>0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
TC: tea catechin extract, GS/OL/CB_1 and GS/OL/CB_2: two mixtures of grape seed, olive leaf and sweet chestnut bark extracts.

5.3.5.3 Lipid oxidation in chicken samples

Lipid oxidation of cooked chicken breast samples stored on control and active pads (TC, GS/OL/CB_1 and GS/OL/CB_2) increased throughout aerobic and modified atmosphere storage (Table 5.5). In aerobic packaging, lipid oxidation of cooked chicken on control pads increased from 0.25 mg MDA/kg (day 1) to 4.46 mg MDA/kg (day 7). On day 7 of aerobic storage, low and high concentration TC pads significantly ($p<0.05$) reduced lipid oxidation of cooked chicken breast samples in dose-dependent manner compared to the controls. The lower level (1.9 mg/cm^2) GS/OL/CB_1 pads reduced ($p<0.05$) lipid oxidation in chicken samples on day 7 of aerobic storage. Both concentrations of GS/OL/CB_2 pads significantly ($p<0.05$) protected the aerobically packed cooked samples against lipid oxidation on day 7 of storage. However, the increased in GS/OL/CB_2 pad concentration resulted in a plateau inhibition effect on lipid oxidation in cooked chicken.

Table 5.5 Effect of TC, GS/OL/CB_1 and GS/OL/CB_2 active pads on lipid oxidation (TBARS, mg MDA/kg chicken) of cooked chicken breast stored in aerobic and modified atmosphere packaging (MAP) (40% CO_2 : 60% N_2) at 4°C for up to 7 and 28 days, respectively.

Treatment	**Concentration (mg/cm^2)**	**Storage time at 4°C, days**							
		1		**4**	**7**		**14**	**21**	**28**
		Aerobic	**MAP**	**Aerobic**	**Aerobic**	**MAP**	**MAP**	**MAP**	**MAP**
Control		0.25 ± 0.07^{a}	0.25 ± 0.06^{a}	2.36 ± 0.21^{a}	4.46 ± 0.04^{a}	0.98 ± 0.10^{a}	0.99 ± 0.17^{ab}	1.01 ± 0.06^{b}	1.78 ± 0.33^{a}
TC	1.9	0.28 ± 0.09^{a}	0.27 ± 0.08^{a}	2.28 ± 0.14^{a}	3.78 ± 0.36^{b}	0.76 ± 0.05^{ab}	0.80 ± 0.03^{bc}	0.98 ± 0.04^{bc}	1.37 ± 0.03^{ab}
TC	3.8	0.25 ± 0.06^{a}	0.23 ± 0.04^{a}	1.40 ± 0.26^{a}	1.79 ± 0.33^{d}	0.71 ± 0.19^{ab}	0.59 ± 0.01^{c}	1.06 ± 0.05^{ab}	1.10 ± 0.16^{b}
GS/OL/CB_1	1.9	0.32 ± 0.05^{a}	0.28 ± 0.07^{a}	1.96 ± 0.58^{a}	3.69 ± 0.04^{bc}	0.70 ± 0.12^{ab}	0.83 ± 0.09^{bc}	0.76 ± 0.01^{c}	1.21 ± 0.17^{b}
GS/OL/CB_1	3.8	0.28 ± 0.06^{a}	0.24 ± 0.04^{a}	1.95 ± 0.39^{a}	4.20 ± 0.24^{ab}	0.57 ± 0.16^{b}	0.86 ± 0.06^{bc}	1.14 ± 0.15^{ab}	1.19 ± 0.06^{b}
GS/OL/CB_2	1.9	0.32 ± 0.03^{a}	0.29 ± 0.04^{a}	2.35 ± 0.55^{a}	3.15 ± 0.06^{c}	0.67 ± 0.08^{ab}	0.74 ± 0.14^{bc}	1.05 ± 0.02^{ab}	1.43 ± 0.16^{ab}
GS/OL/CB_2	3.8	0.35 ± 0.03^{a}	0.36 ± 0.03^{a}	2.31 ± 0.50^{a}	3.67 ± 0.17^{bc}	0.84 ± 0.14^{ab}	1.15 ± 0.05^{a}	1.26 ± 0.14^{a}	1.58 ± 0.23^{ab}

^{abc}Within each column and storage day, mean value (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
TC: tea catechin extract, GS/OL/CB_1 and GS/OL/CB_2: two mixtures of grape seed, olive leaf and sweet chestnut bark extracts.

In MAP, lipid oxidation in cooked chicken on control pads increased from 0.25 mg MDA/kg chicken (day 1) to 1.78 mg MDA/kg chicken (day 28) (Table 5.5). Levels of lipid oxidation were lower than in aerobic packaging due to the low oxygen level present in MAP. Low concentration TC pads reduced the level of lipid oxidation in cooked chicken samples throughout MAP storage, however results were not statistically significant. High concentration TC pads significantly ($p<0.05$) reduced lipid oxidation on day 14 and 28 of MAP storage. The GS/OL/CB_1 pads (1.9 mg/cm^2) significantly ($p<0.05$) reduced lipid oxidation on day 21 and 28, relative to the controls. GS/OL/CB_1 pads (3.8 mg/cm^2) significantly ($p<0.05$) inhibited lipid oxidation of MAP cooked samples on days 7 and 28 compared to controls. Increasing the plant-derived extract concentration in GS/OL/CB_1 pads (from 1.9 to 3.8 mg/cm^2) did not enhance the pad antioxidant activity resulting in a plateau antioxidant effect. Throughout the MAP storage, levels of lipid oxidation of chicken samples stored on GS/OL/CB_1 and GS/OL/CB_2 pads were not significantly higher than those stored on TC pads (on a comparative concentration basis).

It was observed that the protective antioxidant effect of active pads did not occur on days 1 and 4 of aerobic storage, significant inhibition of lipid oxidation was observed from day 7 onwards in both aerobic and MAP storage. Presumably, antioxidant pad function increases with storage time as pads require time to absorb moisture prior to the release of antioxidant compounds from the pad matrix (see Figure 5.1).

5.4 Conclusions

Two mixtures of grape seed, olive leaf and sweet chestnut bark extract were evaluated as antioxidant agents for potential use in antioxidant active packaging to improve shelf-life parameters of cooked chicken, in comparison to tea catechin active pads. Despite of being less potent than tea catechin (TC) extract, the two mixtures of grape seed, olive leaf and sweet chestnut bark (GS/OL/CB_1 and GS/OL/CB_2) active pads had the ability to reduce the levels of lipid oxidation in cooked chicken samples, compared to the controls in both packaging format. The increase in the concentration of plant-derived extract mixtures resulted to plateau inhibition effect on lipid oxidation in cooked chicken samples. This study highlighted the suitability of GS/OL/CB_1 and GS/OL/CB_2 pads to deliver their potencies in improving the oxidative stability of cooked chicken stored in refrigeration.

CHAPTER 6

6 Use of plant extract-enriched 'active' gelatin films to enhance the oxidative stability of cooked chicken breast meat stored in aerobic and vacuum-skin packaging conditions.

Abstract

Two mixtures of grape seed, olive leaf and sweet chestnut bark (GS/OL/CB_1 and GS/OL/CB_2) and tea catechin (TC) extract (positive control) were incorporated into active gelatin films at two concentrations (0.7 and 1.5 mg/ml). The effect of active films on the oxidative stability of cooked chicken breast meat was examined. Film solutions were casted and dried (25°C, 50% RH) for 48 hr. The antioxidant activity (TPC and DPPH), physical properties (thickness, colour, light transmission, opacity, contact angle, water vapour permeability (WVP) and oxygen permeability (OP)) and structural profile of control and active films were investigated. Cooked-sliced-cored chicken breast samples were overwrapped with control and active films and stored in aerobic and vacuum-skin packaging (VSP) at 4°C for up to 6 and 18 days, respectively. The ability of active films for influencing the lighness and lipid oxidation of cooked chicken was investigated. The addition of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts improved the *in vitro* antioxidant activity of the active films in dose-dependent manner ($p<0.05$). Both concentrations of the active films had similar thickness, contact angle and WVP relative to the controls. High concentration TC films had lower ($p<0.05$) OP compared to control films. Colour differences of active films increased in dose-dependent manner ($p<0.05$). The active films efficiently reduced light transmission and increased films opacity due to the present of polyphenolic compounds, especially in GS/OL/CB_1 films. The FTIR spectrum of the active films showed that plant-derived extracts addition slightly modified the structural properties of the protein network (amide A and B). Both concentrations of TC and GS/OL/CB_1 films

reduced the L^* ($p<0.05$) but GS/OL/CB_2 films did not influence the L^* of cooked chicken throughout aerobic and VSP storage. All active films reduced lipid oxidation in aerobic and vacuum-skin packaged samples in refrigerated storage ($p<0.05$). The protective effect exerted by the active films was presumably due to the migration of polyphenol compounds from the films onto the surface of cooked chicken samples. Overall results demonstrated the potential of plant-derived extracts for use as antioxidants in gelatin active films used to stabilise cooked chicken against lipid oxidation.

6.1 Introduction

The high consumption of plastic materials by the food industry has resulted in serious environmental and ecological problems due to their non-degradable nature (Gómez-Estaca *et al.* 2009; Marcos *et al.* 2014). This has lead to numerous research studies aiming to develop biodegradable packaging produced from natural polymer sources (Gómez-Estaca *et al.* 2009). Gelatin is produced from the chemical denaturation of fibrous insoluble protein (known as collagen) obtained from the bones, skin and connective tissues of animal generated during slaughtering and processing (Nur Hanani *et al.* 2014). Gelatin is widely utilised to manufacture biodegradable packaging films (Gómez-Estaca *et al.* 2009). Gelatin films possess good mechanical and gas barrier properties but low water barrier properties (Nur Hanani *et al.* 2012). However, the characteristics and properties of gelatin-based films can be improved by incorporating additives during film manufacture (Nur Hanani *et al.* 2014). For example, the addition of butylated hydroxytoluene (BHT) (0.2 mg/g) improved the water vapour permeability and hydrophobicity of gelatin films due to the dispersion of compounds within the matrix which increase the density of the network (Jongjareonrak *et al.* 2008).

Recently, research efforts are focused on the development of antioxidant active packaging where antioxidant agents are added into the film network to improve the physical, mechanical and antioxidant activity of the resulting films (Wu *et al.* 2013; Li *et al.* 2014). Antioxidant packaging functions to improve the quality of packaged food due to its ability to delay or inhibit lipid oxidation by reducing the oxygen transmission rate, migration of antioxidant agents to the food product and/or reducing the presence of reactive oxygen species which initiate the oxidation processes (Gómez-Estaca *et al.* 2014). The migration of antioxidant agents from the packaging onto food facilitates localisation of antioxidant activity on the food surface where high level of lipid oxidation can occur

(Bolumar *et al.* 2011). Hence, antioxidant-release packaging is a promising technology offering protection to the food surface against lipid oxidation and rancidity (Jongjareonrak *et al.* 2008).

Synthetic antioxidants are frequently used in antioxidant active packaging because of their stability, efficiency and low cost (Siripatrawan and Harte 2010). Due to concerns relating to potential health risks associated with synthetic additives (Siripatrawan and Harte 2010) and a growing consumer demand for clean label food products, there is an urgent need to substitute such additives with natural plant-derived antioxidant compounds (Marcos *et al.* 2014).

Plant-derived extracts containing phenolic compounds, such as tea catechins and mixtures of grape seed, olive leaf and sweet chestnut bark extracts, demonstrated free radical scavenging and electron donating abilities in chapter 5. The incorporation of plant-derived extracts containing polyphenol compounds into gelatin films not only enhances film antioxidant potential, but also the barrier properties of the active films. For example, fish gelatin films containing green tea and grape seed extract (1 mg/ml) and pigskin gelatin films containing hydrolysable chestnut tannins (10 mg/ml) exhibited good *in vitro* antioxidant activity and improved film barrier properties against UV light and moisture (Li *et al.* 2014; Peña-Rodriguez *et al.* 2015). To date, research on the incorporation of olive leaf extract into gelatin films is not available in the scientific literature. However, the addition of tyrosol (47.1 mg/g) (compound typically found in olive leaf) into chitosan-fish gelatin films improved the moisture barrier property of the films due to cross-linking of compounds with the polymer to form a stable network (Benbettaïeb *et al.* 2016). Currently, most of the research of antioxidant gelatin films aims to improve the film characteristics and *in vitro* antioxidant properties but limited studies focus on the effect of active gelatin films in preserving the quality of oxidation-sensitive muscle foods.

In chapter 5, absorbent pads impregnated with tea catechin and mixtures of grape seed, olive leaf and sweet chestnut bark extracts (antioxidant active pads) protected cooked chicken meat against lipid oxidation, mediated through the moisture activated-release of antioxidant compounds upon direct contact of the pads with cooked chicken. This study proved the suitability of plant-derived extracts for improving the oxidative stability of cooked muscle foods when incorporated into antioxidant active pads. Therefore it is interesting to evaluate potential use of the same plant-derived extracts in different antioxidant active packaging and delivery systems, such as gelatin films, to protect chicken meat against lipid oxidation. Benbettaïeb *et al.* (2016) studied the release kinetic of tyrosol and ferulic acid (47.1 mg/g) from chitosan-fish gelatin films into a water medium, and tyrosol possessed a higher release rate and diffusion coefficient than ferulic acid. This was possibly due to the greater ability of ferulic acid to interact with the polymer network which increased the cross-linking density and reduced the diffusion of antioxidant compound from the film into the liquid medium (Benbettaïeb *et al.* 2016). The kinetic release study suggested that the release of active compounds from the film into packaged foods depended on the degree of cross-linking between the active compounds and the polymer network and surface nature (hydrophilic/hydrophobic) of the food surface, in order to elicit an antioxidant effect.

The objective of the study was to evaluate the effect of incorporation of tea catechins (TC) extract and mixtures of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) extract (GS/OL/CB_1 and GS/OL/CB_2) on gelatin film characteristics (thickness, water contact angle, light transmission, opacity, colour, water vapour permeability and oxygen permeability), the structural and antioxidant properties of the active films. The ability of the active films to inhibit lipid oxidation in cooked chicken breast meat was also investigated.

6.2 Material and Methods

6.2.1 Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$), Folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), 2-thiobarbituric acid (TBA) ($C_4H_4N_2O_2S$), trichloroacetic acid (TCA) ($C_2HCl_3O_2$), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) ($C_{18}H_{12}O_6$) and methanol (CH_3OH) were supplied by Sigma-Aldrich Ireland, Ltd., Arklow, Co. Wicklow, Ireland. Beef skin gelatin (Type B) and glycerol were supplied by Sigma-Aldrich, Steinheim, Germany. All chemicals used were of analytical grade. Tea catechin (TC) (81.43%) extract containing epigallocatechin gallate (37.62%), epicatechin gallate (16.22%), epicatechin (11.51%), epigallocatechin (9.64%), catechin (4.82%) and gallocatechin gallate (1.62%) was supplied by New Kinglong Natural Products Co. Ltd, Hunan, China. Two commercial mixes of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) extracts ($GS/OL/CB_1$ and $GS/OL/CB_2$) were supplied by Envirotech Innovative Products Ltd., Dublin, Ireland. The classes of polyphenolic compounds present in the commercial mixtures were reported in Chapter 2.

6.2.2 Plant extract-enriched film manufacture

Film manufacture was based on a method described by Li *et al.* (2014) using a casting technique. Plant-derived extracts (TC, $GS/OL/CB_1$ and $GS/OL/CB_2$) were dissolved in distilled water (40 ml) to produce TC, $GS/OL/CB_1$ and $GS/OL/CB_2$ extract solutions at two concentrations; 0.7 and 1.5 mg/ml. Beef gelatin (3% w/v) and 60 ml of distilled water was added to the plant extract solutions and dissolution was aided by mechanical stirring (45 rpm) resulting in film forming solutions (FFS). Glycerol (30% w/w of gelatin) was added into the FFS and stirred (45 rpm) at 45°C (Hotplate stirrer, Wisd

Laboratory Instruments, Daihan Scientific Co. Ltd., Korea) for 30 min to obtain a homogenous solution and the solution was subjected to ultrasonic treatment (Clifton Digital Ultrasonic, Ultra 8050-H, England) for 15 min to dispel air bubbles. Finally, 10 ml of the solution was applied onto a petri dish (diameter: 92 mm) and dried in a humidity chamber (Binder GmbH, Model KBF, Germany) at 50% relative humidity (RH for 48 hr) at 25°C (Nur Hanani *et al.* 2012) to produce antioxidant active films. The same methodology was used to manufacture control gelatin films (control) without any added plant extracts.

6.2.3 Evaluation of antioxidant properties of plant extract-enriched films

Dried films (10 mg) were dissolved in distilled water (10 ml) at 45°C and centrifuged (Beckman J2-21, Beckman Instruments Inc., CA, USA) at 10,000 g for 10 min at 20°C (Wu *et al.* 2013). The supernatant was collected and used for measurement of the total phenol content and DPPH radical scavenging activity.

6.2.3.1 Total phenol content (TPC)

The total phenol content (TPC) of TC, GS/OL/CB_1 and GS/OL/CB_2 enriched active films was measured using Folin-Ciocalteu method described by Singleton & Rossi (1965) with slight modifications. Film supernatant (0.5 ml) was mixed with 1.25 ml Folin-Ciocalteu reagent (20% in distilled water) in test tubes and held at room temperature. After 5 min, 1 ml of Na_2CO_3 (15% in distilled water) was added and tubes were vortex mixed and incubated in the dark for 2 hr at room temperature. The absorbance of the coloured mixture was measured at 750 nm using a spectrophotometer (Cary 60 UV-Vis Agilent Technology, Australia) against a blank containing all reagents and distilled water. A standard curve of aqueous gallic acid equivalent (0.004 – 0.06 mg/ml) was prepared and results were expressed as milligrams (mg) of gallic acid equivalents (GAE)/g film.

6.2.3.2 DPPH radical scavenging activity (DPPH)

The DPPH free radical scavenging activity of plant extracts-enriched active films (TC, GS/OL/CB_1 and GS/OL/CB_2) was determined as described by Yen and Wu (1999) with slight modifications. Film supernatants (2 ml) were mixed with 2 ml 0.2 mM DPPH in methanol in tubes, vortex mixed and incubated in the dark for 30 min at room temperature. Absorbance measurements were recorded at 517 nm using a spectrophotometer (Cary 60). A standard curve of methanolic Trolox (0.006 – 0.06 mg/ml) was prepared and results were expressed as mg of Trolox Equivalent (TE)/g film.

6.2.4 Characterisation of plant extract-enriched active films

6.2.4.1 Film thickness

Film thickness was measured using a hand-held digital micrometer (Käfer Digital Thickness gauge, Käfer Messuhrenfabrik GmbH & Co., Villingen-Schwenningen, Germany) with 0.001 mm accuracy. Five measurements were recorded at random positions on each film. Results were based on the average of three replicates and reported in micrometres (µm).

6.2.4.2 Surface colour of plant extract-enriched active films

The surface colour of control and active films were measured using a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Japan). The chroma meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400) (Konica Minolta Sensing, Inc., Japan). The chroma meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79, a = -0.11, b = 2.69). The 'L*' value represented lightness and the 'a*' and 'b*' values

represented redness and yellowness, respectively. The total colour difference (ΔE^*) between the active and control films was calculated using following formula (Stancil and Jordan 1985):

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

A ΔE^* value of 3.0 - 6.0 for very distinct, 6.0 - 12.0 for great and a value of > 12 reflected that the colour of active film was very different from the control films (Drlange 1994).

6.2.4.3 Light transmission and opacity measurement of plant extract-enriched active films

The ultraviolet (UV) and visible light barrier properties of control and active films was measured using a spectrophotometer (Cary 60) at wavelengths ranging from 200 to 800 nm. Films were cut into a rectangular size and placed directly into the spectrophotometer test cell. An empty test cell was used as a reference. Opacity was determined according to Li *et al.* (2014) by measuring the film absorbance at 600 nm (Abs_{600}) using the spectrophotometer (Cary 60). The opacity of film was calculated using following equation:

$$\text{Opacity} = \frac{Abs_{600}}{\text{Thickness (mm)}}$$

High opacity value indicates low light transmission of the films (Li *et al.* 2014).

6.2.4.4 Contact angle measurement

The contact angle (θ) of the control and active films was measured using the sessile drop method and a Theta Lite Optical Tensiometer (Biolin Scientific, Finland). A 10 µl droplet of distilled water was deposited onto the surface of the film mounted on a glass

slide using an automatic pipette at room temperature (25°C). The drop contour, θ profile and volume of water were monitored and recorded with a digital camera at 30 frames per second for 10 second during the deposition process. Images were analysed using the OneAttension software (v 2.1) and fitted to the Young-Laplace equation. All values reported were the mean of 120 data points (twenty data points x two independent experiments x three samples x three independent drops). The surface properties of film (hydrophobic or hydrophilic) were based upon the θ value. The θ value > 90° represents a hydrophobic film surface and a θ value < 90° indicates a hydrophilic film surface (Förch *et al.* 2009).

6.2.4.5 Water vapour permeability measurement

The water vapour permeability (WVP) of films were measured using the MOCON PERMEATRAN-W Model 3/33 (Minnesota, USA) and permeability software in accordance to ASTM F-1249 (ASTM 2013). The settings used were 50% RH, continuous and no individual zero mode. An aluminium foil mask with the area of 1 cm^2 was used to fix all films in the test cells.

6.2.4.6 Oxygen permeability measurement

A MOCON OX-TRAN Model 2/21 (Minnesota, USA) and WinPermTM permeability software were used to determine the oxygen permeability (OP) of control and active films in accordance with ASTM D 3985-05 (ASTM 2011) at 50% RH using 100% oxygen (O_2) as the test gas. Setting of continuous and no individual zero mode were used. An aluminium foil mask with an area of 1 cm^2 was used to fix all films in the test cells.

6.2.4.7 Structural analysis

Structural changes of the control and active films were monitored by Fourier Transform Infrared (FTIR) Varian 660 spectrometer (Varian Resolutions, Varian Inc, Victoria, Australia) using a diamond crystal ATR Golden Gate (Specac) attachment. Transmittance spectra were recorded with 32 scans at 4 cm^{-1} resolution in a wavenumber range 500 – 4000 cm^{-1}. Three spectra were obtained for each film from three independent experiments and the average spectra were used.

6.2.5 Effect of antioxidant active films on the quality of cooked chicken breast meat

6.2.5.1 Preparation and packaging of cooked chicken breast meat

Raw chicken breast meat was supplied by Shannon Vale Foods, Clonakilty, Co. Cork, Cork, Ireland. Chicken samples were vacuum packed individually and cooked in circulating water bath (Julabo SW23-GB, Julabo Labortechnik GmBH, Seelbach, Germany) at 85°C for 45 min (internal temperature of 80°C). Following cooking, chicken samples were immediately chilled in ice water (± 0.2°C) for 30 min. Cooked chicken breast samples were subsequently sliced into 6 mm thickness using a conventional meat slicer (Scharfen G330F, Witten, Germany) and cored to produce uniform size cooked chicken breast meat samples (3.5 mm diameter and weight ~6.0 g). Cooked chicken breast samples (surface area of 9.62 mm^2) were wrapped with antioxidant active films as to ensure optimal contact between the cooked chicken and active films.

6.2.5.2 Aerobic packaging

Cooked chicken breast samples wrapped with control and plant extracts-enriched active films were overwrapped with oxygen permeable (6000 – 8000 cm^3 m^{-2} 24 h^{-1} at STP) polyvinylchloride (PVC) film (Musgrave Wholesale Partners, Dublin, Ireland) and placed

in polystyrene/ethylvinylalcohol (EVOH)/polyethylene (PE) trays and stored for up to 6 days at 4°C.

6.2.5.3 Vacuum skin packaging (VSP)

Cooked chicken breast samples wrapped with control and plant extract-enriched active films were placed in PP/EVOH/PE sealing layer black trays (190 mm x 144 mm x 30 mm; ES Plastic GmbH & Co KG, Germany) and vacuum-skin packed using the ILPRA FP Basic VG machine (Vigevano, Italy). The top film (SkinfreshTop 80 co-extruded EVOH/PE, Schur Flexible Dixie GmbH, Germany) shrink-wrapped onto the test samples was sealing layer with an oxygen transmission rate < 5 cm^3 m^{-2} d bar. The VSP samples were stored for up to 18 days at 4°C.

6.2.5.4 Colour measurement of chicken samples

The plant extract-enriched films and packaging (aerobic and VSP) were removed prior to colour measurement. The surface colour of test samples was measured using a Minolta CR-400 Chroma Meter (Konica Minolta). The chroma meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400) (Konica Minolta). The chroma meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79, a = -0.11, b = 2.69). The 'L^*' value represents lightness. Colour measurements on chicken meat samples were recorded on days 1, 3 and 6 and days 1, 6, 12, and 18 for aerobic and vacuum skin packaging, respectively.

6.2.5.5 Lipid oxidation measurement of cooked samples

Lipid oxidation was measured using the 2-thiobarbituric acid assay of Siu and Dapper (1978). The malondialdehyde content was calculated using extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde (MDA)/kg cooked chicken breast. Lipid oxidation measurement on chicken meat samples were recorded on days 1, 3 and 6 and days 1, 6, 12, and 18 for aerobic and vacuum skin packaging, respectively.

6.2.6 Statistical analysis

Statistical analysis was carried out using SPSS 22.0 for Windows (SPSS Statistical Software, Inc., Armonk, NY, USA) software package. All analyses were performed in duplicate and three independent experimental trials were carried out. One way ANOVA was used to examine data from the *in vitro* antioxidant assays (TPC and DPPH) and film characteristics (thickness, colour, contact angle, WVP, OP and opacity) of control and plant extract-enriched active films (TC, GS/OL/CB_1 and GS/OL/CB_2). One way ANOVA was used to examine data from the colour and lipid oxidation of cooked chicken breast meat wrapped in control and plant extract-enriched active films (TC, GS/OL/CB_1 and GS/OL/CB_2) experiment. A full repeated measure ANOVA was conducted to investigate the effects of TC, GS/OL/CB_1 and GS/OL/CB_2 films concentration, time and their interactions for colour and lipid oxidation measurements in chicken meat. TC, GS/OL/CB_1 and GS/OL/CB_2 films concentration represented the 'between-subjects' factor and the effect of time measured using the 'within-subject' factor. Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means. All data is presented as mean values ± standard deviation.

6.3 Results and Discussion

6.3.1 Total phenol content and DPPH radical scavenging activity of the films

The total phenol content (TPC) of TC, GS/OL/CB_1 and GS/OL/CB_2 active films (at both concentration) was significantly ($p<0.05$) higher than the controls (Table 6.1). The TPC of the active films were in the range of 3.19 to 7.90 mg GAE/g film while TPC of control films was 0.65 mg GAE/g film. The significant TPC value of the active films derived from the high TPC of the TC, GS/OL/CB_1 and GS/OL/CB_2 extracts (Chapter 5). It was observed that TPC of active films were significantly ($p<0.05$) proportional to the amount of extracts added into the films. In agreement, Wu *et al.* (2013) reported that 7 mg/ml green tea fish gelatin film (40 mg GAE/g) had higher TPC value than 3 mg/ml green tea fish gelatin films (19 mg GAE/g). At low concentration, the TPC level of TC, GS/OL/CB_1 and GS/OL/CB_2 films was similar. But at high concentration, TC and GS/OL/CB_2 films demonstrated significantly ($p<0.05$) higher TPC than GS/OL/CB_1 films.

The DPPH scavenging activities of the active films (TC, GS/OL/CB_1 and GS/OL/CB_2) was in the range of 15.47 – 40.20 mg TE/g film while the DPPH scavenging activity of control film was 5.73 mg TE/g film (Table 6.1). Both concentration of TC, GS/OL/CB_1 and GS/OL/CB_2 active films possessed significantly ($p<0.05$) greater DPPH radical scavenging abilities than control films, corroborated to good *in vitro* radical scavenging activity of all extracts reported in Chapter 5. The addition of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts improved the antioxidant property of the active films in concentration-dependent manner ($p<0.05$). Similarly, 0.01 mg/ml green tea and grape seed-fish gelatin films had 8 – 10% DPPH scavenging activities while 0.1 mg/ml of the same films produced ~90% scavenging activities (Li *et al.* 2014). The inclusion of 100 mg/g

Table 6.1 Physical and antioxidant characteristic of antioxidant active films containing tea catechin (TC) extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts (GS/OL/CB_1 and GS/OL/CB_2) at 0.7 and 1.5 mg/ml.

Treatment	Concentration (mg/ml)	TPC (mg GAE/g film)	DPPH (mg TE/g film)	Thickness (µm)	Colour differences (ΔE)
Control		0.65 ± 0.05^{a}	5.73 ± 0.54^{a}	44.63 ± 2.77^{a}	
TC	0.7	3.42 ± 0.07^{b}	26.63 ± 2.50^{c}	45.90 ± 1.37^{a}	3.81 ± 0.14^{a}
TC	1.5	7.90 ± 0.87^{d}	40.20 ± 2.51^{d}	44.20 ± 1.66^{a}	4.67 ± 0.11^{b}
GS/OL/CB_1	0.7	3.19 ± 0.20^{b}	15.93 ± 0.72^{b}	44.76 ± 0.65^{a}	13.98 ± 0.23^{e}
GS/OL/CB_1	1.5	5.04 ± 0.58^{c}	28.91 ± 2.49^{c}	45.93 ± 0.71^{a}	25.39 ± 0.02^{f}
GS/OL/CB_2	0.7	4.26 ± 0.50^{bc}	15.47 ± 1.10^{b}	44.80 ± 2.88^{a}	5.43 ± 0.17^{c}
GS/OL/CB_2	1.5	7.03 ± 0.73^{d}	29.26 ± 1.62^{c}	45.66 ± 1.36^{a}	9.68 ± 0.30^{d}

Treatment	Concentration (mg/ml)	Contact angle (°)	Water vapour permeability (WVP) (g.mm m^{-2} day^{-1} kPa^{-1})	Oxygen permeability (OP) (cm^3.mm m^{-2} day^{-1} kPa^{-1})
Control		71.36 ± 3.19^{a}	1.51 ± 0.10^{a}	0.035 ± 0.002^{a}
TC	0.7	77.94 ± 4.71^{a}	1.38 ± 0.06^{a}	0.029 ± 0.000^{ab}
TC	1.5	72.84 ± 5.24^{a}	1.08 ± 0.21^{a}	0.025 ± 0.003^{b}
GS/OL/CB_1	0.7	71.69 ± 4.04^{a}	1.33 ± 0.14^{a}	0.027 ± 0.003^{ab}
GS/OL/CB_1	1.5	72.31 ± 5.94^{a}	1.27 ± 0.24^{a}	0.032 ± 0.008^{ab}
GS/OL/CB_2	0.7	79.73 ± 0.71^{a}	1.21 ± 0.19^{a}	0.026 ± 0.003^{ab}
GS/OL/CB_2	1.5	75.97 ± 2.32^{a}	1.17 ± 0.11^{a}	0.027 ± 0.001^{ab}

^{abcdef}Within each column parameter, mean (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
TC: tea catechin film, GS/OL/CB_1 and GS/OL/CB_2: two mixtures of grape seed, olive leaf and sweet chestnut bark films.

hydrolysable chestnut tannin improved the DPPH scavenging activity (87.1%) of the pigskin gelatin films but the study did not evaluate the effect of concentration on *in vitro* antioxidant activity (Peña-Rodriguez *et al.* 2015).

TC films exhibited greater scavenging capacity as compared to the GS/OL/CB_1 and GS/OL/CB_2 films (on a comparative concentration basis). This is not surprising, as TC extract possesses excellent *in vitro* antioxidant activity as compared to GS/OL/CB_1 and GS/OL/CB_2 extracts (Chapter 5). The significant level of TPC and DPPH radical scavenging activity of the TC, GS/OL/CB_1 and GS/OL/CB_2 films suggested that the plant-derived extracts incorporated in the gelatin film remained functional after film manufacturing. Therefore, it is interesting to evaluate the potency of the active films in improving the oxidative stability of the cooked muscle foods.

6.3.2 Film characteristic of antioxidant active films

6.3.2.1 Thickness, colour, light transmission and opacity

The thickness of control and active films were in the range of 44.2 – 45.9 µm (Table 6.1). The inclusion of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts and their concentrations did not significantly affect the thickness of the active films as compared to the control films. It was reported that green tea and grape seed (1 mg/ml) containing fish skin gelatin films were significantly thicker (140.20 – 156.67 µm) than the control (123.80 µm) (Li *et al.* 2014). Similarly, Benbettaïeb *et al.* (2015) also reported that ferulic acid and tyrosol chitosan-fish gelatin films (50 mg/g) were thicker (72.2 - 76.0 µm) than the control (65.0 µm). The average thickness of 100 mg/g chestnut tannin pigskin gelatin films was 100 µm, but this study did not compare the differences between the control and active films (Peña-Rodriguez *et al.* 2015). The thickness of the active films depended on the materials and

amount utilised, the polyphenol compounds and/or plant-derived extracts incorporated and manufacturing process employed.

The magnitude of total colour differences (ΔE^*) between active films and control films were in the increasing order: TC (0.7 mg/ml) < TC (1.5 mg/ml) < GS/OL/CB_2 (0.7 mg/ml) < GS/OL/CB_2 (1.5 mg/ml) < GS/OL/CB_1 (0.7 mg/ml) < GS/OL/CB_1 (1.5 mg/ml) ($p<0.05$) (Table 6.1). The ΔE^* of both concentration of TC films was classified as very distinct, GS/OL/CB_1 films were very great different and GS/OL/CB_2 films in the range of very distinct and great different to control films. The ΔE^* of active films at 0.7 mg/ml were significantly ($p<0.05$) lower than their counterparts at 1.5 mg/ml. This suggested that the ΔE^* of active films depend on the colour and concentration of plant-derived extracts incorporated. The colour of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts was light brown, dark purple and ochra, respectively which explained why the colour of GS/OL/CB_1 films were greatly different from TC and GS/OL/CB_2 films. The literature contains no information on the colour (L^*, a^* and b^*) of gelatin films containing green tea, grape seed, olive leaf and sweet chestnut bark extracts. However, it was reported that the colour of chestnut tannin-pigskin gelatin film (100 mg/g) and green tea-chitosan film (2 mg/ml) were significantly different from their controls (Siripatrawan and Harte 2010; Peña-Rodriguez *et al.* 2015).

Transmission of UV and visible light at selected wavelength in the range of 200 and 800 nm of control and active films (TC, GS/OL/CB_1 and GS/OL/CB_2) were depicted in Table 6.2. In UV light region, active films (0.7 and 1.5 mg/ml) exhibited low light transmission, especially at 300 and 350 nm, as compared to the control. Both concentrations of GS/OL/CB_1 films reduced light transmission better than control, TC and GS/OL/CB_2 films especially at 350 and 400 nm. It was reported that green tea fish skin gelatin (3 and 7

Table 6.2 Light transmission (%) and opacity value of TC, GS/OL/CB_1 and GS/OL/CB_2 active films at 0.7 and 1.5 mg/ml.

Treatment	Concentration (mg/ml)	Wavelength (nm)								Opacity
		200	300	350	400	500	600	700	800	
Control		0.02	23.90	62.13	78.15	85.15	88.07	90.90	92.22	0.64 ± 0.04^{a}
TC	0.7	0.03	2.33	49.27	72.10	81.46	84.84	89.35	91.65	1.00 ± 0.01^{b}
TC	1.5	0.03	0.19	40.13	67.77	80.63	84.56	89.87	91.92	1.15 ± 0.03^{b}
GS/OL/CB_1	0.7	0.02	6.88	34.86	56.89	69.20	73.13	86.19	90.51	2.12 ± 0.15^{d}
GS/OL/CB_1	1.5	0.02	1.92	20.65	44.04	59.80	64.96	85.26	90.79	3.19 ± 0.19^{e}
GS/OL/CB_2	0.7	0.03	9.97	49.98	68.41	76.93	81.51	86.49	88.74	1.43 ± 0.03^{c}
GS/OL/CB_2	1.5	0.02	3.00	41.00	63.34	74.98	82.14	88.49	91.00	1.20 ± 0.01^{bc}

^{abcde}Within each column, mean (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
TC: tea catechin film, GS/OL/CB_1 and GS/OL/CB_2: two mixtures of grape seed, olive leaf and sweet chestnut bark films.

mg/ml) and grape seed (1 mg/ml) fish gelatin films greatly reduced UV light transmission (Wu *et al.* 2013; Li *et al.* 2014). The authors suggested that the incorporation of plant-derived extracts containing phenolic compounds in film matrix can efficiently prevent lipid oxidation in food system induced by UV light. Grape seed and green tea extracts contain numbers of benzene rings and hydroxyl groups which enhanced the transition of n to $\pi*$ of peptide bonds in chains of gelatin which change the light absorption in 200 to 400 nm region (Bao *et al.* 2009; Li *et al.* 2014).

The opacity of TC, CB/OL/CB_1 and CB/OL/CB_2 films (0.7 and 1.5 mg/ml) were in the range of 1.0 – 3.2, which were significantly ($p<0.05$) higher than the controls (0.6) (Table 6.2). The concentration effect was observed on the opacity of GS/OL/CB_1 films but not on TC and GS/OL/CB_2 films. At high concentration, the opacity of GS/OL/CB_1 films were significantly ($p<0.05$) higher than TC and GS/OL/CB_2 films. The present of polyphenol compounds in the film network resulted to significant increase of the opacity values as observed in gelatin films containing hydrolysable tannins (100 mg/g), green tea (1 mg/ml) and grape seed (1 mg/ml) (Li *et al.* 2014; Peña-Rodriguez *et al.* 2015). The high opacity value of GS/OL/CB_1 films reflects their good light barrier property, corresponding to their ability to reduce UV and visible light transmission (Table 6.2).

6.3.2.2 Contact angle

The contact angle (θ) is defined as the angle formed by the intersection of the liquid interface and the surface interface (Clarke *et al.* 2017). Upon deposition of a drop of distilled water on the smooth film surface, the measured θ of control and active films were in the range of 71.4 – 79.7° (Table 6.1). The θ value smaller than 90° insinuates hydrophilic nature of the surface of control and active films (Förch *et al.* 2009). The inclusion of high concentration of TC and GS/OL/CB_2 extracts decreased the surface wetting property of the

active films (not significant) but the effect was not observed for GS/OL/CB_1 extract. Benbettaïeb *et al.* (2015) explained that the surface wettability depends on the polarity/non-polarity of phenolic compounds and their interaction with polymer network during manufacturing. In spite of our research, the chitosan film containing grape seed (10 mg/ml) reduced the contact angle (46°) of the film as compared to control (53°) due to a high energy of the film which flattened the water droplet (Moradi *et al.* 2012).

6.3.2.3 Water vapour permeability

Water vapour permeability (WVP) evaluates ability of water vapour transported through a film. Low WVP means the film is less permeable to water vapour transmission. The WVP for the control and active films were in the range of 1.08 – 1.51 g.mm m^{-2} day^{-1} kPa^{-1} (Table 6.1). The inclusion of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts at both concentrations reduced the WVP of gelatin films compared to the controls, but the reduction was insignificant. The high concentration of TC active films had lower WVP as compared to the low concentration TC active films. Similarly, Li *et al.* (2014) reported grape seed (1 mg/ml) addition reduced WVP of the fish gelatin films (2.49 x 10^3 g.m m^{-2} day^{-1} kPa^{-1}) but the effect was not significant compared to the controls (2.63 x 10^3 g.m m^{-2} day^{-1} kPa^{-1}). The authors also revealed that green tea (1 mg/ml) incorporation in fish gelatin film significantly reduced WVP of the films (1.81 x 10^3 g.m m^{-2} day^{-1} kPa^{-1}). There is no literature available on the effect of the addition of olive leaf and sweet chestnut bark extracts on the WVP of film. However, Peña-Rodriguez *et al.* (2015) demonstrated that addition of 100 mg/g chestnut tannin in gelatin films increase film resistance against water uptake as compared to control. The addition of polyphenolic compounds reduce the water vapour permeability of the film presumably due to the ability of the compounds to fit into the gelatin matrix and formed cross-link through hydrogen bond or hydrophobic interaction

with reactive group of gelatin (Wu *et al.* 2013). The authors explained that this leads to the increase in cross-link formation within the network which decrease the free volume of polymer matrix and therefore limit the water vapour migration.

6.3.2.4 Oxygen permeability

The oxygen permeability (OP) of control and active films (TC, GS/OL/CB_1 and GS/OL/CB_2) were in the range of 0.025 – 0.035 cm^3.mm m^{-2} day^{-1} kPa^{-1} (Table 6.1). The inclusion of GS/OL/CB_1 and GS/OL/CB_2 extracts, at 0.7 and 1.5 mg/ml, reduced the OP of the active films as compared to the control films (but the effect was insignificant). Similarly, addition of TC reduced the OP values but only incorporation of high concentration of TC significantly reduced ($p<0.05$) film permeability against oxygen relatives to the control films. Low OP reflects film barrier against oxygen migrating through the film. Previous study showed that the inclusion of tea polyphenol (0.5 mg/ml) loaded chitosan nanoparticle in gelatin film improved film permeability against oxygen (12.87 cm^3.mm m^{-2} day^{-1} kPa^{-1}) presumably due to the protein–polyphenol complexes dispersing in the film network which compacted microstructure of the active films thus limit oxygen migration (Bao *et al.* 2009). No literature contains information pertaining the effect of grape seed, olive leaf and sweet chestnut bark extract addition on OP of gelatin film. Benbettaïeb *et al.* (2015) suggested that the addition of phenolic compounds (such as 50 mg/g tyrosol) significantly reduced OP of the chitosan-fish gelatin film possibly due to cross-linking between the compounds and polysaccharide-protein network, tighten the entire matrix and subsequently, restrict oxygen migration through the film.

6.3.2.5 Structural properties

Proteins are comprised of amino acids joined together by amide bonds (Nur Hanani *et al.* 2012). The repetition of polypeptide and protein units give rise to nine characteristic in infrared (IR) absorption bands known as amide A, B, and I−VII (Kong and Yu 2007). Figure 6.1 depicted that gelatin film containing TC, GS/OL/CB_1 and GS/OL/CB_2 extracts (0.7 and 1.5 mg/ml) had similar FTIR spectra to control beef gelatin film with slight shift of absorption bands. The active films (0.7 and 1.5 mg/ml) exhibited slightly broader peak between 3266 – 3304 cm^{-1} (amide A designates stretching vibration of N-H bands) and 3066 – 3113 cm^{-1} (amide B represents stretching of alkyl hydrogen), similar to the molecular organization of fish gelatin film containing green tea and grape seed (1 mg/ml) and hydrolysable chestnut tannin (100 mg/g) pigskin gelatin film (Li *et al.* 2014; Peña-Rodriguez *et al.* 2015). The polyphenol in green tea and grape seed extracts contain numerous O-H and C=O bands which strengthen the stretching vibration of related bands and allow formation of intramolecular and intermolecular hydrogen bonds (Li *et al.* 2014). The absorption bands for amide I, II, III and IV of active films were similar to a control at 1630, 1547, 1238 and 651 cm^{-1}, respectively. Similarly, green tea and grape seed fish gelatin film (1 mg/ml) exhibited peak from 1500 to 1675 cm^{-1} represent stretching vibration of C=O bands and bending vibration of N-H bands in amino group and more intense peak between 1000 and 1300 cm^{-1} due to abundant of C-O and C-C bands (Li *et al.* 2014). The hydrolysable chestnut tannin (100 mg/g) pigskin gelatin film exhibited no changes on peak at 1629 cm^{-1} (amide I) and slight alteration on the absorption bands at 1537 cm^{-1} (amide I) and 1243 cm^{-1} (amide IV) (Peña-Rodriguez *et al.* 2015). It can be deduced that the interaction between phenolic compounds with reactive group of protein results in slight alteration of the molecular organization and intermolecular interaction in the film matrix.

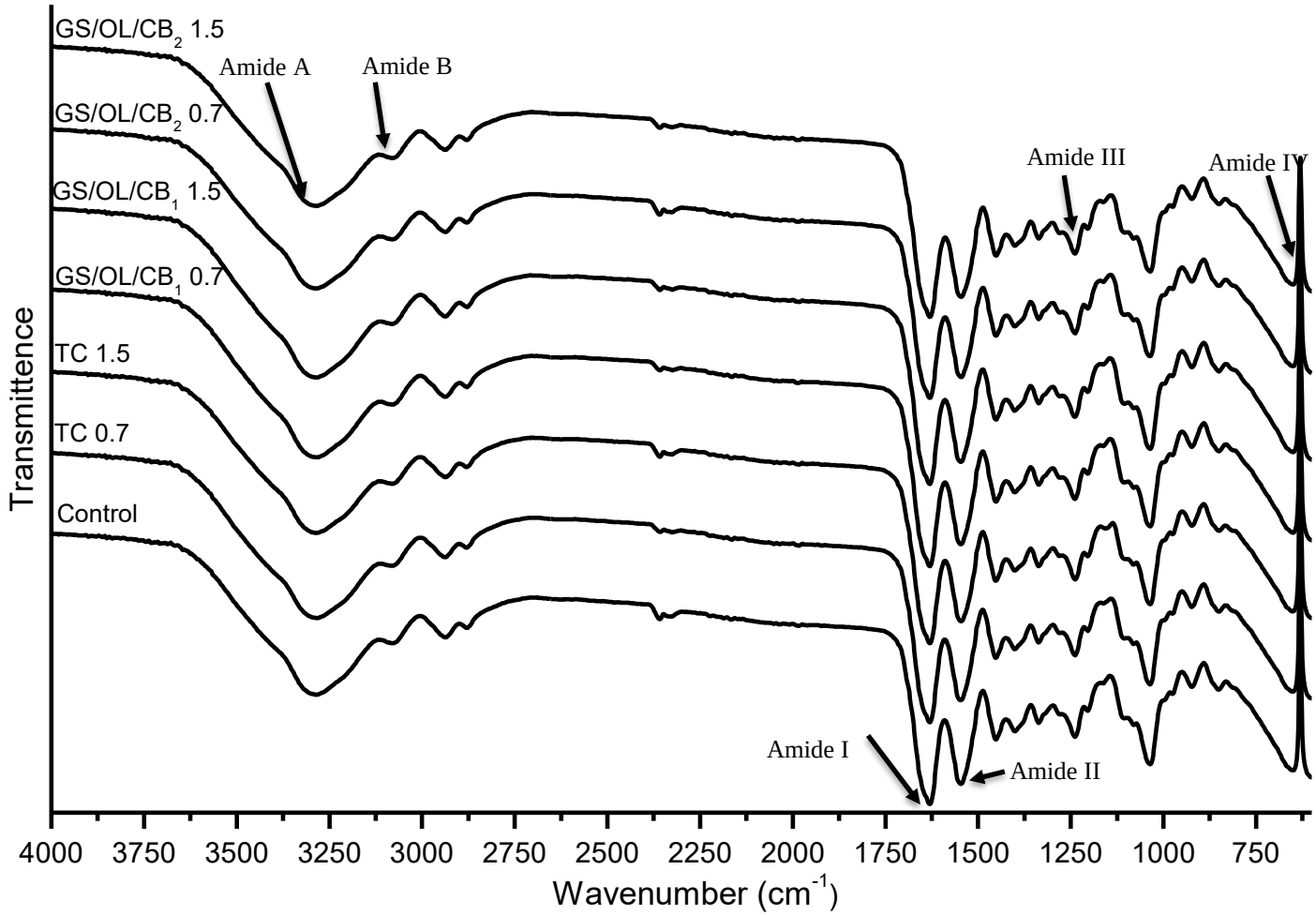


Figure 6.1 Fourier Transform Infra-Red (FTIR) spectra of a control and active gelatin films containing tea catechin (TC) extract and two mixtures of grape seed, olive leaf and sweet chestnut bark extracts ($GS/OL/CB_1$ and $GS/OL/CB_2$) at 0.7 and 1.5 mg/ml.

6.3.3 Effect of active films on the colour and oxidative stability of cooked chicken breast stored in aerobic and vacuum-skin packaging

6.3.3.1 Surface colour of cooked chicken breast

The overwrapping with TC (0.7 and 1.5 mg/ml) significantly ($p<0.05$) reduced the surface lightness (L^*) of cooked chicken samples in both aerobic and VSP storage, but the effect was not concentration-dependent (Table 6.3). This is in agreement to Chapter 5, reported that direct contact between TC active pads lowered the surface lightness of cooked chicken. According to Mitsumoto *et al.* (2005), direct addition of tea catechins (200 and 400 mg/kg) caused discoloration of cooked beef and chicken patties possibly due to the binding of iron present in myoglobin with tea catechins.

The cooked chicken samples overwrapped with 0.7 and 1.5 mg/ml GS/OL/CB_1 films reduced the surface L^* of cooked chicken in concentration-dependent manner ($p<0.05$) throughout aerobic and VSP storage, except on day 12. The discolorations of chicken samples wrapped with high concentration GS/OL/CB_1 films were greater than control and other active films. In fact, the surface of chicken samples wrapped with high concentration GS/OL/CB_1 films stored in aerobic and VSP were significantly ($p<0.05$) darker than those overwrapped with low and high concentration of TC films throughout the storage (except on day 12). This is possibly due to the migration of colour pigment and phenolic compounds from the films onto the chicken surface and potential compounds reaction with chicken component which discoloured chicken samples. In addition, direct addition of grape pomace (10 and 20 mg/kg) affect the lightness of chicken hamburger due to the natural colour of grape pomace (Sáyago-Ayerdi, Brenes and Goñi 2009). It can be inferred that the significant reduction on L^* values of chicken samples presumably due to very great colour different of GS/OL/CB_1 films as compared to control film (Table 6.1).

Table 6.3 Effect of active films on surface lightness (L^*) of cooked chicken breast stored in aerobic and vacuum skin packaging (VSP) at 4°C for up to 6 and 18 days, respectively

Treatment/ Parameter	**Concentration (mg/ml)**	**Storage at 4°C, days**						
		1		**3**	**6**		**12**	**18**
		Aerobic	**VSP**	**Aerobic**	**Aerobic**	**VSP**	**VSP**	**VSP**
Control		83.1 ± 1.3^{a}	84.0 ± 0.7^{a}	81.7 ± 1.3^{a}	81.0 ± 0.9^{a}	83.2 ± 2.0^{a}	82.2 ± 2.6^{a}	84.5 ± 0.6^{a}
TC	0.7	76.2 ± 1.7^{b}	77.0 ± 1.3^{b}	73.9 ± 1.1^{b}	71.5 ± 0.2^{b}	73.4 ± 1.5^{b}	72.8 ± 1.8^{bc}	75.7 ± 0.9^{bc}
TC	1.5	75.8 ± 1.3^{b}	76.9 ± 0.2^{b}	73.7 ± 0.9^{b}	70.5 ± 0.8^{b}	71.8 ± 2.3^{b}	71.4 ± 1.4^{bc}	72.7 ± 1.6^{c}
GS/OL/CB_1	0.7	74.6 ± 0.9^{b}	73.1 ± 3.8^{b}	73.3 ± 2.5^{b}	71.6 ± 0.7^{b}	73.7 ± 1.2^{b}	69.5 ± 6.5^{c}	74.7 ± 3.2^{c}
GS/OL/CB_1	1.5	67.3 ± 2.2^{c}	68.3 ± 1.0^{c}	67.0 ± 1.5^{c}	65.3 ± 1.6^{c}	66.2 ± 2.1^{c}	66.0 ± 3.5^{c}	65.2 ± 2.1^{d}
GS/OL/CB_2	0.7	81.8 ± 0.7^{a}	82.9 ± 0.9^{a}	81.1 ± 1.9^{a}	80.0 ± 1.4^{a}	81.8 ± 2.3^{a}	82.1 ± 0.4^{a}	80.2 ± 1.7^{ab}
GS/OL/CB_2	1.5	81.0 ± 0.7^{a}	82.4 ± 1.4^{a}	80.3 ± 1.3^{a}	79.1 ± 1.4^{a}	80.2 ± 2.2^{a}	79.8 ± 0.9^{ab}	80.8 ± 1.4^{a}

^{abcd}Within each column and storage days, mean (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.

n = 3 independent experiments, measurements recorded in duplicate.

TC: tea catechin film, GS/OL/CB_1 and GS/OL/CB_2: two mixtures of grape seed, olive leaf and sweet chestnut bark films.

Interestingly, the 0.7 and 1.5 mg/ml GS/OL/CB_2 films did not affect the surface lightness of aerobic and vacuum-skin packed samples throughout the storage. The L^* values were similar to those overwrapped with control films. Previous study also showed that GS/OL/CB_2 active pads did not affect the lightness of cooked chicken during refrigerated storage (Chapter 5).

6.3.3.2 Lipid oxidation of cooked chicken breast

The lipid oxidation (TBARS) of cooked chicken samples overwrapped with control and active films (TC, GS/OL/CB_1 and GS/OL/CB_2) increased throughout aerobic and VSP storage (Table 6.4). The cooked chicken samples overwrapped with TC, GS/OL/CB_1 and GS/OL/CB_2 films (0.7 and 1.5 mg/ml) significantly ($p<0.05$) reduced lipid oxidation of the chicken samples (range: 0.41 – 0.91 mg MDA/kg) as compared to the controls (range: 0.96 – 2.35 mg MDA/kg) during 6 days of aerobic storage. High concentration active films produced plateau effect in inhibiting lipid oxidation of cooked chicken samples. No significant reduction in the level of lipid oxidation of cooked samples was observed between different active films on day 1. On day 3, 1.5 mg/ml TC films significantly reduced lipid oxidation of aerobic samples as compared to 1.5 mg/ml GS/OL/CB_2-wrapped samples. At the end of aerobic storage, TC films (both concentration) significantly lowered the lipid oxidation as compared to sample wrapped with 0.7 mg/ml GS/OL/CB_2 films.

The high level of lipid oxidation, especially in control samples, during aerobic storage is due to the presence of oxygen. Overwrapping of cooked samples with the control and active films prevent the direct exposure of oxygen to the surface of food products, but oxygen may permeate through the films and subsequently accelerate lipid oxidation (Smiddy, Papkovskaia, *et al.* 2002). Since the incorporation of plant-derived extracts did

Table 6.4 Effect of active films on lipid oxidation (TBARS, mg MDA/kg cooked chicken meat) in cooked chicken breast stored in aerobic and vacuum skin packaging at 4°C for up to 6 and 18 days, respectively.

Treatment	**Concentration (mg/ml)**	**Storage at 4°C, days**						
		1		**3**	**6**		**12**	**18**
		Aerobic	**VSP**	**Aerobic**	**Aerobic**	**VSP**	**VSP**	**VSP**
Control		0.96 ± 0.13^{a}	0.71 ± 0.02^{a}	1.52 ± 0.17^{a}	2.35 ± 0.23^{a}	0.71 ± 0.02^{a}	1.06 ± 0.06^{a}	1.09 ± 0.24^{a}
TC	0.7	0.53 ± 0.01^{b}	0.45 ± 0.07^{b}	0.59 ± 0.07^{bc}	0.62 ± 0.01^{c}	0.39 ± 0.03^{d}	0.48 ± 0.04^{bcd}	0.51 ± 0.03^{bc}
TC	1.5	0.44 ± 0.01^{b}	0.44 ± 0.04^{b}	0.41 ± 0.01^{c}	0.61 ± 0.01^{c}	0.40 ± 0.06^{d}	0.39 ± 0.02^{d}	0.40 ± 0.02^{c}
$GS/OL/CB_1$	0.7	0.55 ± 0.02^{b}	0.38 ± 0.03^{bc}	0.64 ± 0.09^{bc}	0.87 ± 0.11^{bc}	0.58 ± 0.06^{b}	0.60 ± 0.08^{bc}	0.60 ± 0.04^{bc}
$GS/OL/CB_1$	1.5	0.52 ± 0.03^{b}	0.33 ± 0.02^{c}	0.57 ± 0.10^{bc}	0.70 ± 0.11^{bc}	0.46 ± 0.02^{cd}	0.46 ± 0.03^{cd}	0.46 ± 0.01^{bc}
$GS/OL/CB_2$	0.7	0.55 ± 0.03^{b}	0.39 ± 0.04^{bc}	0.62 ± 0.10^{bc}	0.91 ± 0.01^{b}	0.52 ± 0.01^{bc}	0.61 ± 0.05^{b}	0.71 ± 0.08^{b}
$GS/OL/CB_2$	1.5	0.56 ± 0.03^{b}	0.44 ± 0.03^{b}	0.74 ± 0.13^{b}	0.74 ± 0.04^{bc}	0.46 ± 0.01^{cd}	0.50 ± 0.05^{bcd}	0.58 ± 0.07^{bc}

[abcd]Within each column and storage days, mean (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.
n = 3 independent experiments, measurements recorded in duplicate.
TC: tea catechin film, $GS/OL/CB_1$ and $GS/OL/CB_2$: two mixtures of grape seed, olive leaf and sweet chestnut bark films
TBARS in mg MDA/kg cooked chicken meat.

not significantly reduce oxygen migration through the active films (refer Table 6.1 on OP value), thus the protective effects of the plant extract-enriched films on cooked samples may derived from the *in vitro* antioxidant activities of plant-derived extracts incorporated into the active films (Table 6.1).

In addition, active films overwrap the entire cooked chicken surface to allow optimal migration of active compounds from the films onto the surface of chicken meat samples. In fact, the changes of surface colour of chicken samples were the evident of possible migration of the extracts and/or active compounds. Previous study showed that phenolic compounds (such as tyrosol and ferulic acid) were released from the biopolymer films into the water medium depending on the interaction or cross-link between phenolic compounds and biopolymer network (Benbettaïeb *et al.* 2016). The hydrophilic nature of the film surface (Table 6.1) may contribute to the migration of the active compounds from the films onto the chicken samples (containing 68% water) in order to elicit antioxidant responses.

In VSP, both concentration of TC, GS/OL/CB_1 and GS/OL/CB_2 films significantly ($p<0.05$) lowered lipid oxidation of the cooked samples (range: 0.33 – 0.71 mg MDA/kg) as compared to the controls (range: 0.71 – 1.09 mg MDA/kg) up to 18 days storage. Initially, GS/OL/CB_1 films (1.5 mg/ml) significantly ($p<0.05$) reduced lipid oxidation as compared to chicken samples wrapped with TC (both concentration) and GS/OL/CB_2 (1.5 mg/ml) films. On day 6, both concentration of TC films significantly ($p<0.05$) lowered the level of lipid oxidation in comparison to chicken samples wrapped with low concentration of GS/OL/CB_1 and GS/OL/CB_2 films. The potency of high concentration TC films continued to significantly ($p<0.05$) protect cooked samples against lipid oxidation as compared to those overwrapped with low concentration of GS/OL/CB_1 and GS/OL/CB_2 films (day 12) and those wrapped with low concentration of GS/OL/CB_2 films (day 18).

All active films did not function in dose-dependent manner except on day 6, when 1.5 mg/ml GS/OL/CB_1 films elicited significantly ($p<0.05$) greater protection against lipid oxidation as compared to their counterpart. The level of lipid oxidation of control samples packed in vacuum skin packaging was lower than those packed aerobically due to removal of oxygen from the packaging. However, lipid oxidation still occur in vacuum-packed samples because complete elimination of oxygen from the packaging is difficult (Smiddy, Papkovskaia, *et al.* 2002) and overwrapping of cooked samples may result to small amount of oxygen trapped between the meat surface and the film (Bolumar *et al.* 2011).

The great potency of TC films in lowering lipid oxidation of aerobic and vacuum-packed samples in present study is not surprising, as 0.7 and 1.5 mg/ml TC films possessed significantly greater radical scavenging abilities as compared to the same concentration of GS/OL/CB_1 and GS/OL/CB_2 films (Table 6.1). The potency attributed to the high affinity of tea catechins for the lipid bilayers of muscle and the radical scavenging abilities of tea catechins improve the oxidative stability of cooked muscle foods (Tang *et al.* 2001a).

Although TC films demonstrated great potential as active films, it is also important to highlight the ability of GS/OL/CB_1 and GS/OL/CB_2 films to improve oxidative stability of cooked chicken. In fact, GS/OL/CB_1 films exhibited comparable effect to GS/OL/CB_2 films. Despite the fact that GS/OL/CB_1 extract had lower *in vitro* antioxidant activity as compared to GS/OL/CB_2 extract (Chapter 5) and 1.5 mg/ml GS/OL/CB_1 films had significantly lower TPC than GS/OL/CB_2 films (Table 6.1). It can be deduced that the potency of GS/OL/CB_1 film may be due to enhancement of film opacity and reduction of UV and visible light transmission (Table 6.2). The profound ability of GS/OL/CB_1 film to reduce the amount of light reaching cooked samples may lower lipid oxidation (Wu *et al.* 2013) of oxidation-sensitive foods throughout refrigerated storage.

6.4 Conclusions

Two mixtures of grape seed, olive leaf and sweet chestnut bark extract were evaluated as antioxidant agents for potential use in antioxidant active packaging to improve shelf-life parameters of cooked chicken, in comparison to tea catechin active films. The inclusion of tea catechin (TC) and two mixtures of grape seed, olive leaf and sweet chestnut bark (GS/OL/CB_1 and GS/OL/CB_2) extracts in beef gelatin films reduced UV light transmission, increased the opacity of films and slightly modified the structural properties of the protein network probably due to the interaction between polyphenol compounds with gelatin network. It is important to highlight the ability of the plant extract-enriched active films in improving the oxidative stability of cooked chicken samples stored in aerobic and vacuum-skin packaging conditions. Interestingly, GS/OL/CB_1 and GS/OL/CB_2 films also demonstrated comparable ability in lowering the lipid oxidation in chicken samples compared to TC films. The protective effect exerted by the active films was presumably due to the migration of polyphenol compounds from the films onto the surface of cooked chicken samples. This suggest the potential use of plant derived extracts in gelatin active films to improved lipid stability of cooked chicken samples.

CHAPTER 7

7 General Discussion

Polyphenolic antioxidants have been utilised in muscle foods and packaging systems to delay or inhibit lipid oxidation, thus enhancing quality and shelf-life characteristics. Plant-derived extracts, contain numerous compounds with multi-functional bioactivities such as antioxidant and antibacterial properties. In many instances, plant extracts-enriched food products are termed 'functional foods' as a result of the many bioactive compounds present in plant extracts/functional ingredients. Muscle foods are potential carriers or vehicles for such functional ingredients, resulting in superior quality meat products and economic advantages for the meat industry. The objective of the research presented in this thesis was to investigate the effect of plant-derived extracts on the quality and shelf-life of oxidation-sensitive muscle foods, such as chicken, by incorporating plant extracts into poultry diets pre-slaughter. In addition, extract functionality in active packaging applications (antioxidant active pads and films) was also examined.

Initial studies (Chapter 2) identified phenolic compounds present in commercial grape seed (GS) extract and two mixtures of grape seed (GS), olive leaf (OL) and sweet chestnut bark (CB) (GS/OL/CB_1 and GS/OL/CB_2) extracts and examined the *in vitro* antioxidant and antimicrobial activities of GS, GS/OL/CB_1 and GS/OL/CB_2 extracts. A mechanistic evaluation of the antioxidant activity and potency of the plant-derived extracts (GS, GS/OL/CB_1 and GS/OL/CB_2) in chicken (breast and thigh) muscle model systems was also carried out. The effect of plant-derived extracts on the quality and shelf-life parameters of fresh chicken meat was evaluated where plant-derived extracts were supplemented into chicken diets (Chapter 3). The antioxidant status of chicken blood plasma and the lipid stability of chicken organ (liver, heart and lung) and muscle (breast and thigh) tissues was investigated to determine the fate of supplemental antioxidants present in plant extracts. Furthermore, the potential benefits of *in vivo* supplementation of

GS extract on the quality and shelf-life parameters of cooked chicken muscle was also evaluated (Chapter 4). Plant extract mixtures (GS/OL/CB_1 and GS/OL/CB_2) was selected for use in the development of antioxidant active pads (Chapter 5) and films (Chapter 6) as both mixtures exhibited greater antioxidant potencies than the GS extract alone. The ability of extract mixture to improve shelf-life parameters of cooked chicken, in comparison to tea catechin utilising active packaging systems (pads and films) was examined (Chapter 5 and 6).

The antioxidant activities of GS/OL/CB_1 and GS/OL/CB_2 extracts i*n vitro* and in chicken muscle homogenates was greater ($p<0.05$) than GS extract alone presumably due to the higher levels and diverse range (identified using LC-Q-TOF-MS) of phenolic compounds identified in both extracts. Furthermore, both extract mixtures (GS/OL/CB_1 and GS/OL/CB_2) exhibited moderate antimicrobial activities against Gram positive bacteria while GS extract alone possessed no activity. Overall results demonstrated the potential of plant-derived extracts for use as antioxidant ingredients in chicken muscle.

In Chapter 3, chicken diets were supplemented with GS extracts at three levels (100, 200 and 300 mg/kg (GS100, GS200 and GS300, respectively)) and two extract mixtures (GS/OL/$CB_1$50 and GS/OL/$CB_2$50) at concentration of 50 mg/kg for 35 days pre-slaughter. The concentration of GS extract employed in this study was higher than both extract mixtures as the GS extract exhibited lower antioxidant activity in both test systems (*in vitro* antioxidant assays and chicken muscle homogenates) (Chapter 2). Dietary supplementation did not significantly affect total antioxidant status of the chicken blood plasma. Liver, heart and lung tissue homogenates were subjected to iron ($FeSO_4$)-induced lipid oxidation to determine potential distribution patterns of plant extract antioxidant compounds in other chicken tissues. Although results were not statistically significant ($p > 0.05$), decreased levels of lipid oxidation in the heart homogenates indicated deposition of antioxidant

compounds (GS200) in specific organ tissues. Inclusion of GS extract in chicken diets inhibited lipid oxidation in breast (300 mg/kg) and thigh muscle (100 mg/kg) homogenate systems indicating deposition of antioxidant compounds in chicken muscle tissues.

Dietary supplementation of plant-derived extracts did not significantly affect the proximate composition of raw breast and thigh muscles. The majority of shelf-life parameters (pH, cook loss, texture, microbiology and lipid oxidation), of fresh chicken breast meat stored at 4°C in aerobic and modified atmosphere packs (MAP) (40% CO_2 : 60% N_2), were not influenced by dietary supplementation and packaging formats (aerobic and MAP). Minor significant differences in colour parameters of chicken breast meat samples stored aerobically and in MAP was attributed to variation between chicken breast samples.

Sensory evaluation demonstrated that the highest concentration of GS extract supplementation (300 mg/kg) improved the sensorial attributes of chicken breast in both packaging formats. In the absence of analytical techniques to measure the level of extract compounds deposited in chicken tissues, deposition was confirmed following the observation of an antioxidant effect in chicken muscle homogenates (300 mg/kg). This study demonstrated the suitability and functionality of the GS extract utilised as a functional ingredient in chicken diets to improve chicken meat quality. Although both extract mixtures exhibited superior antioxidant capacity *in vitro* (Chapter 2), a similar response was not observed when incorporated into chicken diets. This could be due to the inclusion level (50 mg/kg) examined in chicken diets or more likely due to the biotransformation of compounds into unavailable forms after ingestion.

In chapter 4, the effect of GS extract in chicken diets (100, 200 and 300 mg/kg) on the shelf-life characteristics of cooked minced chicken breast and thigh meat patties stored in aerobic and modified atmosphere packs (MAP) (40% CO_2 : 60% N_2) at 4°C for up to 14

and 28 days, respectively was examined. Chicken thigh and breast meats vary in composition with respect to iron, lipid and PUFA profile which subsequently influences susceptibility to lipid oxidation. Salt addition and aerobic packaging (oxygen) were utilised in this study, to further promote and accelerate lipid oxidation in cooked chicken in order to examine the thermal stability of deposited antioxidant compounds from GS-enriched chicken diets. GS extract supplementation at all concentrations did not affect the surface lightness and yellowness. But, GS-enriched diets significantly ($p<0.05$) reduced the redness of cooked chicken breast patties stored in aerobically and in MAP. Low (100 mg/kg) and high concentrations (300 mg/kg) supplementation significantly ($p <0.05$) reduced redness of aerobically stored chicken thigh patties. Dietary supplementation enhanced the quality of chicken patties mediated through decreased levels of lipid oxidation in cooked chicken patties. Deposition and retention of thermally stable antioxidant compounds, which remained functional post processing, as well as refrigerated storage is important with respect to processed chicken meat products. However, the GS extract did not improve the microbiological status of cooked chicken patties. This is in agreement with results presented in Chapter 2 where the GS extract displaying no antimicrobial activity against chicken microflora using the disc diffusion assays.

Sensory evaluation demonstrated that the highest concentration of GS extract supplementation (300 mg/kg) improved the sensorial attributes of aerobically stored cooked chicken breast patties, in agreement Chapter 3 where high levels of dietary GS extract enhanced the sensorial characteristics of fresh chicken breast meat. All GS extract supplementation improved selected sensory characteristics of cooked chicken breast patties stored in modified atmosphere packaging and cooked chicken thigh patties stored in both packaging formats. Dietary supplementation of GS extract may prove useful as a strategy for replacing synthetic antioxidants in improving the oxidative stability as well as

enhancing the sensory qualities of cooked chicken breast and thigh meat. Results obtained and presented in chapters 3 and 4 highlight the potential of using GS extract in chicken diets to improve shelf-life parameters of raw and processed chicken meat and associated products.

Antioxidant active packaging is an important emerging technology to enhance the stability of oxidation-sensitive muscle foods. Active packaging technology involves the inclusion of specific compounds into packaging systems to maintain and prolong the quality and shelf-life of food products. Plant-derived extracts containing antioxidant compounds were directly incorporated into food packaging using two methods; impregnation of plant-derived extracts into food absorbent pads to produce active pads (Chapter 5), and addition of plant-derived extracts into edible gelatin film-forming solutions to produce active films (Chapter 6). Mechanisms such as the migration of antioxidant agents into food and localisation of antioxidant activity on the food surface (where high level of lipid oxidation can occur) function to inhibit lipid oxidation in packaged foods. Therefore, the potential use of active packaging to improve quality and shelf-life parameters of cooked chicken breast meat was investigated.

Plant-derived mixtures extracts (GS/OL/CB_1 and GS/OL/CB_2) were selected for use in the development of antioxidant active packaging as both mixtures exhibited greater antioxidant potencies than GS extract alone (Chapter 2). In chapter 5, the focus was on the development and characterisation of GS/OL/CB_1 and GS/OL/CB_2 active pads and their functionality to improve shelf-life parameters of cooked chicken breast in comparison to tea catehin (TC) containing active pads. TC extract, a potent natural antioxidant, was used as positive control as it contains phenolic antioxidant compound classes similar to the two plant extract mixtures.

Initially, the *in vitro* antioxidant activity (TPC, FRAP and DPPH) of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts were evaluated. *In vitro* antioxidant activity for all three assays followed the order: TC > GS/OL/CB_1 > GS/OL/CB_2 ($p<0.05$). Prior to active pad development, commercial micro-perforated low-density polyethylene (LDPE) and cellulose fibres layer absorbent pads were examined for their ability to absorb and diffuse liquid (red dye) onto agar (~98% H_2O). Diffusion of the red dye onto agar overtime suggested that pads impregnated with plant-derived extracts would behave in a similar manner, i.e.: moisture activated release and diffusion of antioxidant compounds from the pad matrix onto the food (where in contact).

The antioxidant active pads were developed by impregnating the micro-perforated LDPE-cellulose fibres layers absorbent pads with TC, GS/OL/CB_1 and GS/OL/CB_2 extracts at two concentrations (1.9 and 3.8 mg/cm^2) and dried at 25°C for 4 days until constant weight was achieved. The colour and level of impregnation of the active pads depended on the natural colour of the extracts and concentration of extracts incorporated into the pad matrix, respectively. The ability of TC, GS/OL/CB_1 and GS/OL/CB_2 extracts to function as antioxidant active pads for improving the quality (pH, colour and lipid oxidation) of cooked, sliced chicken breast meat core samples stored in aerobic and modified atmosphere packs (MAP) (40% CO_2 : 60% N_2) at 4°C for up to 7 and 28 days, respectively was evaluated.

Active pads did not affect the pH of chicken stored in both packaging formats. However, the surface lightness of cooked chicken was significantly influenced (on certain storage days) when stored on TC and GS/OL/CB_2 pads. This may be due to migration of coloured compounds from the plant-derived extracts or an interaction between extract compounds and chicken components (eg: pigments, proteins etc.). In aerobic storage, all low concentration active pads and high concentrations of TC and GS/OL/CB_1 pads

significantly ($p<0.05$) reduced lipid oxidation of cooked chicken, relative to the controls. However, only TC pads significantly ($p<0.05$) inhibited lipid oxidation in cooked chicken in a dose-dependent manner. In MAP storage, TC (3.8 mg/cm^2) and GS/OL/CB_1 (both concentrations) pads significantly ($p<0.05$) protected cooked chicken against lipid oxidation towards the end of storage. Overall results demonstrated potential for the use of GS/OL/CB_1 and GS/OL/CB_2 active pads to protect cooked chicken against lipid oxidation, in comparison to TC extract active pads. Further research is necessary to explore the possibility of incorporating other plant-derived extracts into active pads in order to improve the quality and oxidative stability of cooked chicken.

Numerous research studies have focused on the development of biodegradable packaging materials derived from agricultural, livestock and fishing waste products. Such studies aim to reduce dependency on the use of non-degradable plastic which results in serious environmental and ecological issues. Gelatin films are biodegradable and possess good mechanical and gas barrier properties. Furthermore, addition of antioxidant compounds during the manufacture of gelatin films improved the physical, mechanical and antioxidant activity of the active films. Film containing antioxidants may improve the quality of oxidation-sensitive muscle foods by inhibiting lipid oxidation due to the migration of antioxidant compounds onto the food product and reducing the film oxygen transmission rate. Thus, the development of active gelatin films containing plant-derived extracts (GS/OL/CB_1 and GS/OL/CB_2) and their ability to reduce lipid oxidation in cooked chicken was examined.

In chapter 6, antioxidant active (GS/OL/CB_1 and GS/OL/CB_2) films (0.7 and 1.5 mg/ml) were developed and characterised. Their ability to stabilise cooked chicken against lipid oxidation in comparison to TC active films was examined. Plant-derived extracts were incorporated into beef gelatin solutions (stirred and heated at 45°C for 30 min) which were

casted and dried (25°C, 50% RH) for 48 hr. The physical (thickness, colour, light transmission, opacity, contact angle, water vapour permeability (WVP), oxygen permeability (OP)), structural profile (FTIR spectrum) and antioxidant properties (TPC and DPPH) of manufactured films were measured. Incorporation of GS/OL/CB_1 and GS/OL/CB_2 extracts did not affect the thickness, surface hydrophilicity and WVP of the films in comparison to the control and TC films. However, the higher concentration (1.5 mg/ml) of the TC extract reduced ($p<0.05$) oxygen permeability of the film possibly due to the dispersion of protein-polyphenol complexes in the film network, compacting the microstructure which would limit oxygen migration (Bao *et al.* 2009). Generally, the incorporation of all extracts into gelatin films reduced UV light transmission, increase film opacity and slightly modified the structural properties (FTIR spectrum) of the protein network due to potential polyphenol-protein interactions.

Cooked, sliced chicken breast core samples were overwrapped with control and active films and stored in aerobic and vacuum-skin packaging (VSP) at 4°C for up to 6 and 18 days, respectively. Both concentrations of TC and GS/OL/CB_1 films reduced the lightness ($p<0.05$) but GS/OL/CB_2 films did not affect lightness of cooked chicken throughout aerobic and VSP storage. Overwrapping food with packaging containing plant-derived extracts discoloured chicken samples suggesting possible migration of coloured compounds from the film to the food surface. Evidently, all active films reduced levels of lipid oxidation in aerobic and vacuum-packed samples throughout refrigerated storage ($p<0.05$) compared to the control films. The antioxidant effect exerted by the active films was presumably due to the migration of polyphenol compounds from films onto the chicken surface. It is important to highlight that the significant protective effect of GS/OL/CB_1 and GS/OL/CB_2 films in stabilising lipid oxidation in cooked chicken foods was comparable to potent TC films. The active packaging study demonstrates the potential use of plant-derived

extracts as antioxidant agents in enhancing food packaging characteristics and their functionality in improving the shelf-life parameters of oxidation-sensitive muscle foods such as cooked chicken.

In summary, two mixtures of plant-derived extracts possessed greater antioxidant activities *in vitro* and in the chicken muscle homogenates, compared to the GS extract alone, presumably due to the presence of numerous phenolic compounds. Despite displaying lower antioxidant activity *in vitro*, GS extract exhibited greater protective effects against lipid oxidation *in vivo* (dietary supplementation) compared to the combination plant extract mixtures. This demonstrates that the supplemented extracts were distributed and retained in poultry tissues and remained functional in the resultant chicken meat. In addition, GS extracts not only stabilised raw chicken against oxidation, but also improved the oxidative stability of cooked chicken meat. On the other hand, the two plant extract mixtures examined exhibited potential as active antioxidant ingredients when incorporated into food packaging materials (pads and films). Both extracts enhanced the characteristics of food packaging and also the oxidative stability of cooked chicken meat. Overall, the research presented in this thesis demonstrates the suitability and functionality of the plant-derived extracts in improving the shelf-life quality of muscle foods not only through supplementation of poultry diets but also via active packaging applications.

Overall Conclusions

- Results indicated the potential use of grape seed (GS) extract alone and mixture of grape seed, olive leaf and sweet chestnut bark (GS/OL/CB_1 and GS/OL/CB_2) extracts as natural antioxidant agents for use in muscle foods such as chicken via dietary supplementation and active food packaging applications.
- Mechanistic chicken muscle model systems demonstrated the ability of the selected extracts to inhibit lipid oxidation, highlighting the potential of such extracts for use as natural antioxidant agents in chicken and poultry products.
- Grape seed (GS) extract, when supplemented into chicken diets, enhanced the lipid stability of raw and cooked chicken packaged and stored under refrigerated conditions.
- Combination mixtures of plant extracts are suitable antioxidant agents when incorporated to active food packaging systems such as active pads and films. Packaging material characteristics are also improved as a result of the addition of plant-derived extracts into packaging.

Future Research

This thesis reports preliminary investigations into the use and effect of plant-derived extracts in chicken meat via dietary supplementation and direct addition into food packaging. Evidently, the application of plant-derived extracts as antioxidant ingredients in both dietary supplementation studies and packaging systems improved the oxidative stability of chicken meat. However, more research on the use of novel or specific plant-derived extracts in muscle foods and packaging systems is necessary. Future research needs arising from the work presented in this thesis are as follows:

- Quantification of the phenolic compounds present in grape seed, olive leaf and sweet chestnut bark extracts to provide better a knowledge of the compounds contributing to antioxidant activity and potency.
- Optimisation of grape seed, olive leaf and sweet chestnut bark extracts inclusion levels into poultry diets using Response Surface Methodology prior to dietary supplementation in order to determine an optimum concentration required to enhance the shelf-life characteristics of raw and processed chicken meat.
- Comparing the effect of grape seed extract incorporation via dietary supplementation and direct addition (as antioxidant ingredients) on the shelf-life of raw and cooked chicken patties to determine optimum strategies for improving the quality of chicken meat products.
- Development of other biodegradable and non-biodegradable (such as plastic packaging) antioxidant active packaging systems suitable for use with muscle foods such as chicken meat.

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