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SPANISH SCIENCE RESEARCH COUNCIL

INSTITUTE OF FOOD SCIENCE TECHNOLOGY AND NUTRITION

INTERNATIONAL Ph.D. in

"WELFARE, BIOTECHNOLOGY AND QUALITY OF ANIMAL PRODUCTION"

(XXVII CYCLE)

Related disciplinary scientific section: 07/G1 (Scienze e Tecnologie Animali)

General Coordinator: Prof. Giuseppe Maiorano

Doctorate Thesis Title

EFFECT OF DIETARY POLYPHENOL-RICH GRAPE BY-PRODUCTS ON GROWTH PERFORMANCE, SOME PHYSIOLOGICAL PARAMETERS, MEAT AND MEAT PRODUCTS QUALITY IN CHICKENS

Ph.D. Candidate:
Dr. Maria Nardoia

Supervisors:
Prof. Donato Casamassima
Dr. Agustin Brenes Payà

Co-supervisor:
Dr. Claudia Ruiz-Capillas

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DECLARATION

I hereby declare that the thesis is based on my original work except for citations which have been duly acknowledged. I also declare that this thesis has not been previously or concurrently submitted for any degree or any other institution.

Campobasso, 18/02/2016 Dr. Maria Nardoia

_____

"Stay hungry. Stay foolish"

Steve Jobs (1955-2011)

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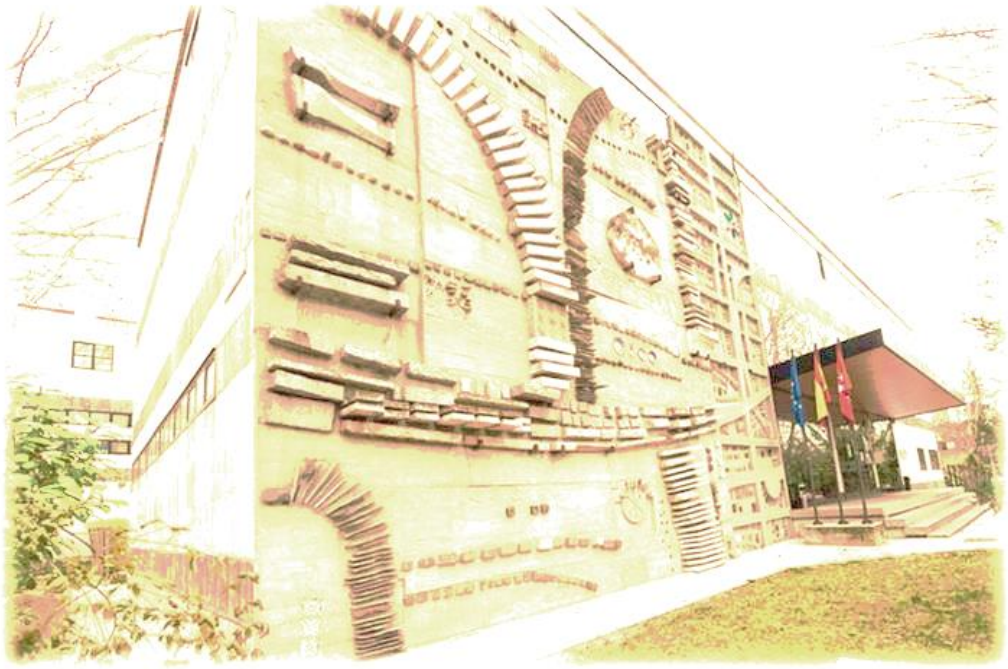
The investigation of the present doctoral thesis was carried out at the Departament of Metabolism and Nutrition and the Department of Products at the Institute of Food Science Technology and Nutrition (ICTAN) of the Spanish Science Research Council (CSIC) of Madrid. The experimental trials of this work were part of the projects AGL2012-31355/GAN, S2013/AGR-2913 (MEDGAN) and the Intramural Project 2014470E073, financed by the Spanish Ministry of Economy and Competivitiness (MINECO, Spain), Comunidad Autónoma de Madrid (CAM) and CSIC, respectively, and coordinated by Dr. Agustin Brenes Payà and Dr. Claudia Ruiz-Capillas.



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ABSTRACT

Five studies were designed to investigate the potential use grape pomace (GP), grape seeds (GS) and grape skins (SS), the major residues from wine-making industry and a good sources of polyphenols, as a cheaper but functionally equivalent product, with antioxidant activity, that could partially replace vitamin E in broiler chickens diet and be able to improve poultry performance and welfare, besides to warrant high-quality, safe and functional meat products. The studies were carried out at the Department of Metabolism and Nutrition and Department of Products of the Institute of Food Science, Technology and Nutrition (ICTAN-CSIC) of Madrid.

The first studies assessed the effect of dietary GS (15 and 30 g/kg), SS (110 g/kg) and GP (37.5 g/kg) and α-tocopheryl acetate (200 mg/kg) inclusion on performance, ileal and excreta content of total polyphenols and tannins, ileal digestibility of protein and ileal and excreta polyphenols digestibility in one hundred and eighty 21-day-old broiler chickens. In addition, plasma and meat α-tocopherol concentration and meat lipid oxidation during refrigerated storage (at 1d and 7d) were evaluated. SS diet reduced daily weight gain ($P<0.01$) and feed conversion ratio ($P<0.001$). α-T diet had no effect on ileal and excreta polyphenols content while significantly higher values were observed in GS, SS and GP groups, compared to the control birds. GS, SS and GP diets increased ileal tannins content, with no effect on excreta content. Protein digestibility significantly decreased in birds fed SS diets. Ileal polyphenols digestibility statistically increased in GS, SS and GP groups, while no differences were observed for excreta digestibility. Plasma α-tocopherol increased significantly in birds fed α-T, SS and GP diets, while γ- tocopherol only in birds fed GP diets. Meat α and γ- tocopherol levels were statistically higher in birds fed α-T diet at 1 and 7 days of storage, compared to the other groups. GP and α-T improved the stability of meat to lipid oxidation by reducing MDA values after 1 day and 7 days of storage.

The second study aimed to investigate the effect of dietary fermented (FS) and unfermented (UFS) grape skin at different doses (30 g/Kg, FS30 and UFS30, and 60 g/kg, FS60 and UFS60) and of α-tocopheryl acetate (200 mg/kg) in one hundred and fifty 21-day-old broilers. The same parameters of the previous experiment and intestinal microflora were assessed, except for plasma and meat vitamin E concentrations.

FS60 and UFS60 negatively affected growth performance and UFS60 significantly decreased protein digestibility. UFS increased ileal and excreta polyphenols content; whereas ileal and excreta tannins content increased in all experimental groups. UFS30 diet statistically increased ileal polyphenols digestibility, while both FS and UFS diets significantly increased the excreta digestibility with higher values in birds fed UFS in comparison to those fed FS. Intestinal microflora was not affected by dietary treatment. Dietary FS and UFS grape skin were not effective as vitamin E in delaying meat lipid oxidation.

The third study investigated the effect of dietary GS (40 g/kg), SS (40 g/kg) and GP (40 g/kg), different combination of GS and SS (20 g/kg GS and 20 g/kg SS; 30 g/kg GS and 10 g/kg SS; 10 g/kg GS and 30 g/kg SS) and α-tocopheryl acetate (200 mg/kg) on the same parameters evaluated in the first experiment, in addition to plasma reactive oxygen metabolites (ROMs). Performance parameters were not affected by dietary treatments. GS, SS and GP diets increased intestinal polyphenols and tannins content. GS and SS combinations decreased ileal proteins digestibility and had no effect on polyphenols digestibility, unlike the other groups. Excreta polyphenols digestibility was statistically higher in all treated groups, compared to the control and vitamin E groups. Plasma α-tocopherol was higher in birds fed α-T, SS and GP, compared to the control ones. GS, SS and GP diets increased plasma ROMs values and meat oxidative stability was improved only in the α-T group.

The fourth study was a continuation of the previous one (third study) and investigated the effect of dietary GS (40 g/kg), SS (40 g/kg) GP (40 g/ kg) and α-tocopheryl acetate (200 mg/kg) on lipid peroxidation levels (TBARS), antimicrobial capacity and physico-chemical characteristic of chicken breast meat patties during refrigerate storage (4°C) during 0, 3, 6 and 9 days. In general, the compositions of the raw meat was similar among groups. The lowest levels of polyphenols were observed in the PE (Control+vitamin E), PSS (Control+Grape skin 4%) and PGP (Control+Grape pomace 4%) patties. Polyphenols levels statistically increased during chilled storage in all samples until day 6. Increased polyphenols content was also observed in cooked patties. PE and PSS patties showed the lowest levels of LAB (lactic acid bacteria). Lower significant TBARS values were detected in PE, PSS and PGP patties. No clear

effect was observed for the color and textural characteristics and the products were acceptable for the panelists.

The last experiment investigated the effect of GS (2%) and SS (2%) direct addition on physico-chemical and sensorial properties of chicken thigh patties during refrigerated storage (4°C). GS and SS addition decreased pH values and reduced lightness, yellowness and redness values, compared with the control sample. Moreover, reduced TBARS levels were observed in relation with the higher total phenolic content also found in cooked patties. The GS and SS patties acceptability was not negatively affected. The use of grape by-products in the development of meat products could have positive effects in the human body and open interesting possibilities in the development of more healthy foods.

RIASSUNTO

Sono stati disegnati cinque studi per investigare il potenziale utilizzo di vinacce (GP), semi (GS) e buccie (SS) di uva, i principali residui derivanti dall'industira di vinificazione e ottima fonte di polifenoli, come un prodotto più economico ed equivalente, con attività antiossidante, che possa sostituire parzialmente la vitamina E come integratore del mangime di polli broiler e che sia capace, allo stesso tempo, di migliorarne le performace ed il benessere, oltre a garantire prodotti salubri e salutari. Gli esperimenti sono stati condotti presso il Dipartimento di Metabolismo e Nutrizione ed il Dipartimento di Prodotti dell'Istituto di Scienze Alimentari, Tecnologia e Nutrizione (ICTAN-CSIC) di Madrid.

Il primo esperimento ha come obiettivo quello di valutare l'effetto dell'integrazione alimentare di GS (15 and 30 g/kg), SS (110 g/kg) e GP (37.5 g/kg) e α-tocoferil acetato (200 mg/kg) sulle performance, il contenuto ileale e fecale di polifenoli e tannini, sulla digeribilità delle proteine, sulla digeribilità ileale e fecale dei polifenoli in cento ottanta polli broiler allevati fino a 21 giorni di età. Inoltre, sono stati valutati il contenuto di vitamina E in plasma e carne e l'ossidazione lipidica della carne conservata in refrigerazione (1d e 7d). La dieta SS ha ridotto l'incremento di peso giornaliero ($P<0.01$) e l'indice di conversione alimentare. La dieta integrata con vitamina E non ha avuto effetto sul contenuto ileale e fecale di polifenoli, mentre nei gruppo GS, SS e GP si sono osservati valori più alti, rispetto al gruppo controllo. Le diete integrate con GS, SS e GP hanno aumentato il contenuto ileale di tannini, senza alcun effetto, invece, su quello fecale. La digeribilità delle proteine è diminuita significativamente nei polli del gruppo SS. La digeribilità ileale dei polifenoli è statisticamente aumentata nei gruppi GS, SS e GP, mentre non si sono osservate differenze nella digeribilità fecale. L' α-tocoferolo plasmatico è stato significativamente maggiore nei polli alimentati con le diete α-T, SS e GP, mentre il γ- tocoferolo nei polli del gruppo GP.

Nella carne i livelli di α- e γ- tocoferolo sono aumentati statisticamente nei polli alimentati con vitamina E, sia ad 1d che a 7d di conservazione, rispetto al gruppo controllo. Le diete integrate con GP e vitamina E hanno miglioramento la stabilità ossidativa della carne riducendo i valori di MDA dopo 1d e 7d di conservazione.

Il secondo esperimento ha avuto come obiettivo quello di studiare l'effetto dell'integrazione alimentare con bucce di uva da vinificazione in rosso (FS) e da

vinificazione in bianco (UFS) a diverse concentrazioni (30 g/Kg, FS30 and UFS30, and 60 g/kg, FS60 and UFS60) e della vitamina E (200 mg/kg) in centocinquanta polli broiler allevati fini a 21 giorni di età. Sono stati valutati gli effetti sugli stessi parametri del precedente esperimento, in aggiunta alla microflora intestinale e fatta eccezione per la concentrazione della vitamina E plasmatica e della carne.

Le diete FS60 e UFS60 hanno influenzato negativamente le prestazioni dei polli e UFS60 ha provocato una diminuzione della digeribilità delle proteine. La dieta integrata con UFS ha aumentato il contenuto ileale e fecale di polifenoli, mentre il contenuto di tannini ileale e fecale è aumentato in tutti i gruppi sperimentali. La dieta UFS30 ha aumentato statisticamente la digeribilità ileale dei polifenoli, mentre sia la dieta integrata con FS che con UFS ha aumentato significativamente la digeribilità fecale dei polifenoli, con valori più alti nei polli alimentati con UFS rispetto a quelli alimentati con FS. La microflora intestinale non è stata influenzata dal trattamento alimentare. L'integrazione alimentare dei polli broiler con FS e UFS non è stata efficace come la vitamina E nel ridurre l'ossidazione lipidica della carne.

Il terzo studio ha valutato l'effetto dell'integrazione alimentare con GS (40 g/kg), SS (40 g/kg) e GP (40 g/kg), diverse combinazioni di GS e SS (20 g/kg GS e 20 g/kg SS; 30 g/kg GS e 10 g/kg SS; 10 g/kg GS e 30 g/kg SS) e α-tocoferil acetato (200 mg/kg) sugli stessi parametri valutati anche nel primo esperimento, oltre i metaboliti reattivi dell'ossigeno (ROMs). Le prestazioni produttive dei polli non sono state influenzate negativamente dal trattamento alimentare. Le diete integrate con GS, SS e GP hanno aumentato il contenuto ileale e fecale di polifenoli e tannini. Le diverse combinazioni di GS e SS hanno diminuito la digeribilità ileale delle proteine e non hanno avuto effetto sulla digeribilità dei polifenoli, diversamente dagli altri gruppi sperimentali. La digeribilità fecale dei polifenoli è stata statisticamente più alta in tutti i gruppi sperimentali, rispetto si gruppi controllo e alimentato con vitamina E. L' α-tocoferolo plasmatico è stato più alto nei gruppi alimentati con α-T, SS e GP, rispetto a quello controllo. Le diete GS, SS e GP hanno aumentato i ROMs del plasma, mentre la stabilità ossidativa della carne è aumentato solo nel gruppo α-T.

Il quarto esperimento è stata una continuazione del precedente (III esperimento) ed ha valutato l'effetto dell'integrazione alimentare con GS (40 g/kg), SS (40 g/kg) e GP (40 g/kg), e α-tocoferil acetato (200 mg/kg) sulla perossidazione lipidica, la capacità

antimicrobica e sulle caratteristiche fisico-chimiche di polpette formulate con la carne del petto di tali polli a 0, 3, 6 e 9 giorni di conservazione a 4°C. in generale, la composizione della carne non ha mostrato differenze tra i gruppi. I più bassi livelli di polifenoli sono stati osservati nei campioni PE (Controllo+vitamin E), PSS (Controllo+GS 4%) and PGP (Controllo+GP 4%).

Il contenuto di polifenoli è aumentato durante la conservazione fino al giorno 6. Alti valori di polifenoli sono stati rilevati anche nelle polpette dopo cottura. Le polpette PE e PSS hanno mostrato il più bassi valori di batteri acido-lattici (LAB). Valori più bassi di TBARS sono stati osservati nelle polpette PE, PSS e PGP. Non si è evidenziato un chiaro effetto per il colore e la tessitura, ed i prodotti sono risultati accettabili per panellisti durante la prova sensoriale.

L'ultimo esperimento ha valutato l'effetto dell'aggiunta diretta del 2% di GS e 2% di SS sulle caratteristiche fisico-chimiche e sulle caratteristiche sensoriali di polpette di carne di coscie di pollo, durante conservazione a 4°C. Si è osservata una diminuzione dei valori di pH, lucentezza, giallo e rosso nei campioni GS e SS, rispetto al controllo. Sono stati registrati valori più bassi di TBARS nei campioni trattati, rispetto al controllo, legati al più alto contenuto di polifenoli, mantenutosi anche dopo la cottura delle polpette. L'accettabilità delle polpette GS e SS non è stata influenzata negativamente.

L'utilizzo dei sottoprodotti dell'uva in prodotti carnei potrebbe avere effetti positivi nell'uomo ed aprire interessanti possibilità nello sviluppo di cibi più salutari.

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CHAPTER 1

GENERAL INTRODUCTION

Worldwide, production of poultry meat and eggs and global consumption of poultry products, especially poultry meat, have increased consistently over the years, and this trend is expected to continue. In all European countries, broiler meat is one of the most important types of meat within poultry meat, and Italy and Spain are among the main producers (EUROSTAT 2014). Chicken meat and product have many desirable nutritional characteristics such as low lipid contents and relatively high concentrations of polyunsaturated fatty acids (Bourre, 2005). In addition, poultry meat is relatively cheap compared to other meats and the consumer preference in food preparations are also in favor of poultry meat.

It is predicted that most increases in poultry production during the next two decades will occur in developing countries, where rapid economic growth, urbanization and higher household incomes will increase the demand for animal proteins. This growth in poultry production is having a profound effect on the demand for feed and raw materials.

Feeding cost represents the major part of total cost in poultry production, and the availability of low-priced, high-quality feeds is critical if poultry production is to remain competitive and continue to grow to meet the demand for animal protein. Minimizing the feed cost provide a compelling reason for exploring the usefulness of locally available, alternative and untraditional cheaper feed ingredients in feed formulations or improving the utilization of common feeds by using some additives. Attention, therefore, should be drawn towards the use of some local by-products, as winery by-products, that could be used as possible optional feed ingredients for poultry and could represent a novel feeding strategy.

A majority of these wastes are valuable to the pharmaceutical industry as they contain polyphenols and antioxidants that can be extracted and sold as supplements. However, when these wastes cannot be sold, a consistent method for their disposal needs to be developed. The treatment and disposal of these materials usually represent a serious environmental problem, due to a high pollution load (high content of organic substances such as sugars, tannins, polyphenols, polyalcohols, pectins and lipids) that

could cause detrimental effects on the flora and fauna of discharged zones (Louli et al., 2004; Schieber et al., 2001). Moreover, plant waste is prone to microbial spoilage; therefore, drying is necessary before further exploitation. The cost of drying, storage, and transport possess economical limitations to waste utilization. Therefore, agro-industrial waste is often utilized as fertilizer. Ideally, these by-products would be composted on-site and used as fertilizer for grape growing; unfortunately, this requires a large amount of organic matter and space for anaerobic composting, not readily available at most vineyards. As such, wine making wastes such as compressed pomace are commonly provided to feedlots so that they can be mixed with feces as an activator and composted to reduce the dry matter and adjust the nitrogen, phosphorus and potassium contents (Ferrer et al., 2001). However, not all products have the appropriate pH and dry matter required for anaerobic composting, such as wine lees. As an alternative form of disposal, they can be used as animal feeds (Nerantzis and Tartaridis, 2006; Arvanitoyannis et al., 2006).

The exploitation of grape by-products as a source of value-added products may be cost-effective and could represent an interesting advance in the maintenance of the environmental equilibrium besides an economic revaluation of the waste raw materials, therefore merits an investigation (Makris et al., 2007).

Throughout the years, poultry breeding and feeding systems have been strongly influenced by consumer's priorities and the poultry industry has changed and adapted to meet the consumer demands of meat products. Nowadays, the idea and interest in finding and using natural products like grape by-products as an alternative to synthetic substances in animal diets is also supported by the growing concerns about public health risks that have heightened consumer awareness on food safety issues, after incidents involving microbiological contamination (such as Salmonella), bovine spongiform encephalopathy (BSE), chemical residues in food (such as dioxins) and the possible consequences of technological interventions (such as genetically modified organisms). Moreover, in recent years, consumers have shown a growing need to have safer and even healthier food products obtained without using of antibiotics or synthetic chemicals and that contain bioactive or functional components which will give additional benefits to their health status (Cofrades et al., 2008), since they are

suitable vehicles for the human beings to carry and deliver the essential nutrients that may improve their wellbeing (Zhang et al., 2010).

The growing demand for food products with an appropriate content and profile of unsaturated fatty acids (UFA) is a trend that is currently influencing the production of poultry meat (Narciso-Gaytàn et al., 2010). In fact, the poultry industry is continuously focused on the development of food products with a modified fatty acid (FA) profile, searching larger n-3 FA content. Meat and eggs rich in n-3 polyunsaturated fatty acids (PUFA) are of interest because of their benefits for cardiovascular related effects (i.e. lowering triacylglycerol and cholesterol levels), inflammatory diseases, behavioral disorders, and mental issues (Hargis and Van Elswyk, 1993; Lands, 2003; Ruxton et al., 2007; Shahidi and Miraliakbari, 2005). For this reason, animal nutrition is currently evolving toward n-3 PUFA-enriched diets to improve animal fat healthfulness (Bourre, 2005) as well as the nutritional quality of lipids in animal products. However, this nutritional strategy also enhances the susceptibility to the lipoperoxidation of the meat because PUFA are highly susceptible to peroxidation. Lipid oxidation can have negative effects on the quality of meat and meat products causing changes in sensory attributes (color, texture, odor, and flavor) and nutritional quality that are highly value by consumers when selecting their purchase (Henchion et al., 2014; Rijswijk and Frewer, 2008; Troy and Kerry, 2010). In addition, lipid oxidation products have harmful biological effects and some have been related to the etiology of various neurodegenerative and cardiovascular diseases as well as different types of cancer (Cohn, 2002; Esterbauer et al., 1991; Esterbauer, 1993; Schroepfer, 2002). Thus, it is important to not only improve the nutritional value of foods but also to minimize lipid oxidation to provide healthy food products. Therefore, an increase in n-3 PUFA intake should be accompanied by diet supplementation with antioxidants.

Alpha-tocopheryl acetate, the synthetic form of α-tocopherol (vitamin E) is the most commonly used antioxidant in animal nutrition and the oxidative stability of poultry meat may be improved by its dietary addition (Avila-Ramos et al., 2012; Carreras et al., 2004). In the feed industry much higher levels of vitamin E (5 to10 times the National Research Council (NRC)-recommended amount; Leeson et al, 2007) are typically added in animal diets to achieve optimized growth performance,

reproduction, and meat quality (Kennedy et al., 1992; Coetzee and Hoffman, 2001; Xiao et al., 2011). Increased prices of vitamin E, which have resulted from the rising cost of raw materials and energy in recent years and the potential environmental impact related to its manufacturing and the growing demand for this functional antioxidant in the feed industry (Feedinfo News Service, 2008, 2009), ensure the necessity of research on cheaper but functionally equivalent products to replace vitamin E. In addition, the bioefficiency of this vitamin is limited when PUFA intake is increased (Allard et al., 1997) and added at high doses, α-tocopherol would be catabolized or excreted in feces and urine (Aurousseau, 2002) and not retained in tissues. On the basis of these observations, and considering the potential antioxidant prooxidant action of vitamin E (Mukai et al., 1993) besides its nonhomogeneous distribution between tissues, there is an increasing interest to improve the endogenous protection against negative effects of reactive oxygen species, especially in young animals, by supplementing various phytogenic antioxidant preparations containing among others flavonoids (Frank et al., 2006; Gobert et al., 2009) that are also readily accepted by both consumers and health authorities (Pokorný, 2007).

By-products obtained from the wine industry, in particular, can be a source of new value added products such as phenolic antioxidant supplements or ingredients for food processing. Using such dietary supplements as antioxidant systems in meat may also enable meat products to become vehicles of bioactive substances as delivering antioxidants (Grashorn, 2007) and plant bioactive compounds (Wallace et al., 2010) with favorable effects on human health, which can be consequently considered as "functional food". Therefore, feeding animals with novel diets, particularly ones that provide potential benefits to both the animal and consumer, is one way to create product differentiation in the meat marketplace.

Antioxidants may be added not only to the feed for the purpose of decreasing autoxidation in the meat of the animal, but also during the manufacturing process of the meat product. Adding antioxidants to the feed have the distinct advantage that they can be more evenly distributed in the meat, whereas addition after harvest requires the disruption of the meat matrix, i.e. it is only possible to add them to a manufactured product.

For more than 50 years, food industries have used several synthetic antioxidants, such as ethylenediaminetetraacetic acid (EDTA), butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl gallate, and combination of these with citric acid and alpha tocopherol and ascorbyl palmitate to control discoloration and prevent/delay lipid oxidation in restructured meat products (Boling et al., 1990; Chastain et al., 1982). Because their antioxidant activity in meat products is well established, the synthetic antioxidants are also commonly used as comparators or controls in studies investigating the antioxidant activity of spices, herbs, and plant extracts. These products are effective and generally inexpensive compared to natural products, however consumers are concerned that these chemicals can be harmful to health due to their suspected toxic properties (Madhavi and Salunkhe, 1995). This has led to the demand and commercialization of "natural food ingredients" of plant origin containing many bioactive compounds with potential health beneficial properties (Ayo et al., 2007; Cofrades et al., 2008; Rojas and Brewer, 2008).

Other two main targets in poultry production are the high growth rate and feed efficiency. In this sense, the gut is a pivotal organ system which mediates nutrient uptake and use by the animals hence, a well-functioning and healthy gut is the cornerstone of the optimum performances of the birds. The importance of intestinal microbiota for the performance of broiler chicken has been the focus of studies for decades and is clear that the balance of intestinal microbiota is important to promote the healthy gut and maximum growth performance of chickens (Kabir, 2009). During the last 50 years the use of antibiotics as growth promoters in poultry feed has been practiced worldwide (Yegani and Korver, 2008). This application has been acknowledged to improve feed efficiency and growth, because antibiotics may reduce the microbial load in the gut leading to more nutrient availability for the host (Brisbin et al., 2008). Beyond the beneficial features, the risk concerning the development of antimicrobial resistance and transference of antibiotic resistance genes from animal to human microbiota led the European Union to ban the application of antibiotics as growth promoters since 1st January 2006, which was followed by the other parts of the world including North America (Yegani and Korver, 2008). Consumers are also starting to demand that meat products be produced without the use of antibiotics as

growth promoters (Lusk et al. 2006) therefore, several alternatives to antibiotics in poultry are under investigation (Dahiya et al., 2006; Zakeri and Kashefi, 2011; Seal et al., 2013).

There are now abundant reports of plant products with bioactivities against a wide variety of pathogenic bacteria. Multiple classes of antibacterial products, including phenolic acids and polyphenols, phenanthrenes, flavonoids, and terpenoids have been described and reviewed (González-Lamothe et al., 2009).

The antimicrobial activities of grape, wine and grape-derived by-products have been widely discussed (Xia et al., 2010; Perumalla et al., 2011) and supplementing feeds with polyphenol rich grape by-products has been shown to alter the intestinal microflora of chickens (Viveros et al., 2011). Dietary polyphenols-rich grape products could be effective in increasing the growth of specific beneficial bacteria while competitively excluding certain pathogenic bacteria. As such, feeding chickens with grape by-products can be advantageous for producers (Viveros et al., 2011).

Ultimately, following the multiple and growing demand of consumers, who are more sensitive to the ethical and cultural aspects of food consumption, there is an increasing interest in researching alternative dietary strategies which can improve animal welfare as well as guarantee higher qualitative standards of food safety, nutritional, and sensory properties (Magdelaine et al., 2008) and for optimization of producers' economic gain.

AIM OF THE EXPERIMENTS

The aim of the different studies were to investigate whether the addition of sustained consumption of grape pomace and their components, seed and skin, as dietary supplements could be included in chickens diets. The especific objectives are the following:

- To assess the effect on growth performance and the ileal and fecal digestibility of protein.
- To evaluate the ileal and excreta content and the digestibility of total polyphenols and tannins.
- To estimate the susceptibility to oxidation of chicken refrigerated meat and plasma and meat α-tocopherol concentration.

- To examine changes in chicken gut microbial communities in response to the addition of grape by-products.
- To evaluate the effectiveness of grape by-products on lipid stability during refrigerated breast chicken patties for increasing the shelf life of the food.
- To incorporate the different grape by-products in chicken meat to enhance health-beneficial properties. Sensory qualities and acceptability were also evaluated.

1.1. Broiler chicken characteristics

1.1.1. Poultry production

Chickens were imported to America from Europe and Asia during the 1800's, where they were raised for eggs and meat in the backyards of most homes. Since the 1960s, the global production of poultry meat has been growing faster than that of any other meat in both developed and developing countries. In Italy were raised 115 million broiler chickens in 2008, 120 million in 2009 and 130 million in 2010 (Faostat, 2012). The significant growth in poultry (especially broiler chicken) production and consumption in the developing countries has important implications for the global trading of all meat products, as well as feeds and related inputs (Landes et al., 2004; Taha, 2003).

Broiler is a type of chicken raised specifically for meat production. Produced by fast-growing breeds with low mortality, broilers can be reared successfully in standard housing conditions on readily available, custom-formulated broiler feed rations.

They are noted for having very fast growth rates, a high feed conversion ratio, and low levels of activity. In the last 50 years, the time taken to produce a chicken weighing 2 kg has been halved, from more than 10 weeks to less than 6 weeks. Initially, selection was for greater growth rate and meat yield, in recent years there has also been selection against susceptibility to certain types of disease.

When compared with other livestock species, broiler meat production has been greater than beef and pork since 1996. Poultry meat is consumed and produced all around the world and, over the last few decades, has increased in popularity in many countries.

Chicken production is concentrated in five major areas: America (43.3% of the world's production), Asia (34.1%), Europe (15.9%), Africa (5.2%) and Oceania (1.4 %) (Food and Agriculture Organization of United Nation, FAO, 2012) data from www.thepoultrysite.com, Global Poultry Trends) (Table 1.1) Therefore, the European Union (EU) is one of the world's top producers in poultry meat and a net exporter of poultry products.

In 2014 the leading countries of European Union in poultry meat production (13.1 million tons) were Poland (13.7%), France (12.7 %), closely followed by United Kingdom (UK) (12.4%), Germany (11.4%) and Spain (11.1%) (Table 1.2).

These five countries ensure 61.3% of the EU production of poultry meat. (http://ec.europa.eu/agriculture/poultry/index_en.htm).

Table 1.1. Chicken meat production (million tonnes). Sources: FAO for chicken meat; USDA for broiler meat

Region	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013E	2014F
Indigenous chicken meat production*:											
Africa	2.8	3.3	3.4	3.7	4.0	4.2	4.5	4.6	4.6	4.7	4.7
Americas	27.1	32.7	33.7	35.0	37.5	36.9	38.6	39.8	40.1	40.6	40.6
Asia	18.6	22.4	23.5	25.0	26.2	28.0	29.2	29.9	31.4	31.8	32.1
Europe	9.3	10.9	10.8	11.6	12.1	13.3	13.9	14.6	15.4	15.9	16.5
Oceania	0.7	0.9	1.0	1.0	1.0	1.0	1.1	1.2	1.2	1.2	1.2
WORLD	58.5	70.2	72.3	76.2	80.7	83.4	87.3	90.1	92.7	94.2	95.8
Broiler meat production (million tonnes):											
WORLD	50.1	63.1	64.3	68.3	72.8	73.6	78.2	81.2	83.2	84.1	85.3

*Meat from slaughter of birds origination in a particular country, plus the meat equivalent of any such birds exported live.
E=2013 and F=2014: 5m estimates and forecasts for chicken meat.
F=2014 USDA forecast for broiler meat

Table 1.2. Poultry meat production by classes, by country, 2014 (1000 tonnes). Source: Eurostat

	Total poultry	Chicken	Turkey	Duck	Other poultry
EU-28	13000.0	10073.8	1867.6	458.7	225.3
Belgium	433.3	425.0	8.1	0.1	0.1
Bulgaria	97.9	71.5	:	22.1	4.3
Czech Republic	149.4	143.9	1.0	:	:
Denmark	143.0	142.8	0.0	0.0	0.0
Germany	1527.0	972.0	466.0	45.0	44.0
Estonia	:	:	0.0	0.0	:
Ireland	:	:	:	:	:
Greece	190.5	187.9	2.5	0.1	.01
Spain	1436.7	1209.1	158.6	4.9	64.2
France	1678.0	1047.0	358.0	234.0	41.0
Croatia	59.1	49.8	:	:	:
Italy	1242.8	919.5	309.9	3.5	9.9
Cyprus	21.7	21.5	0.2	0.0	:
Latvia	28.6	28.6	0.0	0.0	0.0
Lithuania	93.3	86.7	4.5	0.0	2.1
Luxembourg	0.0	0.0	0.0	0.0	0.0
Hungary	430.1	261.3	70.8	72.9	25.2
Malta	3.9	3.9	0.0	0.0	0.0
Netherlands	:	956.1	0.0	:	0.0
Austria	:	97.3	:	:	:
Poland	1804.1	1477.1	265.0	34.5	27.4
Portugal	295.2	248.9	35.3	9.5	1.5
Romania	345.6	:	:	0.0	0.0
Slovenia	59.8	55.6	4.2	0.0	0.0
Slovakia	:	:	:	0.0	0.0
Finland	113.4	104.6	7.3	:	1.5
Sweden	133.7	126.2	3.4	0.0	4.2
United Kingdom	1642.6	1437.6	172.9	32.0	0.0

The EU-28 broiler sector is expected to continue to grow in 2015 and 2016, benefiting from growing domestic demand and because it is cheaper and more convenient (Figure 1.1). Production is also supported by strong export demand. The overall EU-28 production in 2015 encompasses various situations, but broiler meat production is expected to increase in major EU producing countries. With a very limited rebound in the EU-28 economic situation foreseen for 2016 (which favors cheap protein sources and continued strong domestic demand for poultry meat) combined with continued export demand, EU-28 broiler production is expected to grow again in 2016, albeit at a slower rate (USDA 2015).

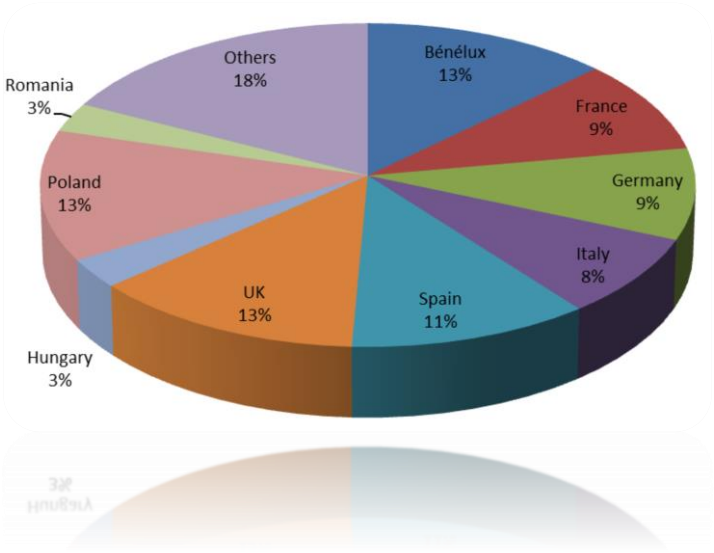


Figure 1.1. EU-27 main broiler meat producing countries (2015). Source: FAS Posts

While all sources show that total meat consumption in the EU-28 has been negatively impacted by the economic downturn, broiler meat, which is the cheapest source of protein, was less affected. However, its consumption growth is less than demographic growth, which means that per capita consumption is basically stable. Several market analyses showed that, while EU-28 consumers generally switched from beef or pork meat to broiler meat, the low income consumers reduced their protein purchases, switching to carbohydrate products (bread, pasta) with the exception of Spain, where pork meat is preferred over broiler meat. In the EU-28, sales of cheaper cuts (legs and wings) also increased faster than sales of more expensive parts, such as breasts or whole birds. This trend is expected to extend into 2016 in the absence of any economic recovery. In several EU countries, such as Germany, France and Poland, the switch to broiler meat is enhanced by the belief that it is a healthier and leaner meat and more convenient to cook and prepare. It is also considered easier to prepare for catering and restaurant use than other meats (USDA, 2015).

Therefore, reasons for the increasing demand of chicken meat are attributed to its healthy and high nutritional profile, sensory properties that make this meat very flexible for any type of home-cooking style as well as for manufacturing processed products, low costs of production, the rapid growth rate of poultry, and the introduction of many new further processed products. In addition, chicken meat is very suitable for quick and easy home-cooking, which is very important in modern societies where

people tend to spend increasingly less time on preparation of meals at home (Petracci et al., 2015).

1.1.2. Poultry gastro-intestinal tract

The digestive tract is also referred to as the gastro-intestinal or GIT tract. Whichever term is used, in birds it begins at the mouth and ends at the cloaca and has several important organs in between (Figure 1.2). In sequential order it is composed of a mouth (e beak or bill), esophagus, crop, proventriculus, ventriculus (gizzard), intestine, ceca, rectum and cloaca. The chicken GIT upper segment comprises crop, proventriculus and the gizzard. The crop is used for food storage and fermentation while digestion starts in the proventriculus, and the gizzard mechanically grinds food and acts as a microbial barrier due to its low pH. After the gizzard the food enters the small intestine that begins at the exit from the gizzard and ends at the junction of the small intestine, caeca and colon . It is made up of the duodenum (also referred to as the duodenal loop), that can be easily distinguished and the lower small intestine. The lower small intestine is composed of two parts, the jejunum and the ileum. The Meckel's diverticulum marks the end of the jejunum and the start of the ileum. The Meckel's diverticulum is formed during a chicken's embryonic stage. In the egg, the yolk sac supplies the nutrients needed for the embryo to develop and grow. Right before hatch, the yolk sac is taken into the navel cavity of the embryo. The residual tiny sac is the Meckel's diverticulum. Much of the digestion and all of the absorption of the nutrients takes place in the small intestine.

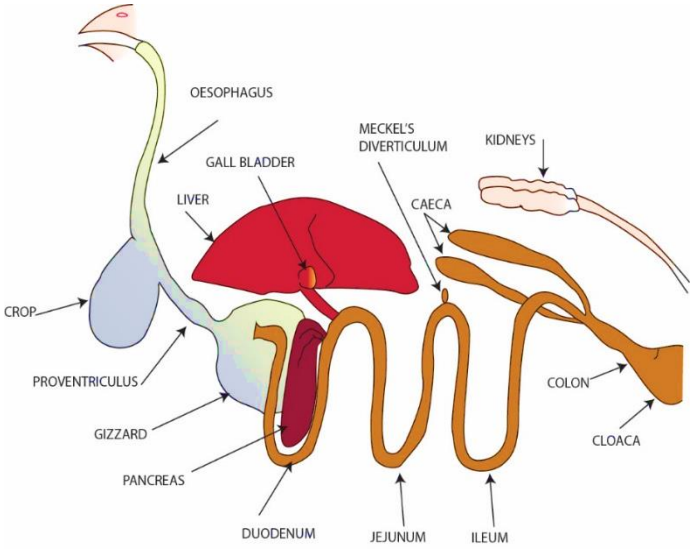


Figure 1.2. Gastro intestinal tract of chickens

Despite the name, the large intestine is shorter than the small intestine. The large intestine is where the last of the water reabsorption occurs. Sometimes this

section is referred to as the colon and the rectum (the rectum being the terminal section).

In mammals, the caecum has a negligible role in digestion; however, in birds, the caeca are an important site of fermentation (Clench and Mathias, 1995), influencing animal health and performance, and so, the caecal microbiota profiles are widely investigated. Caecal microbiota has the ability to digest foods rich in cellulose, starch and resistant polysaccharides (Clench and Mathias, 1995). The ability of many caecal culturable strains to grow on arabinoxylan, a polysaccharide from the plant cell wall, points to the important role of caeca microbiota in digesting grains and cereals (Mead, 1989). Caeca are a major site of water absorption (Clench and Mathias, 1995; Goldstein, 1989; Gasaway et al., 1976) and nutrient transport and absorption (Obst and Diamond, 1989).

The large intestine terminates in the front part of the cloaca. In the cloaca, the digestive wastes mix with wastes from the urinary system (urates). Chickens usually void fecal material as digestive waste with uric acid crystals on the outer surface that is, chickens do not urinate. The color and texture of chicken fecal material can indicate the health status of the chicken's digestive tract: the white, pasty material coating chicken fecal material is uric acid, the avian form of urine, and is normal.

Improving growth performance in chickens has long been one of the most important goals in poultry research and, in this sense the gut is a pivotal organ system which mediates nutrient uptake and use by the animals. Hence, a well-functioning and healthy gut is the cornerstone of the optimum performances of the birds. Of the factors that may be responsible for the gut health and performance of chicken, commensal microbiota in the gut, buried in the mucus layer or adhering to the digestive mucosa, seem to be very important as they may help to direct the development of gut structure and morphology, modulate the immune responses, offer protection from luminal pathogens as well as aid digestion and utilization of the nutrients (Rinttila and Apajalahti, 2013). The chicken GIT harbours a very diverse microbiota that aids in the breakdown and digestion of food and comprised over 900 species of bacteria (Apajalahti et al., 2004; Wei et al., 2013).A wide number of studies have been carried out, culturing on a variety of selective and non-selective media, to characterize and understand the chicken digestive ecosystem (Barnes et al., 1979).

Before the advent of molecular community profiling techniques, culture-dependent methods have indicated that the caecum was dominated by a high density and variability of Gram-positive bacteria, while the small intestine contained a simpler bacterial community dominated by Lactobacilli (Gong et al., 2007).However, traditional methods of classical culturing of digestive microflora only estimated 10–60 % of caecal bacteria (Salanitro et al., 1974; Barnes, 1979) and only around 45 % of chicken intestinal bacteria can be confidently assigned to a known genus (Apajalahti et al., 2004). Therefore, much of the GIT microbiota remains largely unexplored making it a major source of untapped biological potential in terms of newly identified bacteria, encoded enzyme activities and potentially probiotic strains of bacteria

With the rapid advances in the affordability and capacity of DNA sequencing technologies, the sequencing of 16S rRNA genes has rapidly replaced fingerprinting methods as the method of choice for community profiling. In fact, terminal restriction fragment length polymorphism (TRFLP) analysis, also based on 16S gene sequence analysis, indicated that the bacterial communities at different parts of the gut were different, except when comparing jejunum and duodenum (Torok et al., 2008). In a study conducted by Lu et al. (2003), this difference when comparing 16S rRNA gene libraries from the ileum and caecum was confimed. The former contained nearly 70% *Lactobacillus* spp., while the latter had only 8% *Lactobacillus* and was dominated by *Clostridiaceae*-related species. Therefore, the bacterial communities originating from different sections of the chicken GIT are so different that it has been suggested that they should be considered as separate ecosystems (van der Wielen et al., 2002). They are, however, highly connected, and they seed and influence microbiota both up and downstream in the GIT (Sklan et al., 1978). Additionally, the profiles of different GIT sections differ significantly between studies due to differences in bird genetics, sex, diet, use of antimicrobials, housing and also technique-imposed differences such as primers used, method sensitivity, DNA extraction protocol etc. It is therefore difficult to define typical microbial profiles for any sections of the GIT.

1.1.3. Chicken feed

Diet plays an important role in the overall health and performance of an animal. Furthermore, nutrition has a regulatory effect on biological processes in muscle, which can influence the quality of meat and meat products (Anderson et al., 2005). Previous research has primarily focused on the performance of animals in relation to specific dietary compounds, for example, their growth characteristics and carcass traits. However, in the past few decade researchers are focusing on the relationship between feeding bioactive compounds or specific waste products (i.e. winery by-products) and the resulting quality of the end product – meat. Recently, consumers are pushing livestock producers to feed and raise farm animals without antibiotics, hormones, or synthetic feed additives. Therefore, bioactive compounds are used in animal feedstuff to either promote immunity, aid in digestion, and/or improve growth characteristics. When incorporated into feed and food components, the above bioactive compounds have a broad range of effects in animals. Historically, plants have been used for medicinal purposes by humans to treat aliments. Thus, there is global interest in harnessing bioactive properties of plants and their secondary compounds as alternatives to chemical, drugs and growth promoters (Durmic and Blache, 2012). Antibiotic growth promoters in poultry feed increased weight gain, feed utilization, and overall well-being in birds (Gustafson and Bowen, 1997). However, the controversial subject of antibiotics in animal feed and the development of resistant bacteria led to a complete ban on antibiotics in poultry feed by the European Union, with the United States reducing and limiting the amount and type of antibiotics used (Sims et al., 2004). Today, global demands for “antibiotic-free” and “organic” poultry products are directing producers to search for alternative growth promoters. Phytogenic additives are a new class of plant-derived products that are currently being used in animal feed to improve the performance of the livestock/ flock. These compounds are usually derived from fruits, vegetables, grains, spices, herbs, seeds, bark, etc. The use of feed additives is usually subject to restrictive regulations and is generally applied by the farmer to healthy animals for nutritional purposes throughout the entire feeding period (Windisch et al., 2008).

CHAPTER 2

GRAPE BY-PRODUCTS

2.1. Wine production: global and European contributors

Grape crops are one of the main extended agro economic activities in the world with more than 60 million tons produced globally every year, and wine, the product of grape juice fermentation, is one of the most well-known alcoholic beverages distributed around the world, with 271 million of hectoliters (MhL) produced in 2014 according to the last bulletin emitted by Organisation Internationale de la Vigne et du Vin (OIV) (OIV, 2014).

There are three main species of grapes distributed in the world: European grapes (*Vitis vinifera*), North American grapes (*Vitis labrusca* and *Vitis rotundifolia*) and French hybrids (En-Qin et al., 2010). However, in some Central and Eastern European countries, *Vitis rupestris*, *Vitis berlandieri* and *Vitis amurensis* species can be found, but because of their low quality- grape they are not suitable for the winemaking process (FAO, 2013).

The EU (European Union) is the largest wine producer in the world, accounting for about two thirds of global production according to the European Commission's Directorate-General of Agriculture and Rural Development (EUROSTAT) Of the estimated 22.6 million tonnes of grapes produced in the EU-28 in 2014, the vast majority (93 %) was destined for wine production. Italy, Spain and France were the principal wine grape producers in the EU (Figure 2.1).

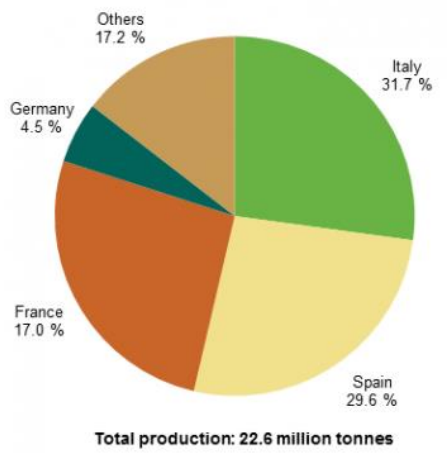


Figure 2.1. Production of grapes for wine use by main producing EU Member States, 2014 (% of EU-28 total harvested production-tonnes) Source: Eurostat (apro_acs_a)

According to its statistics databases of OIV (intergovernmental organization) the world wine production reached 271 MhL in 2014, including the following top five wine global producers: France (46.2 MhL), Italy (44.4 MhL), Spain (37 MhL), USA (22.5 MhL) and finally Argentine (15.2 MhL) (OIV, 2014).

The international scene until 2012 for countries such as China, Chile, Australia and South Africa was greatly positive once they have experimented an increasing (from 41 % for China to over 88 % for Chile) in the total amount of wine produced between 2000 and 2012 (OIV, 2012). On the other hand, countries which were commonly recognized as references because of the high quality of their wines and the quantities produced annually, seems to have decreasing perspectives for the future. This is the case of France, Italy and Spain, which have experimented a declining tendency (28, 22, and 27 %, respectively), considering the same period of time (200-2012) in terms of wine production (OIV, 2012).

In 2014 Italy's wine production, estimated at 4.4 billion liters, was 15 percent less than the previous campaign (5.2 billion liters) and 7 percent below the five-year average, as a result of a rainy summer.

According to the Spanish Ministry of Agriculture, Food, and Environment (MAGRAMA), Spain's 2014 wine production, estimated at 4.2 billion liters, was 22.3 percent lower than the previous record campaign (5.3 billion liters), because of adverse weather conditions during the harvesting period.

The OIV also shared the ranking for the countries which actively participate in the global wine production (Table 2.1)

Table 2.1 Wine production (1000 hL excluding juice and musts). Adapted from OIV (2014)

Country	2010	2011	2012	2013	2014	Ranking 2014
France	44.381	50.757	41.584	42.004	46.151	1
Italy	48.525	42.772	45.616	52.429	44.424	2
Spain	35.353	33.397	31.123	45.650	37.000	3
United states	20.890	19.140	21.740	23.500	22.500	4
Argentina	16.250	15.473	11.780	14.984	15.200	5
Australia	11.420	11.180	12.260	12.310	15.560	6
China	13.000	13.200	13.810	11.780	11.178	7
South Africa	9.327	9.725	10.568	10.980	11.420	8
Chile	8.844	10.646	12.554	12.845	10.029	9
Germany	6.906	9.132	9.012	8.409	9.725	10
Portugal	7.148	5.622	6.327	6.238	5.886	11
Romania	3.287	4.058	3.311	5.113	4.093	12
New Zeland	1.900	2.350	1.940	2.480	3.200	13
Greece	2.950	2.750	3.115	3.343	2.900	14
Brasil	2.459	3.460	2.967	2.710	2.810	15

2.2. Main by-products derived from winery industry

During winemaking steps significant amounts of waste, in their majority solids are also generated (Figure 2.2). The major residues from wine-making activity are represented by: organic wastes (grape pomace, containing seeds, pulp and skins, grape stems, and grape leaves which is separated from the juice in the pressing step (white wine fermentations), or from the wine (in red wine fermentations), wastewater, emission of greenhouse gases (CO_2, volatile organic compounds, *etc*.), and inorganic wastes (diatomaceous earth, bentonite clay, and perlite) (Oliveira et al., 2013). In this regard, it is estimated that in Europe alone, 14.5 million tons of grape by-products are produced annually (Chouchouli et al., 2013).

Figure 2.2. The solid remains of grape: stems, seeds, and skins

After grape juice extraction the remaining solid wastes are currently not valued as highly profitable waste, being mainly directed to composting or discarded in open areas potentially causing environmental problems (Rondeau et al., 2013). Large amounts of pomace (also called grape marc) are produced during a short period of harvesting (August to October), which increases the concentration of pomace per area of landfill. Waste contains significant amounts of organic species (such as sugars, phenolics, polyalcohols, pectins and lipids) with high chemical and biological oxygen demand and is therefore recognized as an environmental pollulant (Lafka et al., 2007).

Large scale production of wines and increased awareness of the potential environmental consequences of inappropriate disposal of winemaking by-products have resulted in the territorial authorities of many mayor wine-producing countries introducing regulations to minimize the negative effects (European Council Regulation (EC) 479/2008) The increasing demand for environment-friendly industrial production in addition to the challenge for gaining operational efficiency and minimizing by-product treatment cost in the wine industry should encourage wine industries to investigate the novel technologies for waste treatment and to identify other value-added products.

The valorization and some industrial uses currently under investigation for winemaking by-products include use as animal feed, as possible nutritive ingredients for value added products, in the production of citric acid, and the use of anthocyanins from grape skins as colorants (Lu et al., 1998). Winery by-products has also been used as a fertilizer, though not very successfully (Su and Silva, 2006). The use of these by-products as fertilizers, in fact, may lead to plant germination problems, due to their high content in phenolic compounds (Kammerer et al., 2004). Pomace has also been used in the production of traditional foods such as molasses and vinegar (Ozkan et al., 2004). In certain cases, grape pomace (mainly the seeds) are used in wood adhesives extractive processes (Ping et al., 2011). The most recent and innovate application is associated to a new pesticide, namely “phytosanitary bioproducts” used for the control of the incidence of diseases in some crops (Benouaret et al., 2014; Goupil et al., 2012).

By-products obtained from the juice and wine industry contain valuable biologically active compounds, a large part of which is polyphenols. It has been reported that polyphenols in grape pomace have a higher antioxidant capacity than in

wine (Su and Silva, 2006). These phenolic compounds have an extremely high market value as food additives, nutraceuticals and cosmeceuticals, due to their biological activity. A variety of health-promoting products obtained from by-products of the grape and wine industry are being introduced into the market (Gomez-Plaza et al., 2006) for prevention of cardiovascular disease, cancers, and other diseases (Figure 2.3).

Figure 2.3. Commercial nutrition supplements and cosmetics from grape extracts.

2.3. Winery By-products: general composition

2.3.1. Grape pomace

Grape pomace is a major by-product of wine making (Figure 2.4) and is obtained after grape processing into juice or wine by means of separating the liquid product from the solid residues. It consists of different amounts of grape skin, pulp, seeds, and, if not removed, stems.Itis estimated that around 20% of the total weight of grapes used for wine is made up of grape pomace. According to Cabanis and Flanzy (2003), for every 100 kg of grape marc there is 70 to 80 kg of grape skins, 15 to 25 kg of seeds and 2 to 3 kg of stems.

Concerning the general composition of grape pomace, the moisture percentage varies from 50% to 72% depending on the grape variety considered and it ripening state. The insoluble residues from this material have a lignin content ranging from 16.8% to 24.2% and a protein content lower than 4%. In respect to proteins, glutamic acid is the major amino acid along with limited lysine, tryptophan and sulfur-containing amino acids (Valiente et al., 1995) Glucose is the major soluble sugar in

grape pomace (Bravo and Saura-Calixto, 1998) which varies by the extraction degree of winemaking (Llobera et al., 2007).

Some studies pointed out that pectin and condensed tannin can be considered as part of dietary fiber, in which branched pectin represents as one-third of carbohydrate (uronic acid as rhamnose, arabinose and galactose) in soluble fiber fraction (Llobera et al., 2007) whereas condensed tannin is related to the resisted protein in insoluble fiber fraction by the protein-binding capacity (Bravo and Saura-Calixto, 1998). In general, peptic substances are the main polymer-type constituent of the cell walls present in grape pomaces, ranging from 37% to 54% of cell wall posysaccharides. Cellulose is the second type of cell wall polysaccharides in abundance in grape pomaces, varying from 27% to 37% (González-Centeno et al., 2010).

Figure 2.4. Grape pomace

2.3.2. Grape skins

Grape skins represent about 5-10% of the total dry weight of the grape berry and act as a hydrophobic barrier to protect the grapes from physical and climatic

injuries, dehydration, fungal infection and climatic injuries and UV light (Pinelo et al., 2006) (Figure 2.5 and 2.6).

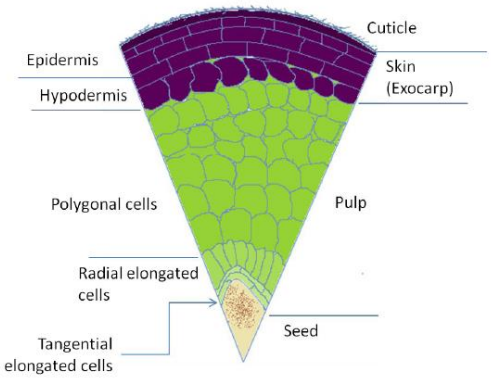


Figure 2.5. Schematical morphology of different grape cells adapted from Ribreau-Gayon, et al. (2004).

Skin cell walls are characterized by the presence of cutin (over 15% of dry weight) the hydroxylated fatty acid which compose the outermost layer, the cuticle, and by insoluble proanthocyanidins (~15%); represent 50% of the dry weight with predominance of cellulose, pectins, hemicelluloses, xiloglucans, arabinoxilans, and manans (Pellerin and Cabanis, 2003), followed by proteins (18.8%), sugars (mainly glucose and fructose, totally 14%) and ash (7.8%). No lignin was detected in grape skins (Mendes et al., 2013). High ash in skins is characterized as potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) (MartinCarron et al., 1997).

The internal layer, the hypodermis, which is the layer closest to the pulp, and which is composed of several cell layers contains most of the phenolics in grape skin (Lecas and Brillouet, 1994).

Figure 2.6. Grape skins

2.3.3. Grape seeds

When considering the separate fractions of grape pomace (seed andskin), the relative proportion of seeds ranges from 38% to 52% of the dry material and account for approximately 17% of the weight of fresh grape pomace (Ghafoor et al., 2009; Fernandes et al., 2013; Toscano et al., 2013) (Figure 2.7). The information available on the composition of grape seeds (*w*/*w*) point out the content of up to 40% fiber, 16% essential oil, 11% protein, 7% complex phenolic compounds like tannins, and other substances like sugars and minerals (Campos et al., 2008).

Phosphorus (P) is the major mineral in the seeds (Saunders et al., 1982). Grape seeds have abundant fat, mostly linoleic acid, followed by oleic, palmitic, stearic and myristic acids (Bravo and Saura-Calixto, 1998)

Figure 2.7. Grape seeds

The chemical composition of grape pomace, seed and skin have been assessed by several authors, as showed in the table below (Table 2.2).

Table 2.1. Chemical composition of grape pomace, seed and skin (g/100g as fed basis). Based of data reported by Brenes et al. (2016).

	Grape pomace	Seed	Skin
Dry matter	90-93	91-93	81-93
Protein	11.2-13.8	9.3-14.6	11.0-13.8
Fat	5.6-11.7	9.5-11.1	3.2-6.3
Ash	2.4-5.8	2.9	6.2-7.5
Fiber	32.5-56.3	41.4	30.6
Neutral detergent fiber	54.2-70.8	50.3-67.0	24.3-70.4
Acid detergent fiber	48-70.4	45.4-57.0	19.3-49.0
Acid detergent lignin	30.7-47.5	21.4-43.7	28.3-43.7
Condensed tannins			
Free	1.6-3.8		
Fiber-bound	1.9-3.4		
Protein-bound	5.6-13.1		
Total	9.1-20.3		
Minerals			
Ca	0.5-0.7	0.5-0.7	4.1-7.0
P	0.2-0.3	0.2-0.4	2.3-2.9
Fe[a]	64-185	45-120	117-398
Cu[a]	65-124	6.4-20	23-124
Zn[a]	18-12	9.5-15.0	18-12
Mn[a]	13-17	11.3-21	13-17
Vitamin E[a]		4.0-22.8	

2.3.4. Grape stems

In traditional wine making, stems were often left with grapes during crushing, pressing, and even during fermentation, especially for the production of red wine (Jackson, 2008). In fact, grape clusters or grape stems constitute a residue of the winery industry (Figure 2.8) partially applied as a source of astringent compounds, mainly represented by proanthocyanidins (LLobera and Cañellas, 2007). However, the modern trend is to remove this material before the vinification steps in order to minimize the excessive uptake of phenols and lipids from vine parts (Jackson, 2008) and to avoid an excessive astringency of the wine or a negative effect on the organoleptic characteristics.

The quantity of stems varies between 1.4% and 7.0% of the raw matter processed (Souquet et al., 2000). Currently, the commercial value of grape stems is low, being mainly used as animal feed or soil amendments. The average moisture percentage of grape stems has been reported as ranging from 55% to 80%, with the

higher variability attributed to the grape variety. The grape stem is rich in dietary fiber which constitute up to 77% of its dry matter mainly neutral sugars and lignin (43.4% and 31.6% of the dry matter, respectively) (LLobera and Cañellas, 2007). LLobera and Cañellas (2007) found that the lignin in the grape stem contains important amounts of condensed tannins. Concerning the carbohydrate composition, cellulose is the predominant component followed by pectin (Gonzalez-Centeno et al., 2010).

Figure 2.8. Grape stems

2.4. Phenolic compounds in grape by-products

2.4.1. Structure and classes of polyphenols

Phenolic compounds are organic chemicals characterized by the presence of at least one aromatic ring with one or more hydroxyl groups attached and can be classified in different ways because they are constituted in a large number of heterogeneous structures that range from simple molecules to highly polymerized compounds. According to their carbon chain, phenolic compounds can be divided into 16 major classes (Harborne, 1989). The term “polyphenols” should be used to define phenolic compounds with more than one phenolic ring and devoid of any nitrogen-based functional group in their most basic structural expression (Quideau et al., 2011).

That definition leaves out all monophenolic structures, such as hydroxytyrisol or gallic acid, which can be either metabolites of polyphenols or biogenetic precursors (Quideau et al., 2011) and can share with polyphenols many of their proprieties and characteristics (Dixon, 2004), being thus generally related with the polyphenol research and colloquially included under the polyphenol term. Polyphenols are classified into families according to their carbon atoms-ranging from simple small single aromatic-ring structures to the complex and weighty condensed tannins (Figure 2.9) (Gonzalez-Castejon and Rodriguez-Casado, 2011; Harbone, 1989; Seabra et al., 2006).

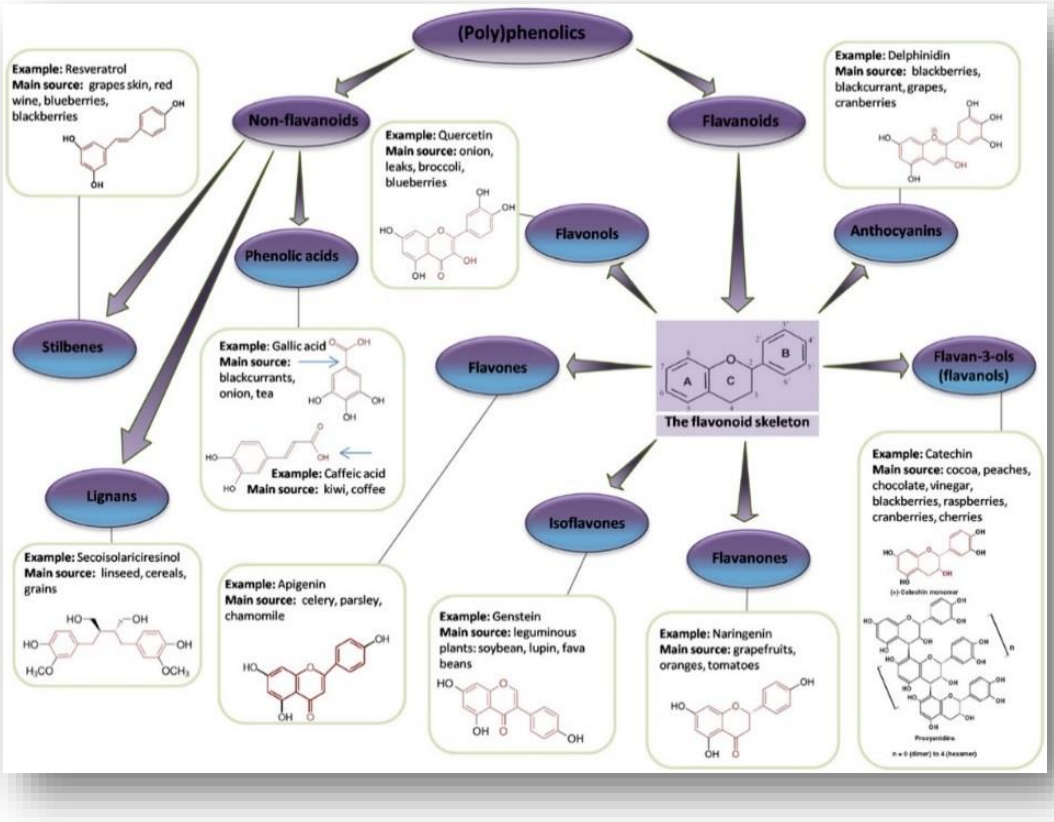


Figure 2.9. Classification of polyphenols

Following the flavonoids and non-flavonoids classification proposed by Crozier et al. (2009), dietary non-flavonoids are characterized by phenolic acids that possess one carboxylic acid functional group and are divided into hydroxycinnamic acids and hydroxybenzoic acids. The hydroxybenzoic acids are more common than hydroxycinnamic acids, and they mainly include gallic acid, as the precursor of

hydrolysable tannins, hydrixycinammates (C_6-C_3) and their conjugated derivatives, and stilbens (C_6-C_2-C_6), *p*-coumaric, caffeic, chlorogenic acid, ferulic and sinapic acids. These acids are rarely found in the free form, except in food that undergone freezing, sterilization or fermentation. The bound forms are glycosylated derivatives or esters of quinic acid, shikimic acid and tartaric acid (Vermerris and Nicholson, 2006). Nonetheless, this classification omits a sub-class of phenolic compounds named lignans, a phenolic family formed by two phenylpropanoid units linked by a hydrogen bridge, these being the monomeric and dimeric forms of hydroxycinammic acid and cynammic alcohol (Chesson et al., 1997).

Dietary flavonoids are the most numerous, widespread and best studied phenolic compounds. Flavonoids are characterized by a C_{15} phenylchromane core, composed of two aromatic rings linked by a three carbon bridge (C_6-C_2-C_6) (Crozier et al., 2009; Passamonti et al., 2009) and are sub-classified into sevensubclasses: flavonols (e.g. quercitin), flavanols (e.g. catechin, epicatechin), anthocyanins (e.g. cyanidin-3-O-glucoside), flavones (e.g., luteolin and apigenin), flavanones (e.g. narinigenin), flavanonols (e.g. taxifolin), and isoflavones (e.g. genistein or daidzein) (Bravo, 1998; Hallman and Katan, 1997; Harborne and Baxter, 1999; Williams et al., 2004) which are sometimes classified into an independent sub-category apart from flavonoids (Gonzalez-Castejon and Rodriguez-Casado, 2011) (Figure 2.10). Moreover, most flavonoids in foods are conjugated to a carbohydrate moiety, representing a wide range of combinations depending on the flavonoid, its linkage and the linked mono- and disaccharide (Passamonti et al., 2009).

The majority of flavonoids present a hydroxylation pattern, usually in 4', 5- and 7- position, or glycosilation pattern that reflects a biological strategy in plant cells to increase their water solubility. The presence of methyl groups or isopentyl units may give a lipophilic character to flavonoid molecules (Crozier et al., 2009).

Finally, tannins are complex phenolic compounds of high molecular weight and are defined as either galloyl esters and their derivatives, in which galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores (gallotannins, ellagitannins and complex tannins, respectively), or they are oligomeric and polymeric proanthocyanidins that can possess different interflavanyl coupling and substitution patterns (condensed tannins) (Khanbabaee and Van Ree, 2001). They can

be classified into three groups: condensed tannins, complex tannins and hydrolysable tannins, which include gallotannins and ellagitannins (Jankun et al., 1997). With more detail, condensed tannins are constituted by subunits of flavan-3-ols monomers with a polyol core (referring to a compound with multiple hydroxyl groups) substituted by 10-12 gallic acid residues; ellagitannins are also hydrolysable tannins, complex (poly)phenolics that can be degraded into smaller units, mainly sugars and phenolic acids, derived from pentagalloylglucose but, unlike gallotannins, they contain additional C-C bonds between adjacent galloyl moieties in the pentagalloylglucose molecule and complex tannins are defined as tannins in which a catechin unit is bound glycosidically to either a gallotannin or an ellagitannin unit (Wilfred and Nicholson, 2006).

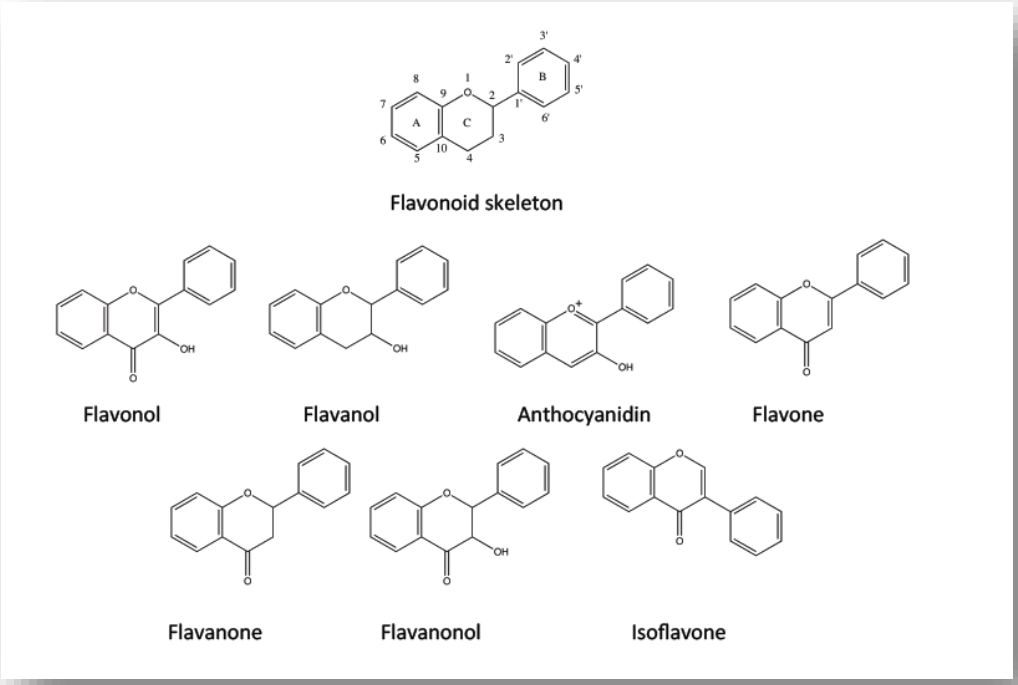


Figure 2.10. Basic structural scheleton of flavonoids

Finally, as to the location in the plant (free in the soluble fraction of cell or bound to compounds of cell wall), together with the chemical structure of these substances, phenolic compounds may also be classified as: *soluble* (such as simple phenol, flavonoids and tannins of low and medium molecular weight not bound to

membranes compounds) and *insoluble* (essentially constituted by condensed tannins, phenolic acids and other phenolic compounds of low molecular weight bound to cell wall polysaccharides or proteins forming insoluble stable complexes). This classification is useful from the nutritional viewpoint, to the extent that the metabolic fate in the gastrointestinal tract and the physiological effects of each group will depend largely on their solubility characteristics. Insoluble phenolic compounds are not digested and may be partially or fully recovered quantitatively in the feces, while a part of the soluble can cross the intestinal barrier and be found in the blood, unchanged or as metabolites (Sánchez-Moreno, 2002).

2.4.2. Chemical characterization of polyphenols

The biological properties of polyphenols and their health benefits have intensified research efforts to discover and utilise methods for the extraction, separation and identification of these compounds from natural sources. Despite a great number of investigations, the separation and quantification of various polyphenolics remain difficult, especially the simultaneous determination of their different structural groups. Quantification of phenolic compounds in plant materials is influenced by their chemical nature, the extraction method employed, sample particle size, storage time and conditions, as well as assay method, selection of standards and presence of interfering substances such as waxes, fats, terpenes and chlorophylls. A number of spectrophotometric methods have been developed for quantification of plant phenolics. These assays are based on different principles and can be classified as either those which determine total phenolic content or those quantifying a specific group of class of phenolic compounds (Ignat et al., 2011; Naczk & Shahidi, 2006). The Folin–Ciocalteu (FC) assay is widely used for determination of total phenolics. This assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid reagent to form blue coloured complexes that are determined spectrophotometrically at 760 nm (Dai & Mumper, 2010; Singleton et al., 1999).

The FC assay is operationally simple, reproducible and convenient since the reagent is commercially available, the procedure is rather standardized, and the absorption of the product at a long-wavelength minimizes interferences from the

sample matrix. Nevertheless, the original assay is time-consuming (2 h) which makes its implementation for routine analysis difficult. Moreover, it is performed in aqueous phase, thus it is not applicable for lipophilic compounds/matrices.

However, due to interferences created by factors like the nature of the dissolvent, the pH value and the presence in the sample of other compounds that absorb UV-VIS, such as fats, vitamins and amino acids, these methods are not very accurate. In recent years, most work on the analysis of polyphenols has been based on the use of methods of chromatographic separation (particularly high-performance liquid chromatography or HPLC), followed by structural characterization using mass spectrometry (MS). This methodology (HPLC-MS) is currently used to quantify extractable polyphenolic compounds. However, the quantification of more complex compounds, such as non-extractable polyphenols, continues to be based on the use of less accurate spectrophotometric

2.4.3. Polyphenolic compounds of grape by-products

The various groups of phenolic compounds present in winery by-products are not uniformly distributed in the plant material, being distributed within subcellular compartments (Pridham et al., 1960; Bengoechea et al., 1997) as part of the different metabolic pathways involved in the plant cell defense to stress and pathogens (Naczk et al., 2004). The average distribution of polyphenolic compounds in grape berries is about 1% in the pulp, 5% in the skin, and approximately 62% in the seeds (Thorngate and Singleton 1994) (Figure 2.11)

Considerable contents of polyphenols (over 10% on dry bases) are retain in grape pomace, depending on the type of grape (white or red), the part of the tissue (skins, seeds, etc.), as well as the processing conditions (e.g., contact time between skins and must) (Guendez et al., 2005; Makris et al., 2007).

The polyphenol composition of each part of the GP varies depend on the varieties of grapes and is influenced by the growing location, maturity and the time of fermentation (Fuleki and Ricardo da Silva, 1997; Kennedy et al., 2000; Shi et al., 2003; Montealegre et al., 2006).

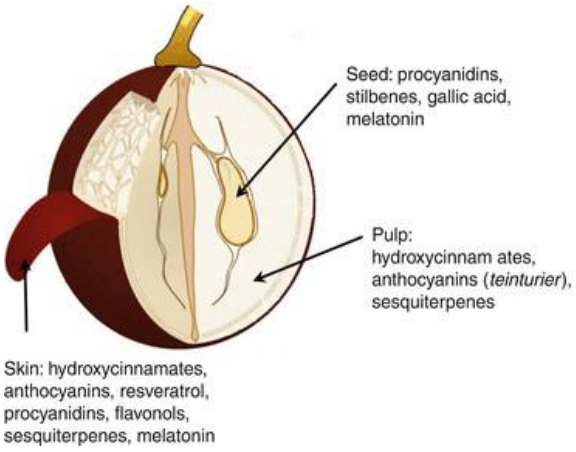


Figure 2.11. Polyphenols in grape berries

Phenolic compounds in by-producs of wine-making can be divided into two large groups: non flavonoids and flavonoids. Non flavonoids have simpler structures than flavonoids and include phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) and stilbens (Pinelo et al., 2006). Primarily, they are stored in cell vacuoles of grape cells and can easily be released by crushing. Gallic acid is described as the most abundant ***hydroxybenzoic acid*** derivative in grape stems, skins and, seeds, followed by syringic acid in grape stems, and protocatecuic acid in grape seeds and skins (Anastasiadi et al., 2012; Apostolou et al., 2013). Gallic acid is especially relevant due to its role as a precursor of hydrolysable tannins (Kallithraka et al., 2009). The protocatechuic acid is the most abundant hydroxybenzoic acid in grape seeds and pomace from red varieties, with higher concentration than the other hydroxybenzoic acid derivatives. Seeds from white varieties showed high contents in both gallic acid and protocatechuic acid with no significant differences between them (Montealegre et al., 2006).

Hydroxycinnamic acids are found in all parts of grape fruit, with the highest recorded content being in the external tissues of the ripe fruit (grape skins). The concentration in hydroxycinnamic acids generally decrease during the ripening process, however, the total amount of this phenolic class increases proportionally to the fruit size. The main hydroxycinnamic acids found in grapes and wines are caftaric, *p*-coutaric, and fertaric acids (Teixeira et al., 2014).

Concerning to grape skins, the data available on the hydroxycinnamic acids in red and white varieties suggested the existence of critical differences. Whereas skins from white grapes are dominated by the content in *cis*-coutaric acid and *trans*-caftaric

acid (Di Lecce et al., 2014) this residue from red grapes, in addition to displaying much lower concentration of this type of phenolics, is mainly represented by chlorogenic acid (Rockenbach et al., 2011a) In this way, no hydroxycinnamic acids have been described in seeds from white varieties whereas in those from red ones they were represented by chlorogenic acids in a relevant concentration (Rockenbach et al., 2011b)

Grape stems from both red and white varieties showed *trans*-caftaric acid as the most abundant compound in this class (Anastasiadi et al, 2012; Apostolou et al., 2013).

Flavonoids are found primarily in the skins and seeds of the grapevine fruit. The most common flavonoids found in grapes are anthocyanins (3-*O*-monoglucosides or 3,5-*O*-diglucosides of malvidin, cyanidin, peonidin, delphinidin, pelargonidin and petunidin, as well as their acetyl-, p-coumaroyl- and/or caffeoyl-esters), flavonols (3-*O*-glycosides of quercetin, kaempferol, myricetin, laricitrin, isorhamnetin and syringetin), flavanols or flavan-3-ols [(+)-catechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate, (-)-epigallocatechin], dihydroflavonols (astilbin and engeletin), proanthocyanidins and tannins (Ali et al., 2010; Ananga et al., 2013; , Nassiri-Asl et al, 2009; Xia et al., 2010; Ananga et al., 2012; He et al., 2010)

Most of the flavonoids are found primarily in the outer epidermal cells (the grape skin), whereas about 60%–70% of total polyphenols are stored in grape seeds (Ali et al., 2010; Nassiri-Asl et al., 2009; Tsao, 2010).

Anthocyanins are the highly soluble flavonoids directly responsible red or pink color in grape berries, and therefore found only in red grape varieties. Grape skin is the specific part of grape with major content in these bioactive colored flavonoids, this tissue being the most responsible for the transferring of pigments to the wine (Di Lecce et al., 2014).

Malvidin-3-*O*-glucoside is the main anthocyanin found in grape skin and pomace (Amico et al., 2004; 2008). The second most abundant anthocyanin in these tissues is peonidin-3-*O*-glucoside (Ky et al., 2014). Other minor anthocyanins, like 3-caffeoylglucosides of peonidin, cyaniding and delphinidin, have also been reported in grapes (Vidal et al., 2004). Grape anthocyanin composition depends not only on the

maturity but also different vine growing parameters like soil and climate together with practices such as pruning, fertilization, or watering (Gonzalez-Neves et al., 2002).

The separate materials of vinification wastes (stems, seeds, skins, pomace, and leaves) show differences concerning the individual ***flavonols*** as well as to their relative proportions and, in general depends on grape varieties and cultivars. In fact, grape by-products from red varieties present higher amounts of these compounds than white variety byproducts. Quercetin, kaempferol and isorhamnetin derivatives are found in both red and white grapes, whereas myricetin derivatives are found only in red varieties (Zhu et al., 2012; Castillo-Muñoz et al., 2007). In general quercetin-3-*O*-glucoside and quercetin-3-*O*-glucuronide are the predominant compounds present in grape pomace (Ruberto et al., 2007; Amico et al., 2004; Amico et al., 2008).

Concerning to red grape stems, it has been observed that a wide variety of flavonols, with remarkable contents of quercetin derivatives, are mainly represented by quercetin-3-*O*-glucuronide (Souquet et al., 2000; Negro et al., 2003). Other major flavonols described in grape stems are quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, and quercetin-3-*O*rutinoside (Negro et al., 2003; Apostolou et al., 2013). The stems from red and white varieties showed a similar profile of flavonols, but the plant material from white varieties present a much lower concentration of compounds, as said above.

Flavanols are the major compounds responsible for the astringency, bitterness, and structure of wines (Montealegre et al., 2006; Burdock, 2005) and, in the grape, are the compounds found in the greatest proportion, which include simple monomers of (+)-catechin, and its isomer (−)-epicatechin and (−)-epicatechin-3-*O*-gallate, as well as oligomeric procyanidins (from 2 to 5 units) and polymers (more than 5 units), commonly known as condensed tannins or proanthocyanidins (Crozier, 2003). The dimeric procyanidins are often referred as B-series, and the trimeric procyanidins as C-series. They accumulate in the grape seeds but are also found in the skin of the grape berries (Cantos et al., 2002). Five different dimers (procyanidin B1, B2, B3, B4 and B5) and two trimers (C1 and C2) were identified from grape skin and seeds (Shi et al. 2003).

The quantity, structure, and degree of polymerization of grape proanthocyanidins differ, depending on their localization in the grape tissues (Ricardo

da Silva et al., 1991; Ricardo da Silva et al., 1992; Prieur et al., 1994; Escribano-Bailon et al, 1992; Escribano-Bailon et al, 1995; Souquet et al., 1996; Jordão et al., 2001a). While seed tannins are oligomers and polymers composed of the monomeric flavan-3-ols (+)-catechin,(-)-epicatechin, and (-)-epicatechin gallate linked by C4-C8 and/or C4-C6 bonds (B type) (Prieur et al., 1994), skin tannins also contain (-)-epigallocatechin and trace amounts of (+)-gallocatechin and(-)-epigallocatechin gallate (Escribano-Bailon et al, 1995; Souquet et al., 1996). The seeds contain higher concentrations of monomeric, oligomeric, and polymeric flavan-3-ols than the skins (Bourzeix et al., 1986; Ricardo da Silva et al., 1992, Escribano-Bailon et al, 1995, Sun et al., 1999; De Freitas et al., 2000; Jordão et al., 2001b). However, the skin tannins have a much higher degree of polymerization than that from the seeds (Souquet et al., 1996; Labarbe et al., 1999; Kennedy et al., 2001), a lower amount of gallates, and are more easily transferred into wine (De Freitas et al., 2000). The average polymerization degree (mDP) for skin tannins is approximately 28, with 80 being the maximum DP detected, and the percentage of gallates in the tannins is only 5.16% (Souquet et al., 1996; Yilmaz & Toledo, 2004).

Monomeric and oligomeric proanthocyanidins are certainly soluble in the organic solvents usually used for polyphenols extraction, but a major proportion of high-molecular-weight proanthocyanidins an polyphenols complexed with protein or cell wall polysaccharides remain insoluble (Wollgast and Anklam, 2000). The quantification of non-extractable polyphenols needs hydrolysis of winery residual to release the bound phenolics from cell wall or protein after soluble polyphenols are extracted (Ignat et al., 2011).

Catechin is the major compound in all winery residues from both red and white grapes, and the concentrations in grape stems are higher than those described in skins and seeds. In white varieties flavanols represent 46% to 56% of total phenolics, whereas in red grapes they represent between 13% and 30% of total phenolic content (Cantos et al., 2002).

Concerning red varieties, the highest content in condensed tannins is represented by the procyanidin dimer B3 (Anastasiadi et al., 2012; Apostolou et al., 2013). Data from white grape residues shows that procyanidin B1 is the major compound.

Stilbenes are non-flavonoid compounds and can be found in wine by-products, mostly in grape skin, but also in stems and seeds but in lower abundance (Liu et al., 2013; Kataliníc et al., 2010). To date, the presence of *trans*-piceid, *trans*-resveratrol, *trans*-resveratrol-3-*O*-glucoside, *cis*-resveratrol-3-*O*-glucoside, and ε-viniferin has been described. Resveratrol is present in the different organic residues of vinification procedures (Lima et al., 1999; Moreno-Labanda et al., 2004; Püssa et al., 2006). The data available on the concentration of the separate stilbene derivatives indicates that, in red grapes, the highest content corresponded to *trans*-resveratrol and ε-viniferin in grape stems which largely surpasses the concentration recorded in seeds and pomace. On the other hand, grape stems from vinification processes developed on white grapes present lower concentrations of both *trans*-resveratrol and ε-viniferin. In grapes, *trans*-resveratrol is distributed mostly in the skin (Jeandet et al., 1991).

2.4.4. Factors affecting grape by-producs polyphenols: winemaking process

As said above, the composition of the residue of the grape has significant variations depending on grape variety and technology applied during the winemaking steps. Regardless of the grape processes, the composition of grape pomace is closely defined by basic unit operations, namely destemming/crushing, maceration, and pressing. White wine making differs from reds one (Figure 2.12). White wine is produced through "fermentation off skins" (named white method) in which grapes are pressed, separating juice from grape pomace and therefore from skins, before fermentation; although, some types of white wines perform a short maceration at low temperatures, also called cold soaking. Unlike white wine, red wine is produced through "fermentation on skins" (named red method) in which the solid residues (grape pomace) are separated from the liquid product (must wine or wine), after fermentation and maceration.

The objective of fermentation and maceration is to permit the juice and skin contact to allow for diffusion of skin and seed components, namely phenols and aroma into the must. Maceration in wines is an operation where temperature and time are the main variables defining the composition of grape pomace. As said above, red winemaking maceration takes place simultaneously with fermentation.

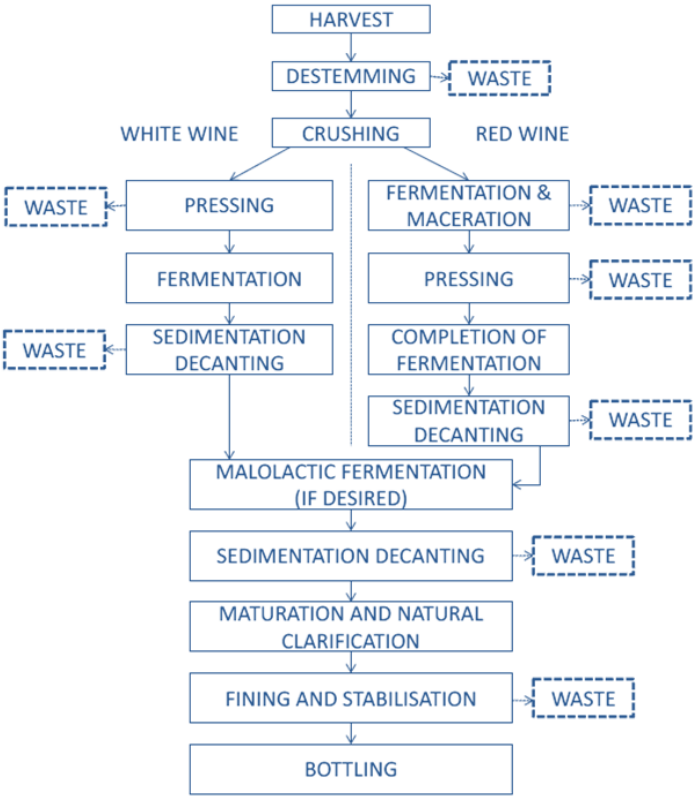


Figure 2.12. Diagram of grape waste generated in the process of making red and white wine (adapted from Arvanitoyannis et al., 2006; Devesa-Rey et al., 2011)

This implies that grapes are subject to biochemical changes produced by the metabolism of *Saccharomyces cerevisiae* yeasts, which consume glucose and fructose, producing ethanol and CO_2 in an exothermic process. The temperature rise during fermentation (up to 30 °C) increases the solubility of phenolic compounds, enhancing extraction of several compounds and therefore producing more exhausted grape marc. Also, the longer maceration time involves a sequential and selective extraction of compounds due the increasing ethanol concentration (Boulton et al., 1996).

During fermentation grape skins are displaced to the top of the tanks forming a *cap* or solid phase that reduce contact with the juice. *Cap management* operations such as punching down and pumping wine over the top of the tank enhance the release of compounds from grape skins. Rotary tanks equipped with baffles and other automated devices are also used to increase the release of compounds into the wines. Other enological techniques, as must freezing and thermovinification, use extreme temperatures (0-2 °C and 60-70 °C respectively) to achieve degradation of grape skin cells, thus releasing a higher amount of compounds (Pinelo et al., 2006).

Obiouvsly, limited extraction of grape compounds during the wine making process results in a high phenolic content in the grape pomace.

2.3.5. Digestion, absorption and metabolism of grape polyphenols

To explain the biological effects of polyphenols, it is assumed that they are bioavailable and are effective in reaching target tissues. It is therefore important to fully understand how they are absorbed, metabolized and eliminated from the body. There is considerable controversy surrounding current studies on the absorption and metabolism of polyphenols and results are therefore inconclusive. Studies on absorption are made difficult by the molecular complexity of the extracts or polyphenol-rich feed owing to factors like their level of polymerization and conjugation with other phenols. Most polyphenols are present in food in the form of esters, glycosides or polymers that cannot be absorbed in their native form. These substances must be hydrolysed by intestinal enzymes or microbiota before they can be absorbed. Once ingested, polyphenols are recognized by the body as xenobiotics, and their bioavailability is therefore relatively low in comparison to micro- and macronutrients.

The metabolization of polyphenols takes place through a sequence of reactions common to all of them. This is similar to a metabolic detoxication to reduce their potential cytotoxic effect by increasing their hydrophilicity and facilitating urinary or biliary elimination (Manach et al., 2004). It is the chemical structure of polyphenols, rather than the concentration, that determines the rate and extent of absorption and the nature of the metabolites circulating in the plasma. Depending on their degree of structural complexity and polymerization, these compounds may be readily absorbed in the small intestine (i.e., low-molecular-weight polyphenols such as monomeric and dimeric structures) or reach the colon almost unchanged (oligomeric and polymeric polyphenols) (Monagas et al., 2010). It has been estimated that only 5–10% of the total polyphenol intake may be absorbed in the small intestine. After absorption, these less complex polyphenol compounds may be subjected to hydrolysis and biotransformation in the enterocytes and then the hepatocytes, resulting in a series of water soluble conjugate metabolites (methyl, glucuronide and sulphate derivatives) being rapidly released into the systemic circulation for further distribution to organs and excretion

in urine (Manach et al., 2004). A proportion of these metabolites enters the duodenum by means of the bile and is subsequently hydrolysed by bacterial enzymes (mainly β-glucuronidase) in the large intestine. This enterohepatic recycling may lead to a longer presence of polyphenols within the body. However, the remaining polyphenols (90–95% of the total polyphenol intake) may accumulate in the large intestinal lumen where, together with conjugates excreted into the intestinal lumen through the bile, they are subjected to the enzymatic activities of the gut microbial community, generating metabolites such as aromatic acids (hydroxyphenylacetic, phenylpropionic, phenylbutyric acids, phenyl valerolactones and others) (Selma et al., 2009; Sánchez-Patán et al., 2012). All these microbial-derived phenolic metabolites may be absorbed or excreted in the faeces. When absorbed, they reach the liver through the portal vein where they may be further subjected to extensive metabolism (including glucuronidation, methylation, sulphation or a combination of these) until they finally enter the systemic circulation and are distributed to the organs or eliminated in the urine. The gut microbiota are therefore responsible for the extensive breakdown of the original polyphenolic structures into a series of low-molecular-weight phenolic metabolites which, being absorbable, may actually be responsible for the biological activity derived from polyphenol-rich food consumption, rather than the original compounds found in foods. The concentrations of polyphenols reached after their consumption varies significantly according to the nature of the polyphenol and the food source. Plasma concentrations in intact flavonoids rarely exceed 1μM and the maintenance of a high polyphenol concentration in plasma requires repeated ingestion over time; in fact, maximum concentrations are most often reached 1-2 h after ingestion (Manach et al., 2004). Polyphenols and their derivatives are eliminated chiefly in the urine and the bile (Figure 2.13).

Until now, research on the digestibility of polyphenols from grape by-products in domestic animals has been lacking, as have studies on their effect on the digestibility of other nutrients. Recent work carried out in our laboratory shows that concentrations of up to 6% of grape pomace (GP) and 0.25% of grape seed extract (GSE) can be used in chicken feed without any modification in productivity parameters, the size of the digestive organs or the ileal digestibility of protein and amino acids (Brenes et al., 2008; Chamorro et al., 2013). Studies using spectrophotometric techniques on the

digestibility in birds of non-extractable polyphenols present in GP (Brenes et al., 2008) show that the ileal and faecal digestibility of hydrolysable polyphenols (56% and 73%, respectively) is greater than that of condensed tannins (14% and 47%, respectively).

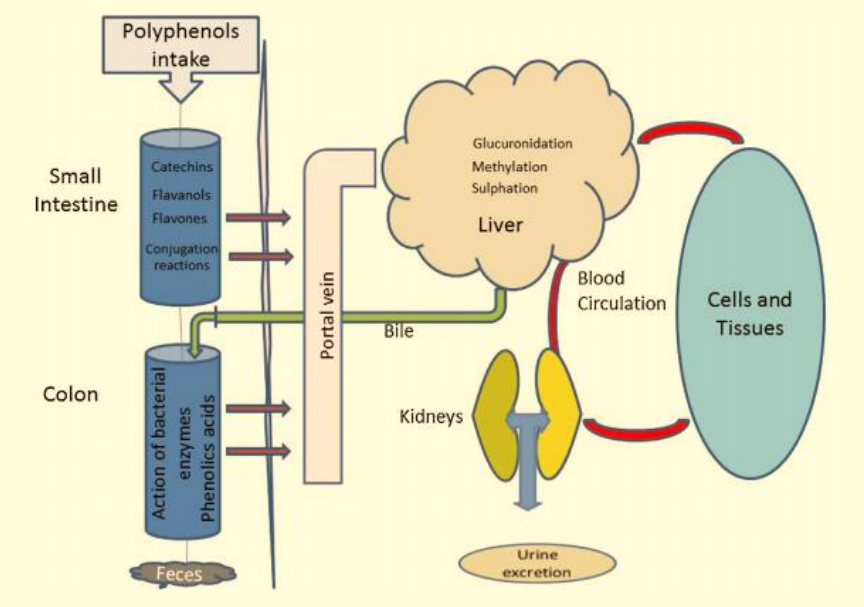


Figure 2.13. Schematic description of metabolic fate of dietary polyphenols (from Brenes et al., 2016)

On the other hand, greater digestibility of the total extractable polyphenols in the birds' diets was observed when commercial polyphenolic grape extracts (PGE) were used, (Brenes et al., 2010), with values of up to 69% obtained at faecal level. Liquid chromatography techniques in combination with mass spectrometry, which allows the different polyphenolic compounds present in grape by-products to be identified and quantified, have recently been used to demonstrate that extractable polyphenols are highly digestible in birds at both ileal and faecal level (Chamorro et al., unpublished). The results also show that digestibility of these compounds depends on their degree of polymerization (which is greater in monomers [catechin and epicatechin] than in dimers [B1 and B2 procyanidins]), and their degree of esterification (greater in free compounds than in those esterified with gallic acid). It has also been shown that the inclusion of polyphenolic grape extracts at concentrations of 5 g/kg in birds' diets can reduce the digestibility of other nutrients like fat (Brenes et al., 2008), protein and certain amino acids, such as proline and cystine (Chamorro et al., 2013).

The bioavailability of procyanidin is largely influenced by its structure. For this reason, there is increasing interest in how dietary flavonoids are broken down into simple phenolic compounds. Strategies to encourage hydrolysis of the more complex polymeric structures of polyphenols have been studied in vitro with the aim of improving the digestibility of polyphenols present in grape by-products. These have included the study of thermal treatment (furnace and autoclave) and the addition of enzymes (pectinase, cellulose and tannase) to degrade or convert the polymeric structures into compounds with a lower molecular weight (monomers and oligomers). Results reported by Chamorro et al. (2012a) show that heating conditions (dry or wet heat) had a considerable impact on the hydrolysis of procyanidins. The effectiveness of the thermal treatment varied according to whether polyphenols were present in free form (GSE) or whether they were bonded or linked to other structures (GP). Furnace thermal treatment did not modify the polyphenol compounds of these grape products. However, the effect on individual compounds in GSE was more severe with autoclave treatment, leading to extensive hydrolysis of catechin, epicatechin, gallocatechin, PB1 and PB2. In the case of GP, autoclave treatment increased gallic acid, gallocatechin and epigallocatechin. These modifications suggest that during autoclave treatment the more highly polymerized molecules seem to be changed to relatively less polymerized molecules. At the same time, the addition of commercial cell-wall-hydrolysing enzymes (pectynolitic and cellulolytic activities) was used to release cell wall complex polysaccharides present in GP, facilitating the release of certain nutrients entrapped by the cell wall. Tannase or tannin acyl hydrolase (EC, 3.1.1.20) was also used to catalyse hydrolysis of the ester and depside bonds present in hydrolysable tannins or gallic esters in GP and GS (Chamorro et al., 2012b). The results obtained in this study demonstrate that the use of pectinase and tannase in both grape by-products and pectinase in GP changed the galloylated form of catechin to its free form, releasing gallic acid. Pectinolytic enzymes also broke down the GP plant cell-wall matrix, releasing monosaccharides and facilitating polyphenol extraction.

The applicability of these findings with enzymes in vitro needs to be supported by experiments in vivo. The inclusion of enzymes (carbohydrases and tannase) in chicken diets containing GP (5% and 10%) modified the polyphenol polymeric structures present in the ileal content, with increased concentrations of gallic acid,

catechin and epicatechin in birds fed carbohydrases, and of gallic acid, catechin, epicatechin, procyanidins PB1 and PB2 and epicatechin gallate with the addition of tannase. These findings show that the inclusion of enzymes in diets containing GP increased the amount of total polyphenol released in the intestine, although this effect was not accompanied by an increase in the birds' performance (Chamorro et al., 2015).

2.4.6. Biological activities of grape by-products polyphenols

To date, there is a great deal of evidence concerning biological activities, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and cardiovascular protection activities, in support of phenolic compound use in pharmaceutical, food, and cosmetic industries (González-Centeno et al., 2012; Yilmaz et al., 2011).

Finding and utilization of plant compounds as an alternative to chemical or synthetic antimicrobials and antioxidants to combat the foodborne pathogens, inhibiting lipid oxidation and thus extending the shelf life is an increasing trend in the food industry, and the presence of both antioxidant and antimicrobial properties in a single molecule makes them more effective and better suited as food preservatives**.**

2.4.6.1. Antioxidant properties

One of the most cited biological activities attributed to the phenolic compounds is based on their antioxidant capacity.

Their specific chemical structure allows them to reduce oxidative stress through numerous mechanisms (Heim et al., 2002; Procházková et al., 2011). For example, it was reported that, *in vitro*, flavonoids can prevent injury caused by free radicals by the following mechanisms:

(1) direct scavenging of reactive oxygen species (ROS),

(2) activation of antioxidant enzymes (Nijveldt et al., 2001),

(3) metal chelating activity (Ferrali et al., 1997),

(4) reduction of α-tocopheryl radicals (Hirano et al., 2001; Heim et al., 2002)

(5) increase in uric acid levels (Lotito et al., 2006),

The chemical functional group and structure is OH for antioxidant capacity of phenolic compounds. The number of OH group (hydroxyl group) and its position on the ring of molecule determined the antioxidant capacity of flavonols (Arora et al.,

1998). Thus, whereas hydroxyl (–OH) groups enhance the activity, their substitution by carboxyl ($–OCH_3$) groups diminishes the antioxidant potential (Di Majo et al., 2008).

In different parts of grape, the highest antioxidant capacity was found in grape seeds, followed by skin, and the flesh displayed the lowest antioxidant capacity (Pastrana-Bonilla et al., 2003).

Many researchers have tried to discover which phenolic compounds and chemical structure(s) are mainly responsible for the antioxidant activities of grape extracts. The result suggested that perhaps the antioxidant capacity of phenolics has a concentration saturation limit, and above this limit, the activity could not increase further with the concentration (Dani et al., 2009). However, the relationship between phenolic compounds and antioxidant capacity was inconsistent among the results from different studies, which indicated that, besides the concentration, the antioxidant capacities of phenolic compounds were affected by other factors (Radovanovic et al., 2009; Di Majo et al., 2008).

Anthocyanins contribute more to the antioxidant capacity of fruits (90%) than flavonols, flavan-3-ols, and phenolic acids (10%) (Jakobek et al., 2009). On this matter, grape skins have been highlighted for their antioxidant and anti-glycation activities because of their anthocyanins and proanthocyanidins content (Poudel et al., 2008; Rockenbach et al., 2011).

Furthermore, the in vitro antioxidant activity could be increased by polymerization of flavonoid monomers, e.g. proanthocyanidins (also known as condensed tannins), the polymers of catechins, are excellent in vitro antioxidants due to the high number of hydroxyl groups in their molecules. The antioxidant capacity of proanthocyanidins depends on their oligomer chain length and the type of ROS with which they react (Spranger et al., 2008). The glycosylation of flavonoids reduces their in vitro antioxidant activity when compared to the corresponding aglycons (Pinelo et al., 2006; LLobera et al., 2007; Souquet et al., 2000; Corrales et al., 2008; Figuerola et al., 2005). The results from a study carried out by Spranger et al. (2008) showed that procyanidin polymers with higher degrees of polymerization had higher antioxidant activities. However, Faria et al. (2006) showed that in five fractions of different degrees of procyanidins polymers, the second degree fraction displayed the highest

antioxidant capacity (scavenging peroxyl radicals). A similar result was obtained by Soobratteea *et al*. (2005), who showed that the most antioxidative compound in various phenolics was procyanidin dimer, and the decrease in antioxidant capacity was in order of procyanidin dimer, flavanol, flavonol, hydroxycinnamic acids and simple phenolic acids. Diphenols are more effectively antioxidant than simpler phenols due to stabilization of the phenoxy-radical through hydrogen bonding (Amico et al., 2008). The high molecule weight compounds might be as important as the monomer flavanols such as catechin, which have been demonstrated high antioxidant potential in phenolic compounds (Yilmaz and Toledo 2004). Furthermore, the antioxidant activity of a sample could be synergic effect among several compositions, rather than a single compound (Dopico-Garcia et al., 2008; Monagas et al., 2005).

Other possible mechanism by which flavonoids act is through interaction with various antioxidant enzymes.Flavonoids are able to induce phase II detoxifying enzymes (e.g. NAD(P)H-quinone oxidoreductase, glutathione S-transferase, and UDP-glucuronosyl transferase), which are the major defense enzymes against oxidative stress.

Nagata et al. (1999) described that the protective activity of quercetin and catechin against hydrogen peroxide cytotoxicity in cultured rat hepatocytes BL-9, which are cells highly expressing cytosolic glutathione peroxidase (GPx), was related to the activation of GPx.

In addition, administration of the flavonoid-rich fraction along with a high fat diet caused a significant increase of SOD (superoxide dismutase), CAT (catalase) and GPx activities in rat erythrocytes (Kaviarasan et al., 2008).

Specific flavonoids are known to chelate iron and copper, thereby removing a causal factor for the development of free radicals. Quercetin was able to prevent oxidative injury induced in the erythrocyte membrane by a number of oxidizing agents which cause release of iron in its free, redox active form (Ferrali et al., 1997). Pietta (2000) proposed that the binding sites for trace metals in the molecule of flavonoids are the catechol moiety in the ring B, the 3-hydroxyl and 4-oxo groups in the heterocyclic ring C, and the 4-oxo and 5-hydroxyl groups between the C and A rings.

The catechol moiety in the B ring has been shown to be important for Cu2+-chelate formation and thus being the major contributory site of the metal chelation

(Brown et al., 1998). Quercetin, in particular, is known for its iron-chelating and iron stabilizing properties. Morin and quercetin were shown to form complexes with Cd(II) and exhibit strong antioxidant activity in the in vitro studies.

The α-tocopherol represents a major antioxidant in cell membranes. Hirano et al. (Hirano et al., 2001) suggested that flavonoids can act as hydrogen donors to α-tocopheryl radical, which is a potential prooxidant. It has been reported that polyphenols (quercetin, (+)-epicatechin and (+)-catechin), owing to their one-electron reduction potentials, may spare vitamin E, to delay lipid oxidation, and to regenerate tocopherol in rat and human models (Frank, 2005). Iglesias et al. (2012) demonstrated that the antioxidant mechanism of grape procyanidin might be explained by its capacity to repair the oxidized α-tocopherol and to delay the ascorbic acid depletion of muscle tissues.

Flavonoids (kaempferol, morin, myricetin and quercetin) showed a varying protective activity against depletion of α-tocopherol in LDL, with kaempferol and morin being less effective than myricetin and quercetin (Zhu et al., 2000). Catechins may be evenmore effective than ascorbate in regeneratingα-tocopherol in micellar solution (Mukai et al., 2005). Similarly, the addition of green tea catechin extracts (epigallocatechin, epigallocatechin gallate, epicatechin, and epicatechin gallate) demonstrated a gradual regeneration of α-tocopherol in human LDL (Zhu et al., 1999).

Interestingly, there are the great discrepancies between plasma or serum total antioxidant capacity and plasma concentrations of flavonoids. Lotito and Frei (2006) suppose that the large increase in plasma total antioxidant capacity observed after the consumption of flavonoid-rich foods is not caused by the flavonoids themselves, but is likely the consequence of increased uric acid levels, which is a major contributor to plasma total antioxidant capacity. Cao et al. (1998) described the significant increase in plasma or serum urate after consumption of strawberries, spinach or red wine. Similar increase of plasma or serum urate was described after drinking of port wine (Day and Stansbie, 1995), French Bordeaux (Maxwell and Thorpe, 2000), tea or coffee (Natella et al., 2002). Thus, several studies indicate that the consumption of flavonoid-rich foods may increase plasma urate, although the underlying mechanism still remains unclear.

2.4.6.2. Antimicrobial effect

Antimicrobial activities of grape, wine and grape-derived byproducts have been widely discussed (Georgiev et al., 2014; Perumalla et al., 2011; Xia et al., 2010). There is an emerging consensus that gut microbiota may play a crucial role in the potential health benefits of polyphenols (Crozier et al., 2009). The microbiota present in the intestinal tract could metabolize dietary polyphenols into more bioactive compounds with different physiological significance and could also modify the composition and/or activity of the intestinal bacteria population (Bustos et al. 2012). Thus, dietary phenolic compounds are often transformed by gut microbiota and gut microbial population is modulated by dietary polyphenols in a two-way phenolic-microbiota interaction. Polyphenols and their derivatives affect the intestinal ecology as a significant proportion of them are not fully absorbed but are metabolized in the liver, excreted through the bile as glucuronides and accumulated in the ileal and colorectal lumen (Tzounis et al., 2008). Substantial levels of unabsorbed dietary phenolic compounds exert significant effects on the intestinal environment by suppressing or stimulating the growth of some of the components of intestinal microbiota. It has been shown in numerous in vitro studies (Papadopoulou et al., 2005; Özkan et al., 2004; Rodríguez-Vaquero et al., 2007b; Gañan et al., 2009; Silván et al., 2013) that flavonoids present in grape by-products have the capacity to inhibit the growth of certain organisms, such as *S. aureus*, *E. coli*, *C. albicans* and *Campylobacter*. Likewise, certain polyphenolic compounds, such as resveratrol, hydroxytyrosol, quercetin and phenolic acids, also have shown to possess antimicrobial capacities against certain intestinal pathogens like *Salmonella* and *Helicobacter pylori* (Daroch et al., 2001; Just and Daeschel, 2003). Grape polyphenols also inhibit the growth of different pathogens, polymeric flavonoids (procyanidins) showing greater activity than monomeric flavonoids (Mayer et al., 2008). Cueva et al. (2010) also showed in vitro that microbial biotransformation of dietary phenolic compounds (phenolic acids) selectively influenced intestinal bacteria species and could affect the diversity and metabolic activity of the intestinal microbiota. Until now, only a limited number of specific bacterial species capable of dietary polyphenol degradation have been identified, for example *Eubacterium ramulus* and *Flavonifracter plautii*, previously known as *Clostridium orbiscindens* (Braune et al.,

2001; Schoefer et al., 2002). In a recent study Kemperman et al. (2014) used a simulator of the intestinal microbial ecosystem to show that grape polyphenols cause changes in the gut microbial community by inhibiting Firmicutes and promoting Proteobacteria.

CHAPTER 3

APPLICATIONS OF GRAPE BY-PRODUCTS BIOLOGICAL ACTIVITIES IN ANIMAL NUTRITION

3.1. Supplementation of grape by-products to the diet as antioxidants

Nutrition has a strong impact because an insufficient intake of antioxidants, a high intake of pro-oxidants, or both, may lead to oxidative stress. Animal nutrition is currently evolving towards n-3 polyunsaturated fatty acid (PUFA) to improve animal fat healthfulness but this nutritional strategy has been associated with an increase in lipoperoxidation in subcutaneous and intramuscular lipids. Dietary PUFA are a group of pro-oxidants known to increase oxidative stress *in vivo* in pigs (Salobir et al., 2005), chicks (Gao et al., 2010) and hens (Cherian and Hayat, 2009). Increasing the level of unsaturation in the muscle membrane by dietary manipulation increases the susceptibility of meat to oxidative deterioration during storage, the consequence being a reduction in both flavour and nutritional value (Enberg et al., 1996).

Endogenous defence mechanisms are inadequate for complete prevention of oxidative damage. As discussed above, vitamin E is the antioxidant most commonly used in animal nutrition but this has certain drawbacks, including its synthetic origin, its limited bioefficiency when n=3 PUFA intake is too high (Allard et al.,1997), its potential pro-oxidant action (Mukai et al., 1993), and its nonhomogeneous distribution between the tissues. Therefore, there is a growing interest in the nutritional aspect of polyphenolic compounds in light of their antioxidant capacity and they may become an important alternative as a partial substitute for vitamin E in animal diets. The antioxidant potential of grape seed polyphenols is 20 times higher than vitamin E and 50 times higher than vitamin C (Carpenter et al., 2007).

Natural antioxidants can be applied either through dietary or technological strategies to reduce or prevent oxidative processes in meat. The optimum dose of inclusion of polyphenols in animal diets is difficult to define due to the different composition of phenolic compounds present in these by-products. Nevertheless, the use of plant-rich polyphenols seem to be a promising strategy for improving products quality. In dietary manipulations, antioxidants are introduced into the muscle via the

animal feed. Various authors have reported that inclusion of natural antioxidants in animal diets not only slows down oxidation, but also greatly improves meat quality when compared to diets with no antioxidants. It has been shown that the addition of polyphenolic grape extract in monogastric diets, especially oligomeric flavanols, enhances oxidative stability in chicken and turkey meat (Lau and King, 2003; Rababah et al., 2006).

Studies carried out and published by some authors (Goñi et al. 2007; Brenes et al., 2008; Brenes et al., 2010) also showed that increasing concentration of GP up to 60 g/kg and GSE up to 3.6 g/kg improved the antioxidant activity of the diet, ileal content and faeces. Likewise, we also observed an enhance oxidative stability in chicken meat products (TBARS, thigh and breast) during the refrigeration process. Similar increment was obtained when vitamin E was added to the rations. The concentration of vitamin E in the liver also increased with the addition of GP, which could be due to a vitamin E saving effect. A reduction in α-tocopherol deposition in chicks fed unsaturated diets was also reported by Surai and Sparks (2000) and Sijben et al., (2002). The increased vitamin E content in the liver with the inclusion of GP could be due to the saving effect of GP in the intestine. Less vitamin E would be destroyed through oxidation, resulting in greater amounts of the vitamin being absorbed. It has also been reported in rat and human models that, owing to their 1-electron reduction potentials, polyphenols may save vitamin E to delay lipid oxidation and regenerate tocopherol (Frank, 2005).

The inclusion of GP and GSE in chicken diets significantly improved oxidative stability (TBARS) and radical scavenging capacity (ABTS) in raw breast meat and cooked chicken patties (Sayago-Ayerdi et al. 2009 a,b , Selani et al. 2011). Similar results have been reported by Brannan (2008) and Lau and King (2003) in chicken thigh meat during refrigerated storage and by Mielnik et al. (2006) in turkey meat. Sahin et al. (2010) and Liu et al. (2014) reported that the inclusion of resveratrol in quail diets enhanced the antioxidant activity status of birds and eggs and reduced oxidative stress in heat-stressed chickens by increasing serum growth hormone concentrations and modulating the expression of heat shock genes in organs of the immune system.

A reduction of plasma TBARS values (Zhang et al., 2014) and a modification in the fatty acid pattern in subcutaneous fat (Yan and Kim, 2011) have also been reported in pigs fed diets containing a plant extract including GSE (2g/kg) and fermented GP diets (30g/kg), respectively. However, the addition of GSE (0.7 g/kg) did not affect the oxidative stability and quality of raw and cooked pig meat (O'Grady et al., 2008).

All these studies, mainly in chickens, suggest that polyphenols present in grape by-products are absorbed, distributed and retained and remain functional at sufficient levels to contribute to PUFA protection in membranes and modulate antioxidant activity in muscle tissue. These bioactive compounds could therefore reduce the amount of additives like vitamin E but also improve vitamin E status.

Part of the relationship between polyphenols and their antioxidant activity involves their transition metal-chelating potential. As dietary polyphenolic compounds show metal chelating activity (Cook et al., 1995), a high intake of these bioactive substances may have consequences for the iron status. They chelate iron by forming insoluble complexes with iron ions in the gastrointestinal lumen, thereby making the iron unavailable for absorption. Iron is also implicated in the initiation process of PUFA peroxidation (Hatcher et al. 2009). In this respect, it has been shown that the inclusion of grape polyphenols in chicken diets reduces the plasma iron concentrations associated with lower oxidation of meat (Chamorro et al., 2013). In line with this result, Marouani et al. (2007) and Lee et al., (2010) showed that the dietary addition of tea polyphenols and tannic acid significantly reduced plasma iron in rats and pigs. The antioxidant effect of grape seed proanthocyanidins was also demonstrated by Naidoo et al. (2008) and Wang et al. (2008) in chickens infected with different dosages of Eimeria tenella. Results showed an increase in plasma superoxide dismutase content and a decrease in malondialdehyde (MDA) and plasma nitric oxide concentration, indicating that GSE was able to restore the balance of antioxidant-oxidant status, which had been disturbed by oxidative stress after parasite infection.

There are also references showing that the addition of grape seed by-products modifies the fatty acid composition of meat. In this respect, the dietary inclusion of a fermented GP product for pigs increased the total PUFA and PUFA/SFA ratio in the subcutaneous fat of the Longissimus muscle (Yan and Kim, 2011). Information on the

supplemental effect of GP on the fatty acids profile of chicken meat is not available. Changes in the muscle fatty acid proportion (reduced SFA and increased PUFA) following the dietary addition of different bioflavonoids (genistein, hesperidin, gallic acid) have been reported in chickens (Kamboh and Zhou, 2013; Jung et al., 2010). Recent results from Chamorro et al. (2015) confirm that birds fed vitamin E and GP diets showed higher meat PUFA content. This effect was also correlated with a reduction in the susceptibility of the chicken meat to lipid peroxidation. Previous results indicated that monomers were better digested and that the use of tannase and pectinase released gallic acid, catechin and epicatechin (Chamorro et al., 2015). However, birds fed with a GP diet supplemented with enzymes (tannase) reversed the beneficial effect observed in GP diets. Although the inclusion of enzymes in GP diets hydrolysed the complex polyphenols into compounds with low polymerization, thereby improving the amount of available bioactive substances present in the gut, they are less active against oxidation and evidently no additional protective effect on oxidative stability was observed. These results suggest that simple phenols generated by the action of enzymes are less active than the more complex ones present in GP.

Although several studies have shown that dietary polyphenols have a beneficial effect on the oxidative stability of meat, the mechanism of their action remains to be established. The direct antioxidant activity of dietary polyphenols implies that they are absorbed through the monomeric form and by the metabolites generated by the intestinal microbiota (Luciano et al., 2009). Different authors have recently shown the transmission of dietary phenolic compounds in lambs' meat (Moñino et al., 2008; Luciano et al., 2011) and frankfurters from free-range-reared pigs (Estévez et al., 2007), using rosemary and tannin-rich by- products (quebracho, grapes, nuts and citrus fruits). Moreover, Gladine et al. (2007) reported the presence of five different phenolic compounds in plasma, including epicatechin, and unknown phenolic compounds in sheep that received GS and peel extract directly into the rumen. Nevertheless, the effect of dietary procyanidins on meat oxidative stability may also be indirect, through preservation of the oxidation of other dietary bioactive compounds in the gastrointestinal tract and in meat (for example, fatty acids and vitamins E and C), chelation of the pro-oxidant compounds, and the ability to modulate the activity and

gene expression levels of relevant endogenous antioxidant enzymes (Sgorlon et al., 2006; Larrosa et al., 2010).

3.2. Direct addition of grape by-products to meat products

In recent years, much attention has been paid to developing meat and meat products with physiological functions to promote healthy conditions and prevent disease. Technological strategies involve the application of antioxidants directly into meat and meat products or the coating of packaging materials with plant extracts to improve their oxidative stability. The effectiveness of GSE as a food ingredient has been tested in various systems, including sunflower oil, fish oil, fish, seaweed oil emulsion, turkey, chicken, beef, pork and fish meats (Ahn et al., 2002, 2007; Lau and King, 2003; Hu et al., 2004; Pazos et al., 2005; Mielnik et al., 2006; Rababah et al., 2006; Shaker, 2006; Bañon et al., 2007; Brannan & Mah, 2007; Brannan, 2009). There is also abundant evidence demonstrating the ability of these extracts to delay lipid oxidation in meat during storage. In raw meats, GSE have been shown to be effective in reducing the amount of primary (hydroperoxides and hexanal) and secondary products of oxidation (thiobarbituric acid reactive substances [TBARS]), chicken meat (Lau and King, 2003; Shirahigue et al., 2011), pork (Brannan and Mah, 2007; Carpenter et al., 2007; Rojas and Brewer, 2008; Lorenzo et al., 2014) and fish (Pazos et al, 2005; Sánchez-Alonso and Borderías, 2008; Sánchez-Alonso et al., 2008). In cooked meats, GSE have also been shown to be effective in reducing oxidative rancidity and the formation of volatile compounds in turkey breast meat (Mielnik et al., 2006), chicken breast and thigh meat (Rababah et al., 2006; Sayago-Ayerdi et al., 2009ab; Brannan, 2009; Selani et al., 2011), pork patties (Brannan and Mah, 2007; Rojas and Brewer, 2007, 2008; Sasse et al., 2009), These results indicate that grape polyphenols could be used as a natural ingredient to prevent oxidation and as a functional ingredient in healthy food design. A few studies published in recent years have tried to explain the mechanisms involved in antioxidant effectiveness in muscle foods. Recent data using molecular dynamic simulations with biomembranes suggests that the effects of GSE components over free radicals in fish muscle may be due to inhibition of the propagation of free radicals in the lipid bilayer, reduction in the

contact of pro-oxidant compounds, such as Fe or haemoglobin, and the localization of polyphenols close to active points of oxidation (Sirk et al., 2009; Maestre et al., 2010).

Information on the transference of phenolic compounds in muscle is limited, particularly with respect to the effect of the dietary administration of polyphenols on the presence and concentration of phenolic compounds in animal tissues.

3.3. Antimicrobial activity and modulation of gut microbiota

There have been very few studies on the interaction of polyphenol compounds with intestinal microbiota in animal nutrition. In vivo studies have shown that resveratrol has great potential as an antibiotic alternative for reversing the adverse effects of weaning stress on the growth performance, immunity and microbial environment in *E. coli* and *Salmonella* challenged piglets (Ahmed et al., 2013). Likewise, the addition of GSE in weaned pigs reduces E. coli-induced diarrhoea (Verhelst et al., 2014). Fiesel et al. (2014) showed that feeding GS and grape marc meal extract altered the microbial composition, with a reduction in *Streptococcus* spp. and *Clostridium* in the faecal microbiota. However, other authors (Zhang et al., 2014) did not observe any differences in the microbial count in the faeces and caecum of weaned pigs fed an extract containing polyphenols, including GSE. Studies conducted in rats (Dolara et al. 2005; Larrosa et al., 2009; Pozuelo et al., 2012) reported increases in the colonic populations of *Bacteroides, Lactobacillus* and *Bifidobacterium* that were associated with the dietary inclusion of grape seed polyphenols. An ecological shift in the microbiome, with a dramatic increase in *Lachnospiraceae, Clostridales, Lactobacillus* and *Ruminococcacceae*, was observed in female pigs fed GSE (1%) diet (Choy et al., 2014). Evidence of this effect has also been observed with the use of tea polyphenols in pigs and calves, with a significant increase in the *Lactobacilli* count, a decrease in total bacteria and *Bacteroidaceae* and a tendency towards a decrease in *C. perfringens* (Hara et al., 1995; Isihara et al., 2001). The inhibiting effect of polyphenolic compounds on bacteria could be due to mechanisms related to their capacity to adhere to cellular membranes, interact with bacterial enzymes and sequester metallic ions from the substrate (Scalbert, 1991; Cushnie and Lamb, 2011). The results obtained by Viveros et al. (2011) on the use of GP and grape extract in birds' diets also demonstrated and confirmed the antibacterial effect of polyphenols

found in these by-products with respect to certain intestinal bacteria. This effect differs depending on the segment analysed (ileum or caecum). The inclusion of these by-products in birds' diets exerted an antimicrobial effect on *Clostridium* in the ileum, while in the caecum it was associated with an increase in populations like *Lactobacillus* and *Enterococcus*. This potential prebiotic effect was confirmed in the same study, using molecular techniques (TRFLP) that allow global changes in microbial populations to be analysed. These results reflected an increase in the level of biodiversity and the frequency of detection of certain bacteria with the capacity to degrade phenols, as well as other unidentified organisms in the caecum of birds fed with these by-products.

CHAPTER 4

CHICKEN MEAT AND MEAT PRODUCTS QUALITY

4.1. Importance of chicken meat and meat products

The chicken meat consumption is generally well above the global average. The poultry meat uptake per person will increase faster than that for pork and beef as poultry consumption will rise by some nine per cent between 2013 and 2022, compared with gains of three to four cent for beef and pig meat respectively. The increase of this meat is in relation to the increase of the global demand for inexpensive proteins particularly among developing countries as a result of population growth and rising consumer incomes. On the other hand, the increasing demand of chicken meat is attributed mainly to the quality of this meat; its healthy and high nutritional profile, sensory properties that make it very flexible for any type of home-cooking style as well as for manufacturing processed products and the low costs of production (Petracci et al., 2013), and also because of the absence of cultural or religious obstacles (Valceschini, 2006).

Besides, chicken meat is widely used for the elaboration of many meat products such as chicken frankfurter, chicken bologna, chicken meat balls, chicken ham, chicken burgers, chicken longganisa, etc. Manufacturers endeavour to make chicken and turkey sausages similar to red meat sausages in taste and flavour, but point out the health benefits of poultry products (low fat, low cholesterol, etc.) (Heinz and Hautzinger, 2007).

4.2. Proximate composition

In general, meat is a good source of proteins, lipids, carbohydrates, as well as many other essential compounds (vitamins, minerals, trace elements, etc.). In addition, poultry meat well fit the consumer demand for a low-fat meat (3-8%), low sodium (0.09%) and cholesterol levels (Suchý et al., 2002) with a low energetic value (519–741 kJ/100 g).

Poultry meat belongs to a group of high-quality meats because of its valuable dietetic and nutritional properties. The chemical composition and the ratio between muscles and fat in carcass are distinctive quality parameters. Content of muscle tissue

of the carcass varies between 40-70%. In the case of broiler crosses muscle tissue proportion in the pectoral muscle is from 94 to 98% and in the leg from 92 to 97%. Poultry meat consists of dry matter and water. The water represents approximately 75% of the muscle (although different cuts may have more or less water) and 20-25% of muscle is composed by protein with the remaining 5% representing a combination of fat (1.2-2.5%), carbohydrate and minerals (Maiorano et al., 2012). Dry matter contains inorganic substances (ash) and organic substances such as nitrogen-containing substances (proteins, amides) and non-nitrogen substances (sugars, starches, fat and organic acids). Proteins are the most important components of meat from both the nutritional and technological point of view Steinhauser et al. (2000). The proteins are "highly nutritious" and contain essential amino acids, however individual parts of the carcass differ in protein content. In comparison with dark-red thigh muscles, light-red breast muscles contain more proteins, less fat and less myoglobin per 1 g of muscle tissue. The highest portion of proteins can be found in breast muscles from chicken and turkey. According to Simeonovová (1999) breast muscles contain approximately 22.00 % of proteins while thigh muscles, which contain more fat, consist of ca 17.20 % of proteins.

Fat (esters of fatty acids and glycerol) represents the highest portion of all lipids (99 %) present in meat and is very important from a sensory aspect since it is a source of many aromatic substances affecting the meat taste. The remaining part consists of phospholipids and other associated substances. Based on a single value for a 100g lean fillet serve, lean chicken breast has relatively higher proportions of monounsaturated fatty acids (>20% compared to beef and tuna) and polyunsaturated fatty acids (>30% compared to beef, lamb and pork) (Rule et al., 2002; Probst, 2009). Abdominal fat represents a relatively high portion of the total body weight of chickens causing a significant loss during carcass processing.

Gender is one of the factors affecting fat accumulation during growth. As a result of different growth intensity, females accumulate more fat in comparison with males. Intramuscular fat increases energy value, improves the taste, but too much body fat inhibits gastric acid secretion and complicates protein digestibility (Jukna et al., 2007). Consumers prefer lean meat with reduced content of fat. However, overmuch low intramuscular fat content in worse taste qualities of meat (Valsta et al., 2005; Jukna

et. al., 2010). Intramuscular fat is the most variable part of the meat. Its coefficient of variation is several times higher than other meat characteristics

Although the level of ash represents only a very low portion of the total weight (ca. 1 %), it is a significant criterion for evaluation of the mineral content in muscles. In fact, chicken meat is also a major source for phosphorus, iron, copper, zinc, and selenium besides vitamin B1, niacin, B2, B6 and B12 and vitamin A.

4.3. Quality evaluation

The chicken meat and meat products are highly perishable. They are deteriorated very quickly due to internal factors (composition, kind of meat etc) and external factors (handled, processing conservation, etc.). The meat is an ideal medium for bacterial growth because of its high moisture content, richness in nitrogenous compounds (essential amino acids, proteins), minerals, vitamins and other growth factors. Furthermore, meat pH is favourable for the growth of micro-organisms. The water activity (aw) of poultry meat is about 0.98 to 0.99 depending on if and how long the meat has been stored in dry air. The pH of chicken breast muscle is 5.7 to 5.9, while that of leg muscle is 6.4 to 6.7 (Bhaisare et al., 2014). Both poultry muscle and skin are excellent substrates for supporting the growth of a wide variety of microorganisms (ICMSF, 2005). The results of deterioration is the loss of the quality and a short-life of these products.

The quality concept of food is a fairly broad definition. According to the Standard ISO 9000:2000, the quality is defined as "The totality of features and characteristics of a product, process or service that bear on its ability to satisfy stated or implied needs" (FAO 2004).

The quality of meat in general and particularly of poultry meat can be assessed from different points of view. From the standpoint of consumer interests and the slaughter industry, from this last point broilers should have not only high slaughter yields and desirable carcass conformation scores etc (daily weight gain; feed intake, feed conversion ratio). The poultry meat and meat products could be examined for nutritional, sensory, physico-chemical and microbiological evaluation to assure their quality (Touraille, 1994; Jassim et al., 2011; Ingr, 1989).

4.4.1. Physico-chemical methods

There are different physico-chemical methods of assessment of the chicken meat and meat products quality. The composition of this meat and products are also analyzed with different phicycochemical methods such as protein content in the diet, in the ingredients supplemented in the diet, and in meat and patties samples, by using a Nitrogen Determinator LECO FP-2000 for protein. The quality of poultry meat gathers others quantifiable properties of meat such as water holding capacity, texture (shear force), drip loss, cooking loss, pH, collagen content, protein solubility, cohesiveness, and fat binding capacity, color, etc which are indispensable for processors involved in the manufacture of value-added meat products (Allen et al., 1998). A basic understanding of the live production and processing factors that influence these poultry meat quality attributes, especially color and texture, is necessary to produce consistently high quality poultry products.

4.4.1.1. Color

Among these quality attributes, color is the most critical for the selection of many food commodities, including poultry products. Color has long been known to be a major selection criterion for fresh poultry and meat products as well as for final consumer's product satisfaction or rejection of the products.

Among the factors affecting poultry meat, color include the nature and reactions of the major meat pigment, myoglobin, as well as effects of nitrates and nitrites, ovens and environmental gasses (primarily carbon monoxide and nitric oxide), age, sex, and strain of animal, scalding temperature, irradiation, cooking temperature, storage, canning, processing additives, pre-slaughter conditions, haemachromes, and cytochrome C reactions on final meat color.

The major contributing factors to poultry meat color are myoglobin content, chemical state of the haem structure, and meat pH. Myoglobin content has been shown to be primarily related to species, muscle, and age of the animal. Muscle pH has been shown to be primarily related to the biochemical state of the muscle at time of slaughter and following rigor mortis development. Both of these factors contribute to meat color and the occurrence of meat color defects. The various ionic and covalent complexes of both the ferrous and ferric state of the haem with oxygen and other compounds to

form the basic meat colour variations from the purplish red of deoxygenated myoglobin to the bright red of oxymyoglobin to the brown/gray of metmyglobin are well established. The reactions with various nitrogen compounds and heat to form stable nitrosyl haemachrome complexes produce the desirable pink colour of cured red meats or the undesirable pinkness of some poultry products (Bard and Townsend, 1978).

Muscle pH and meat colour have consistently been reported to be highly correlated, (inversely correlated) especially when wide ranges of meat colour are examined. Higher muscle pH is associated with darker meat while lower muscle pH values are associated with lighter meat. The effect of pH on meat colour is complex. One effect, as noted earlier, is that many of the haem-associated reactions are pH dependent. In addition, muscle pH affects the water binding nature of the proteins and therefore directly affects the physical structure of the meat and its light reflecting properties (Briskey, 1964). Also, pH affects enzymatic activity of the mitochondria1 system thereby altering the oxygen availability for haem reactivity (Ashmore et al., 1972 and Cornforth and Egbert, 1985).

Color of meat can be evaluated using different subjective or objective systems. For the objective assessments usally are study the most popular is the CIE LAB, defined by the Commission Internationale de I'Eclairage (CIE) (1978). In the CIE LAB, the L* value is an expression of the lightness of the surface ranging from 0-100 (black to white), a* value indicates red, ranging from negative to positive (green to red), and b* value also ranging from negative to positive, which stands for blue to yellow (Barbut, 2002) (Figure 4.1 and 4.2). For this measure are use different equipment such as Chroma Meter (Konica Minolta Business Technologies, Tokyo, Japan), Hunter L, a, b; Munsell (hue, lightness, and chroma). A wide range in lightness (L*) of breast meat has been reported by different researchers ranging from 35 to 71(Barbut, 1997; Woelfel et al., 2002; Wilkins et al., 2000; Anadon, 2002; Petracci et al. 2004; Lesiow et al., 2007).

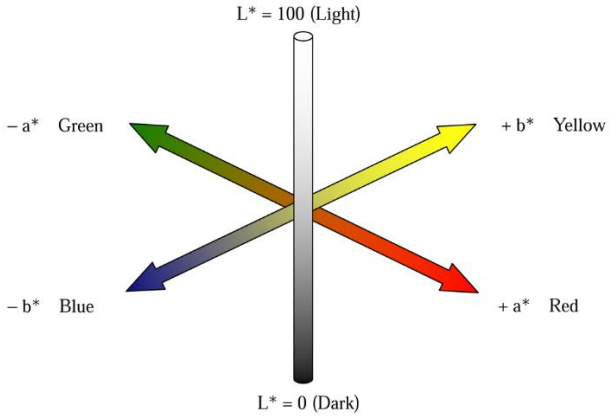


Figure 4.1. CIE LAB color space

Figure 4.2. CIE LAB color measuring instrument spectrophotometer

4.4.1.2. Texture

Texture is probably the single most critical quality factor associated with the consumers' ultimate satisfaction with a poultry meat product. The two major contributors to poultry meat tenderness are the maturity of the connective tissues and contractile state of the myofibrillar proteins. The first, maturity of the connective tissue involves the chemical cross bonding of the collagen in the muscle. Since collagen cross-linking increases with age, meat is generally tougher from older animals. The second factor, the contractile state of the myofibrillar proteins, is primarily a function of the rate and severity of rigor mortis development. As the modern broiler industry developed and began to dominate the chicken meat market, the issue of age related toughness (connective tissue cross-linking) has virtually disappeared. Except for spent hen and older bird utilization, or for specialty markets such as for capons, age related connective tissue toughness is not a major factor in broiler meat quality since the market age of broilers is less than 7 to 8 weeks of age. The myofibrillar protein impacts on ultimate meat tenderness are primarily a function of the biochemical predisposition of the muscle at the time of slaughter, the rate and severity of rigor mortis development, and the physical handling of the carcass and muscle during rigor development. With traditional broiler industry production practices, processing, and the predominant marketing of whole carcasses the negative impact of the myofibrillar

protein reactions were not thought to have a major impact on meat quality. However, in recent years with the dramatic increase in cut up, deboned meat, and further processed products, the demands are placed on the slaughter plant to cut up and debone the carcasses as fast as possible. If the carcass is cut-up into parts, or more importantly, if the breast meat is removed from the carcass prior to the completion of rigor mortis, the muscles will contract unimpeded by the normal skeletal restraint, the muscle fibres will contract and shorten the muscle, and the resulting meat will be less tender. Although the predominant marketing of young broilers minimizes age associated toughness, the economic incentive to cut-up and debone broilers earlier in the processing scheme has resulted in an increased incidence of tough broiler breast meat. During the past 20 years, intensive research efforts have been focused on determining the live bird and processing factors which affect breast meat tenderness. The ultimate goal has been to develop slaughter methods which would allow for acceleration of post-mortem rigor mortis such that carcasses could be cut-up and deboned as soon after slaughter as possible.

Tenderness is also associated with the pH and other properties like the water hold capacity, cook loss etc. in meat even with defect like poultry meat defects including PSE (pale, soft, exudative) and DFD (dark, firm, dry) (Allen et al. 1997 and 1998) and with the time of postmortem.

The evaluation of texture may be controlled by objective or subjective methods of assessment. The texture could be evaluated objectively through the Texture profile analysis (TPA), shear force, Kramer shear force (KSF), Warner-Bratzler (WB) shear etc. using different instrument like TA-XT plus Texture Analyzer or Instron (Herrero et al., 2008) (Figure 4.3).

Lyon and Lyon (1996) reported that the average Warner-Bratzler (WB) shear value for intact broiler breasts deboned at 2 h postmortem was among 4.49 kg- 10.12 kg of force for breast. In chicken products like chicken balls the hardness, cohesiveness, springiness and shear force were ranged between 3.73-5.73, 0.55-0.69, 11.40- 13.71, 31.27-53.77 and 0.51-1.28, respectively (Huda et al., 2009).

Figure 4.3. TA-XT plus Texture Analyzer

4.4.1.3. Lipid oxidation

Oxidation in lipid has been demonstrated as the main, non-microbial cause of quality deterioration during processing and storage. Currently, lipid oxidation is one of the biggest economic problems in the meat industry since decreases the market value of meat and meat products (Contini et al., 2014; Sample, 2013, Palmieri & Sblendorio, 2007). (Kolakowska & Bratosz, 2010). The undesirable effects of lipid oxidation on food quality are reflected on the sensory attributes (development of off-odor, off-flavor, discoloration, undesirable texture), nutritional value (loss of PUFA, vitamins, antioxidants and damage of proteins, amino acids, formation of protein radicals, lipid-protein interactions), toxicity (generation of hydroperoxides, aldehydes, epoxides, dimers, oxysterols, *trans* fatty acids, Maillard type products) and technological suitability (decrease of emulsifying activity of protein and protein solubility) etc. (Kolakowska, 2002).

The rate of lipid oxidation in fresh and cooked meat products depends on various internal factors such as fat content, fatty acid composition mainly high concentrations of unsaturated lipids, heme pigments, iron content, metal catalysts ntioxidants, and a range of oxidizing agents in the muscle tissue (Addis, 1986; Du et al., 2000; Choe and Min, 2006; Min et al., 2008). These factors are also influenced by

animal breed and species, muscle types and anatomical location (Min et al., 2008). Moreover, the type of diet consumed by animals during the production phase has a great influence on the susceptibility of meat to oxidation postmortem. Poultry meat tissues have low lipid content but contain relatively high polyunsaturated fatty acids (Igene and pearson, 1979, Pikul et al., 1984) that are the main oxidation substrates, with several double bonds that are thermodynamically favored sites for attack by lipid peroxyl radicals (Gardner, 1989). Zhang et al., (2011) reported an increase in lipid and protein oxidation in the breast muscles of birds that had been fed a dietary oxidized oil diet compared to antioxidant-supplemented and control diets.

The external conditions such as exposure of meat to oxygen, light and temperature, as well as preservative and processing techniques, such as chilling, freezing, additives (salt, nitrate and spices), cooking, irradiation, high pressure and packaging, could play important roles in lipid oxidation process (Ahn et al., 2009)

Autoxidation is considered the major mechanism that causes lipid oxidation in food and meat products and is initiated by reactive oxygen species (ROS) (Gray and Monhan, 1992; Min and Ahn, 2005). Autoxidation mechanism is well studied and summarized into three main steps: initiation, propagation and termination (Gray and Monahan, 1992; Min and Ahn, 2005; Laguerre et al., 2007) (Figure 4.4)

The initiation step is the rate-limiting, but very important in lipid peroxidation process, this usually starts by reactive oxygen species that abstracts hydrogen atoms from the site of a fatty acid chain and form a lipid free radical (L·). This lipid free radical will react rapidly with oxygen to form a peroxyl radical (LOO·). The peroxyl radical (LOO·) formed in the presence of oxygen abstracts hydrogen atom from another hydrocarbon chain, and yields hydroperoxide (LOOH) and a new free radical (L·), which later decompose to produce the volatile aromatic compounds that give meat its perceived off-flavours and rancid odour (Chaijan, 2008) and participate in propagation chain reaction (Pearson et al., 1977; Enser, 1987). Lipid peroxyl radical and alkoxyl radical can also abstract hydrogen atom from another lipid molecule and initiate and propagate the chain reaction (Addis, 1986; Coyle and Puttfarcken, 1993; Min and Ahn, 2005). The final step is called termination step in which free radicals such as lipid peroxyl radicals (LOO·) react with each other to form non-radical products such as aldehydes, alkanes and conjugated dienes. Formation of aldehydes has been found to

be directly related to the deterioration of meat colour and flavour, protein stability and functionality (Lynch et al., 2001; Min & Ahn, 2005). The consequence of aldehydes has also been associated with atherosclerosis, putative mutagens and cancer formation in the body (Duthie et al., 2013).

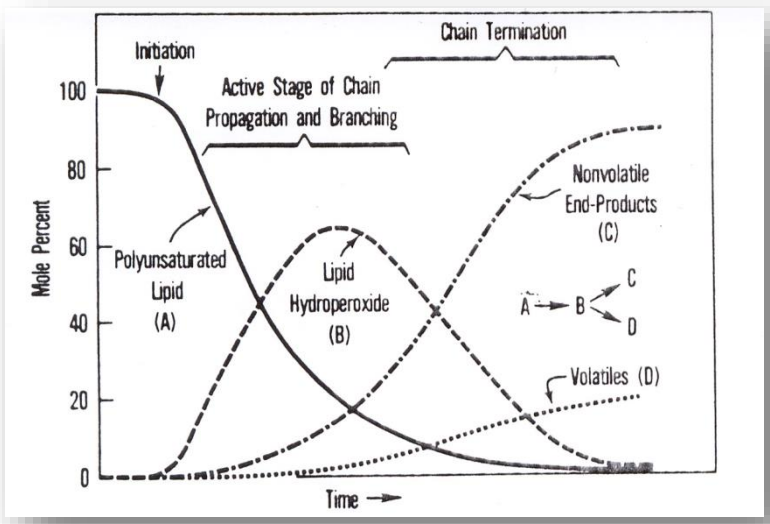


Figure 4.4. Hypothetical autoxidation of a polyunsaturated lipid as a function of time (Gardner, 1983)

Oxidation in meat is usually assessed by measuring the amount of peroxide value (PV), thiobarbituric acid-reactive substances (TBARS), sulphydryl and carbonyl group generated during the process. This analysis is carried out using spectrophotometric or chromatographic (head space gas chromatographic (GC), high-performance liquid chromatography (HPLC) and liquid chromatographic mass spectrophotometer [(LC–MS) and 2,4 dinitrophenylhy-drazine (DNPH)] methods. Recently, studies on protein–lipid oxidation have been conducted at a molecular level using mass spectrophotometry (MS) and liquid chromatography–tandem mass spectrophotometer (LC–MS/MS) with proteomic tools to better understand the mode of mechanism in relation to meat quality.

To avoid oxidative process is essential finding a good balance between prooxidative/antioxidative factors of muscle food products, preserving the natural antioxidant stability and manipulating animal diet with antioxidants supplementation or adding antioxidant compounds directly during processing.

4.4.1.3.1. Antioxidants

An antioxidant can be defined as: "any substance that, when present in low concentrations compared to that of an oxidizable substrate, delays or inhibits the oxidation of that substrate" (Murthy, 2001). Antioxidants may protect cells from the damage caused by free radicals. They trap the reactive substrate radicals and peroxide radicals before they can react with oxygen or substrate (Figure 4.5)

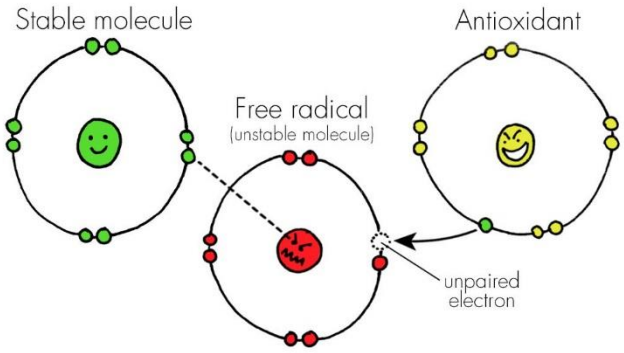


Figure 4.5. The antioxidant behavior

The antioxidant system basically works at three different levels of defense: the first level that is responsible for the prevention of free radicals formation by antioxidant enzymes such as superoxide dismutase (SOD) and glutation peroxidase (GP_X); the second level that combats the production of free radicals and is made of chain-breaking antioxidants such as vitamin E, β-carotene, vitamin A, vitamin C and uric acid and the third level that activated for eliminating or repairing the molecules damaged by free radicals. It primarily consists of enzymes such as lipases, proteases, nucleases and various transferases (Surai 2002; 2006; 2016; Niki 2014).

Antioxidants are increasingly important additives in food processing. They are widely used as food additives to prevent rancidity and also in animal diets. Antioxidants can potentially promote meat tenderness as well. Tenderness and associated juiciness (water-holding capacity) are important quality parameters of meat as well. More recent research has suggested a new role of antioxidants in inhibiting cardiovascular disease and cancer.

The two major types of antioxidants found in food are natural antioxidants and synthetic antioxidants.

4.4.1.3.1.1. Synthetic antioxidants

Synthetic antioxidants as their name suggests are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation (Shahidi, 1992). Synthetic antioxidants include butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), 2,4,5-trihydroxybutyrophenone (THBP), propyl gallate (PG), octyl gallate (OG), nordihydroguaiaretic acid (NDGA) and 4-hexylresorcinol (4HR) (Guan et.al., 2005; Nazni et.al., 2013;Guo et.al., 2006, Ruiz-Capillas and Nollet, 2016).

The use of synthetic antioxidants in food is classified with a E number and their use in food products is regulated and controlled by regulatory laws of a country or international standards. In the United States the use of antioxidants is subject to regulation under the Federal Food, Drug and Cosmetic Act, Meat Inspection Act, Poultry Inspection Act, and other state laws (Mikova, 2001; Shahidi & Zhong, 2005). In the European Union, regulation of antioxidants is stipulated by the European Parliament and Council Directive No. 95/2/EC of 20 February 1995 on food additives other than color or sweeteners. Another organization that regulates the use of antioxidants is the Codex Alimentarius, which is a collection of internationally adopted standards. Codex Alimentarius permits only the use of those antioxidants which have been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and these may be used only in foods standardized by Codex (Mikova, 2001; Davidson and Naidu, 2000). The Food and Drug Administration (FDA) requires that their presence be mentioned on food labels along with an explanation of their intended usage (Newell-McGloughlin and Burke, 2014.). The permissible levels of synthetic antioxidant in food is decided based on the fat content in the recipient food item, and are usually limited to 0.02% total antioxidants (PFA, 2008).

Although there are many compounds that have been proposed to possess antioxidant properties to inhibit oxidative deterioration, only a few can be used in food products.

The synthetic antioxidants when used within the recommended levels have a powerful in protecting product quality during processing and distribution by preventing the lipid deterioration and increasing the shelf life. However, in last decade

the use of the excess antioxidants added to food might has been related with toxicological effect in the consumer and thus endanger their health (Mattila and Kumpulainen, 2001; Shalini, 2012). In this sense has been fixing the daily intake of antioxidants like BHT and BHA, and they have been estimated to be about 0.1 mg/kg. At larger doses of about 500 mg/kg i.e. 5000 times the normal intake, both BHT and BHA have been shown to exert pathological, enzyme or lipid alterations or carcinogenic effects (Madhavi et al., 1996, Olsen et al., 1986). Even though at current levels of intake the synthetic antioxidants seems to pose no reasonable threat to health, but long term chronic ingestion of these antioxidants may aid in modifying the acute toxicity of several carcinogenic and mutagenic chemicals and lead to chronic side effects. Moreover, the effects exerted by the antioxidants also depend on their pattern of metabolism and the excretion of the metabolic products (Nagy, 1980; Sharoni et al., 2000; Mattila and Kumpulainen, 2001).

This toxicological problem in relation with the synthetic antioxidant has been promoted the use of natural antioxidants by consumers and the meat industry (Karre et a., 2013).

4.4.1.3.1.2. Natural antioxidants

The consumer demands are increasingly focusing on minimally processed food products, with less use of synthetic additives and at the same time without compromising food safety. Various Natural antioxidants are using in the food industry and for dietary of animal such a as Tartaric acid, Ascorbic Acid (vitamin C), Vitamin E and Similar Compounds, Citric Acid and Citrate, different Phenolic Compounds as Gallic, Caffeic, Ferulic and p-Coumaric Acids, flavonoides etc.The use of this natural antioxidant are also regulate by the legislation and classify with the number E (Davidson and Naidu, 2000, Ruiz-Capillas and Nollet, 2016).

The vitamin E is the more common natural antioxidant use in food and in animal dietary. Vitamin E is the standard term used to describe at least eight naturally occurring compounds that exhibit the biological activity of α-tocopherols (Jensen and Lauridsen, 2007). This group comprises α β, γ, δ - tocopherol and tocotrienol (Figure 4.6).

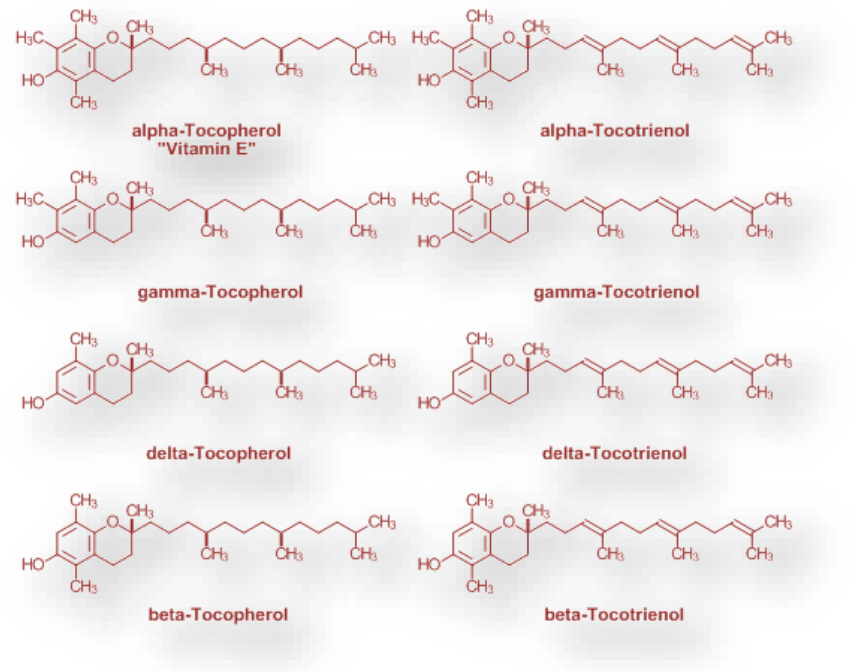


Figure 4.6. Vitamin E family

Vitamin E is a vital component of biological membranes with membrane-stabilizing properties and potent antioxidant activity. In poultry, vitamin E deficiency causes a wide variety of disorders (nutritional muscular dystrophy, erythrocyte hemolysis, exudative diathesis, cerebellar encephalomalacia etc.). (Green, 1972; Combs, 1981; Nair, 1972). Therefore their dietary supplementation is necessary to maintain their optimal health and high productive and reproductive performances. This also includes positive effects on male and female reproduction, immune-competence, effective growth and development, high quality of eggs and meat as well as decreased negative consequences of various stresses (Surai, 2002, 2006, 2014; Surai and Fisinin 2015).

Vitamin E cannot be synthesized by animals and has to be supplied by the diet, thus its presence in body tissues is a reflection of dietary availability. Dietary vitamin E is commonly supplemented in the diet as α-tocopherol acetate, which is characterized by great stability during storage, feed processing, and passage through the forestomach of the animal (Mitsumoto, 2000; Brenes et al., 2008; Brenes et al., 2010). The National Research Council's Committee on Animal Nutrition, USA,

provided the nutrient requirements for poultry species including the vitamin E. According to its recommendations, poultry feed can be supplemented with 10 IU of vitamin E per kg feed (1 IU = 0.67 mg dl-α-tocopheryl acetate) for chickens aged up to six weeks, 5 IU/kg feed for chickens aged over six weeks, (NRC, 1994).

On the other hand administration of vitamin E to poultry also improves both body fat stability and meat quality such as water retention, lipid oxidation, cholesterol oxidation and color stability (Morrissey et al., 2000; Chen et al., 2008; Ripoll et al. 2013). In general, enhancing diets with antioxidants and optimizing nutrient intake not only could reduce lipid oxidation but also may improve water-holding capacity and textural traits of meat. Vitamin E (α-tocopherol) is perhaps the best researched dietary antioxidant. It is an essential nutrient for the growth and health for animal species by functioning as an antioxidant in various biological systems. α-Tocopherol neutralizes free radicals slowing the propagation of lipid oxidation of the highly unsaturated fatty acids in the cellular and subcellular membranes (Burton and Traber, 1990) (Figure 4.7). Early studies have demonstrated delayed lipid oxidation and color deterioration of beef from cattle fed vitamin E supplementation diets (Morrissey et al., 2000). Dietary vitamin E supplemented lamb showed delayed metmyoglobin formation during storage compared to unsupplemented lamb and that the length of the finishing period feeding (supplemented with vitamin E) directly influenced muscle vitamin E content (Ripoll et al. 2013). Gao et al. (2010) also reported that feeding high levels of α- tocopherol lowered thiobarbituric acid-reactive substances production in the tissue and plasma of oxidatively stressed broilers. Similarly, Xiao et al. (2011) and Li et al (2009) reported decreased lipid and protein oxidation, and improved tenderness, respectively, in chickens fed a vitamin E supplemented diet.

On the other hand also has been study the directly incorporation of α-tocopherol into the meat products like pork patties through processing, where also was observed some protective effect (Chen et al., 2008); however, the effect was not nearly as much as that through dietary method.

The use of dietary antioxidants has a distinct advantage over incorporation of antioxidants to meat through processing because dietary antioxidants absorbed by the bird can be effectively distributed in muscle (meat) both inside the cell and at the

membrane, which is not possible if the antioxidants are incorporated into meat through blending and mixing.

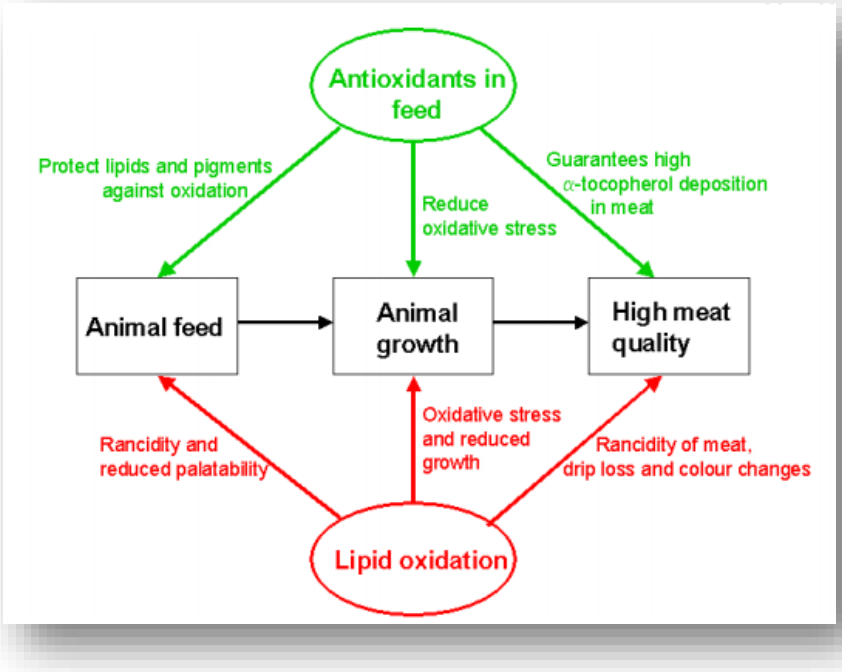


Figure 4.7. Schematic overview of interaction between meat quality, lipid oxidation and antioxidants in animal diets

In recent years, special attention has been paid to a number of medicinal plants that could be used as potential sources of antioxidants for muscle food preservation and nutritional quality improvement. In contrast to synthetic antioxidants, the use of natural antioxidants from spices is increasing since their application is less stringently regulated in most countries around the world. More of these compounds present also antimicrobial properties. Phenolic compounds are the major constituents of plant materials that contribute to their antioxidant and antimicrobial capacity. In addition, most of the plant materials (herbs and spices) possess relatively high chemical nutrients (such as protein, fat, carbohydrate), mineral contents (calcium, potassium, iron, phosphorus) and less anti-nutritional properties.

Several authors have reported the efficacy of different plant products for reducing lipids and protein oxidation, discolouration and microbial growth in some types of meat (Camo et al., 2008; Fasseas et al., 2007; Zinoviadou et al., 2009). The use of natural compounds such as organic acids and essential oils has been identified

for decontamination of beef, pork and poultry products against *Salmonella* (Mani-López, et al., 2012; Sant'Ana et al., 2014). The effectiveness of medicinal plants for example: *Artemisia absinthium*, *Hypericum perforatum*, *oleoresin rosemary*, *Origanum vulgare*, *Satureja horvatii*, *Syzygium aromaticum*, *Fatsia* spp., and olive among others, against microbial growth in meat and meat products has been reported in several studies (Kim, et al., 2013; Kurcubic et al., 2014; Sanchez-Muniz et al., 2012). Krishnan et al. (2014) found a stronger antimicrobial effect of the combination of *S. aromaticum*, *Cinnmomumcassia* and *O. vulgare* extracts in chicken meat than individual spices, and they attributed this to synergistic actions of each specific compounds present in the mixed spices. The presence and level of concentration of different phytochemical compounds such as phenolic, flavonoid, alkaloids, saponins, tannins, carvacrol, terpenes, and thymol among others, have been recognized as the potential source of antimicrobial activities in plant materials (Sharma et al., 2012). In the meat and chicken products, rosemary and sage extracts found to be effective in reducing the rancidity and improve shelf-life (Murphy et al., 1998). In 2010, the European Union authorized the use of rosemary extracts as new food additives for use in foodstuffs under Directive 95/2/EC and assigned E 392 as its E number (European Union directives 2010/ 67/EU and 2010/69/EU) and the applications specified by the directives include meats. With the approval of carnosic acid and carnosol-based rosemary extract as a safe natural alternative to synthetic antioxidants, a new trend for "natural products" has emerged.

4.4.2. Microbial analysis

The microbial contamination in the chicken meat and meat products is the main reason of delay the shelf life of these products. The initial contamination of poultry meat may occur in various stages, including slaughtering, cutting, processing, storage and distribution of meat. Sources may include water, facilities, equipment and manipulators. The skinning stage is particularly important, due to the high microbial load on the leather surface. Fecal material, which may contain deteriorative microorganisms and pathogens, can also serve to as a source of microorganisms. The economic and public health consequences of the presence of microorganisms in food depend on the species and quantity present. The number of microorganisms present in

the product determines whether the contamination will cause microbial deterioration or disease (Fleet, 1999).

The rate of growth of microorganisms in a food item depends on the characteristics of the food itself such as the chemical structure, pH level, presence of in inhibitors and competing microorganisms, and water activity as well as the environmental conditions such as the temperature and relative humidity of the environment and the air motion. Meat spoilage is the most frequently caused by the following groups of bacteria: 1) *Pseudomonas* spp. 2) *Enterobacteriaceae* 3) *Brochothrix thermosphacta* 4) *Lactic acid bacteria (*Barros-Velazquez, 2015; Pennacchia et al. 2011).

Different measures have been taken to minimize growth of organisms in meat chicken and meat chicken products the more common was additives and preservation technologies. The more usual technologies is the chilling during distribution channels, chilling is normally used to preserve the meat to keep the meat qualities in a fresh-like state, while freezing is an alternative for longer storage or distribution time. Even in chilled storage, the microbial growth continues to spoil the product or endanger the food safety. Freezing stops the microbial growth, which can be resumed later in subsequent thawed storage.

Vacuum packaging and modified atmosphere also have proven to be efficient in extending shelf life, preserving the sensory characteristics inherent to the product for a period sufficiently long for its turnover. Vacuum packaging is an anaerobic/microaerobic microsystem that delays the growth of aerobic bacteria such as *Pseudomonas* and promotes lactic acid bacteria, which have a lower potential for deterioration and limited growth at low temperatures (Sarantópoulos et al., 2001). Vacuum packaging, which is used in the conditioning of whole pieces or small parts, aims to protect the meat product from contact with oxygen from the air. Oxygen promotes the growth of aerobic microorganisms, which can change the odor, color and appearance of meat products, cause oxidative rancidity of the fats, change the meat pigments and destroy vitamins and flavors (Sarantópoulos and Soler, 1991). Under aerobic conditions, *Pseudomonas* spp. can dominate the meat quickly and contribute to its deterioration (Gill and Newton, 1978; Lebert et al., 1998). Vacuum-packaged meats are generally quite stable at low temperatures (Labadie, 1999). Despite the

increased shelf life, fresh meat packed under different technologies will deteriorate after some time.

In some case for preservation are usually used additives or preservatives such as sulphites, nitrates and nitrites, benzoates, sorbates, parabens, acitic acid, lactic acid, etc . (Ruiz-Capillas and Jimenez Colmenero, 2008, Ruiz-Capillas and Nollet, 2016). These food additives have various permitted uses, their primary function is as a preservative to prevent or reduce spoilage, but are also used because of their food technology effects. They help stabilize product colour and inhibit discolouration, thereby improving the oxidation and sensorial appearance and flavour of many foods during preparation, storage and distribution. Therefore, many commonly consumed food and beverages contain varying amounts of these preservatives added during processing or conservation.

Generally, as comment before the use of these additives in normal or general conditions is not a problem for the consumer. However, the use of synthetic compounds have significant drawbacks, such as increasing cost, handling hazards, concerns about residues on food and threat to human environment. In fact, some of them, as sulphites, have been associated with allergic reaction and food intolerance symptoms, being potentially toxic, and that is why they can be considered a hazard to human health (Ruiz-Capillas and Jimenez Colmenero, 2008). Therefore, in recent years because of the great consumer awareness and concern regarding synthetic chemical additives, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds, as those of botanical origin.

Main natural compounds are essential oils derived from plants, extracts of spices and herbs (e.g., basil, thyme, oregano, cinnamon, clove, and rosemary), enzymes obtained from animal sources (e.g., lysozyme, lactoferrin), bacteriocins from microbial sources (nisin, natamycin) etc. The use of these natural antibacterial compounds is widely reported in the literature to improve the shelf life of meat (Jamilah et al., 2008; Jałosńska and Wilczak, 2009). The use of bay essential oil combined to MAP (modified atmosphere packaging) without oxygen (20% CO2—80%N2) was suggested to control *L. Monocytogenes* and *E. coli* growth and also to extend the shelf life of naturally contaminated ground chicken meat (Irkin and Esmer, 2010). Moreover, the addition of essential oils of marjoram and rosemary to beef

patties formulated with mechanically deboned poultry meat at a concentration of 200 mg/kg reduced lipid oxidation and improved the sensory characteristics (Mohamed and Mansour, 2012). A relevant preservation effect for fresh chicken breast meat, stored at 4°C, was obtained by dipping meat in oregano oil, prior to packaging under MAP (Chouliara et al., 2007). Fratianni et al. (2010) also proposed use of thyme and balm essential oils to decrease the natural microflora of chicken breast meat. In particular, balm essential oil significantly limited growth of *Salmonella* sp., whereas thyme essential oil effectively inhibited growth of *E. coli*.

Also grape by-products which represent a good source of polyphenols has been tested with antimicrobial function. It has been shown in numerous in vitro studies (Papadopoulou et al., 2005; Özkan et al., 2004; Rodríguez-Vaquero et al., 2007; Gañan et al., 2009; Silván et al., 2013) that flavonoids present in grape by-products have the capacity to inhibit the growth of certain organisms, such as *S. aureus*, *E. coli*, *C. albicans* and *Campylobacter*.

The safety and hygienic quality of meat are largely determined by the presence of these microorganisms (total number of mesophyllic aerobic bacteria, anaerobic bacteria, coliform, *Enterococcus*, *S. aureus*, *Salmonella*, *Bacillus cereus*, *Clostridium perfringens* (Filimon et al., 2010). With microbial quality change and spoilage, the physical, chemical, microbiological and sensory attributes also change, and sometimes their interrelationship is used to determine meat shelf life. This control is equally important to producers, retailers and consumers (Mead et al., 2004).

4.4.3. Sensory analysis

The major poultry meat quality attributes are appearance, texture, juiciness, flavor, and functionality. Of these, the most important have been appearance and texture since they most influence consumers' initial selection and ultimate satisfaction with traditional poultry meat products. Although juiciness and flavor are extremely important, except for isolated defects they are most often more a function of preparation than of the product itself. With the increasing trends in further processing, meat functionality and all of the sensory quality attributes have increased in relative importance. Complex products such as sausages, hamburgers, marinated fillets; breaded and cooked products, etc. require an understanding of the contribution of

poultry meat of these products as well as their influence on sensory proprieties of the food.

The sensory evaluation is a subjective method that involves the discrimination and description of sensory components of products by a trained panel (Murray et al., 2001) (Figure 4.8). It is defined as "a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of food and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing (Stone et al., 2012).

Trained panelists should be capable of identifying and quantifying the specific attributes, and providing information regarding the instrumental and sensory measurements of foods which have a fundamental role in the food preference determination (Lyon and Lyon, 2001; Touraille, 1994; Gigaud et al., 2008). Some attributes are especially important in chicken such as color, tenderness, juiciness and flavor (Touraille, 1994, Clinquart, 2000; Santé et al., 2001).

The sensation of juiciness is composed of two organoleptic components. First the impression of wetness during the first few chews produced by the rapid release of meat fluid; the second is a sustained juiciness largely due to the stimulatory effect of fat or salivation. Tenderness and juiciness are closely related and, in general, the more tender the meat, the more readily juices appear to be liberated during eating. Flavour and odour are closely related. Generally, flavour is linked to water-soluble materials, and odour is related to fat-soluble volatile elements. Flavour, which comprises mainly the two sensations of taste and aroma or smell, has been found to be one of the most important factors affecting consumers' meat-buying habits and preferences even before the meat is eaten (Shahidi, 1989; Sitz et al., 2005). Lipids play a vital role in the flavour development of poultry meat (Perez-Alvarez et al., 2010). Lipid degradation, mainly the oxidation of the fatty acid components of lipids, results in several hundred volatile compounds and the possible development of off flavours.

Figure 4.8. Test rooms of CSIC for sensory evaluation

CHAPTER 5

AIM OF DISSERTATION

The general aim of this study was to assess the potential use of grape pomace, grape seeds and grape skins, the major residues from wine-making industry and a good sources of polyphenols, as a cheaper but functionally equivalent product, with antioxidant activity, that could partially replace vitamin E in broiler chickens diet and be able to improve poultry performance and welfare, besides to warrant high-quality, safe and funtional meat products.

There is no information in literature about the separated effect of the principal components present in grape pomace, ie skin and seed. To this end five experiment were designed to:

- ✓ Evaluate, in the I, II and III experiment of this thesis, the effect of different concentrations of grape pomace, grape seed and grape skin, different types of grape skin (fermented and unfermented), and the effect of vitamin E (200 mg/kg) inclusion in a corn-soybean basal diet on growth performance, ileal and excreta total polyphenols and tannins content, ileal digestibility of protein and ileal and excreta digestibility of total polyphenols in broiler chickens. The effect on chickens plasma and meat oxidative status was also assessed;

- ✓ Estimate, in the IV experiment, the effect of dietary grape pomace, grape seed and grape skin in improving the shelf-life of meat patties elaborated with the breast meat from chickens fed these diets, and to assess the effect on their physicochemical and sensorial proprieties;

- ✓ Assess, in the V experiment, the effect of grape seed and grape skin directly added to chicken meat patties on their physicochemical and sensorial proprieties.

CHAPTER 6

MATERIAL AND METHODS

6.1. Solvents and reagents

Gallic acid, Folin–Ciocalteau reagent, α-tocopherol, trolox, butylated hydroxytoluene, and 1,1,3,3-tetraethoxy propane were obtained from Sigma–Aldrich (St. Louis, MO). Acetone, butanol, hexane, trichloroacetic acid, thiobarbituric acid, chloridric acid, ethylenediaminetetraacetic acid, sodium carbonate, acetonitrile, NaCl, and methanol were obtained from Panreac (Castellar del Vallés, Barcelona, Spain).

6.2. Proximate composition

Chemical composition was determined in GS, SS and GP grape ingredients, diets, meat and patties.

In GS, SS and GP grape ingredients and in diets, dry matter (DM) (930.15), crude protein (CP) (976.05), crude fiber (978.10) and ash (942.05) were analyzed according to the methods of the AOAC (1995). Crude fat was determined by extraction in petroleum ether after acidification with 4 N HCl solutions (Wiseman et al., 1992). The AIA (Acid insoluble ash) contents of diet, ileal content and excreta were measured after ashing the samples and treating the ash with boiling 4 M HCl (Siriwan et al., 1993).

In meat and patties, moisture (Method 925.09) and ash (Method 942.05) were analyzed in quadruplicated according to the methods of the AOAC (2005). Fat content was evaluated in triplicate according to Bligh & Dyer, (1959) method (Figures 6.1 and 6.2). Protein content was measured in quadruplicated using a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St Joseph, MI, USA).

Figure 6.1. Fat exctaction from chicken meat samples

Figure 6.2 Fat exctaction from chicken meat samples, before and after rotavapor

6.3. Tested products

The grape by-products used in the present study were different for each experiment, therefore their composition is described individually.

I Experiment

Red GP (Vitis vinifera var. Cencibel) was obtained from Grupo Matarromera (San Bernardo-Valbuena de Duero, Valladolid, Spain) (Figures 6.3 and 6.4.). Skin and grape seed were obtained from Hermanos Delgado (Socuéllamos, Ciudad Real, Spain). Proximate composition of SS, GS and GP is shown in Table 6.1.

Figure 6.3. Grape pomace

Figure 6.4. Grape pomace after grinding

Table 6.1. Proximate composition of grape seed (GS), grape skin (SS) and grape pomace (GP)

Item	g/100g of DM[1]	
	GS	SS
Crude fiber	25.28±1.87	14.40±1.33
Protein	19.64±0.41	16.27±0.00
Total polyphenols	8.23±0.16	2.35±0.14
Condensed tannins	1.15±0.20	1.24±0.10

[1] Data are the mean of four determinations ± SD

II Experiment

Fermented (FS) and unfermented (UFS) grape skin was obtained from Explotaciones Hermanos Delgado S.L. Socuéllamos (Ciudad Real, Spain). UFS was taken at the beginning of the wine making process from the grape pomace generated from white grape cultivars. FS was taken after the fermentation process had finished and obtained from different red grape cultivars. Proximate composition of FS and UFS is shown in Table 6.2.

Table 6.2. Proximate composition of fermented (FS) and unfermented (UFS) grape skin

Item	g/100g of DM[1]	
	FS	UFS
Crude fiber	14.4 ±1.9	12.2 ± 0.4
Protein	16.30 ± 0.0	10.00 ± 0.1
Total polyphenols	2.30 ± 0.1	6.56 ± 0.2
Condensed tannins	1.24 ± 0.2	0.61 ± 0.1

[1] Data are the mean of four determinations ± SD

III Experiment

Red GP (Vitis vinifera var. Cencibel), was obtained from Grupo Matarromera (San Bernardo-Valbuena de Duero, Valladolid, Spain). SS and GS were obtained from Hermanos Delgado (Socuéllamos, Ciudad Real, Spain). Proximate composition of SS, GS and GP is shown in Table 6.3.

The same grape by-products were also used in the IV experiment.

Table 6.3. Proximate composition of grape seed (GS), grape skin (SS) and grape pomace (GP)

Item	g/100g of DM[1]		
	GS	**SS**	**GP**
Crude fiber	25.28±1.34	14.40±1.87	33.39±0.84
Protein	17.86±0.04	10.96±0.00	11.37±0.29
Total polyphenols	5.32±20	6.24±10	3.24±20
Condensed tannins	1.11±0.1	1.20 ± 0.2	0.91±0.1

[1] Data are the mean of four determinations ± SD

V Experiment

SS and GS were obtained from Hermanos Delgado (Socuéllamos, Ciudad Real, Spain).

6.4. Birds and diets

Birds and diets were different for every experiment, therefore are individually described for each experiment. The α-tocopheryl acetate (αT) used in the diets was provided by DSM Nutritional Products Iberia S.A. (Alcalá de Henares, Madrid, Spain).

I Experiment

A total of one hundred and eighty 1-day-old male broiler Cobb chicks (Figure 6.5 and 6.6) were obtained from a commercial hatchery. The birds were housed in electrically heated starter battery brooders in an environmentally controlled room with 23 h of constant overhead fluorescent lighting during three weeks. The chicks were allocated to 30 pens (Figures 6.7. and 6.8.), each pen containing six chicks, to receive 6 dietary treatments during 21 days with five replicates per treatment. Diets in mash form (Figure 6.9) and water were provided ad libitum. Celite (Celite Corp., Lompoc, CA), a source of acid insoluble ash, was added at 10 g/kg to all diets as an indigestible marker. The diets were stored in a dark and cool dry location during the experimental period. All diets were formulated to meet or exceed the minimum National Research Council (NRC, 1994) requirements for broiler chickens. Experimental procedures were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes.

Figure 6.5. One day old broiler chickens

Figure 6.6. One day old broiler chickens allocated in the cages

Figure 6.7. Experimental chickens allocated in cages with respective diets

Figure 6.8. Experimental chickens allocated in cages

Figure 6.9. Chickens diets preparation

Ingredients and nutrient composition of diets are shown in Table 6.4. Experimental diets were as follows:

1. Control corn soybean diet (C);
2. C + Vitamin E (200 mg/kg of α-tocopheryl acetate) (C + Vit. E);
3. C + 15 g/kg of grape seed (C+ 15 GS);
4. C + 30 g/kg of grape seed (C + 30 GS);
5. C + 110 g/kg of grape skin (C + 110 SS);
6. C + 37.5 g/kg of grape pomace (C + 3.75 GP).

At the end of the experimental period, birds were weighed and feed consumption was recorded for feed efficiency computation.

Table 6.4. Ingredients and nutrient composition of experimental diets (g/kg as fed)

Ingredients	Control	Control + Vit E*	Control + 15 GS[1]	Control + 30 GS	Control + 110SS[2]	Control + 37.5 GP[3]
Corn (8.1% CP)	409.90	409.90	417.40	414.90	362.80	409.20
Soybean (48% CP)	383.00	383.00	373.00	373.00	359.10	371.00
Sunflower oil	100.00	100.00	100.00	100.00	100.00	100.00
Salt	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate	17.90	17.90	17.90	17.90	17.90	17.90
Calcium carbonate	14.20	14.20	14.20	14.20	14.20	14.20
Vitamin-mineral premix[4]	5.00	5.00	5.00	5.00	5.00	5.00
DL-Methionine	2.00	2.00	2.00	2.00	2.00	2.20
Straw	55.00	55.00	42.50	30.00	15.00	30.00
Grape seed	0.00	0.00	15.00	30.00	0.00	24.38
Grape skin	0.00	0.00	0.00	0.00	110.00	13.13
Celite[5]	10.00	10.00	10.00	10.00	10.00	10.00
Analyzed composition						
Total polyphenols	1.70	1.70	2.10	2.90	2.90	2.60
Condensed tannins (mg /100g)	0.075	0.070	0.171	0.232	0.406	0.241
Crude protein	218.00	207.00	207.00	202.00	201.00	211.00
Calculated composition						
AME[6] (Kcal/Kg)	3106	3106	3113	3119	2963	3099
Ether extract	122.20	122.20	123.00	123.80	126.50	121.60
Crude fiber	48.00	48.00	47.40	46.60	45.80	46.50
Lysine	12.40	12.40	12.30	12.20	12.16	12.18
Meth+Cys	8.80	8.80	8.80	8.82	8.77	9.00
Calcium	10.45	10.45	10.50	10.60	11.10	10.80
Available P	4.50	4.50	4.50	4.50	4.50	4.50

[1]GS = Grape seed.
[2]SS = Grape skin.
[3]GP = Grape pomace.
[4]Vitamin-mineral mix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B12, 12.5 _g; riboflavin, 5.5 mg; Ca panthothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; delquin, 125 mg; DL-Met, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; and NaCl, 2,500 mg.
[5]Celite Corp, Lompoc, CA.
[6]AME = apparent metabolisable energy; calculated values (FEDNA Tables, 2003).
*Vitamin E: 200 mg/kg

II Experiment

A total of one hundred and fifty 1-day-old male broiler Cobb chicks were obtained from a commercial hatchery. The birds were housed in electrically heated starter battery brooders in an environmentally controlled room with 23 h of constant overhead fluorescent lighting during three weeks. The chicks were allocated to 30 pens, each pen containing five chicks, to receive 6 dietary treatments during 21 days with five replicates per treatment. Diets in mash form and water were provided ad libitum. The diets were stored in a dark and cool dry location during the experimental period. Celite (Celite Corp., Lompoc, CA), a source of AIA, was added at 10 g/kg to all diets as an indigestible marker. All diets were formulated to meet or exceed the minimum NRC (1994) requirements for broiler chickens. Experimental procedures were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes.

Ingredients and nutrient composition of diets are shown in Table 6.5. Experimental diets were as follows:

1. Control corn soybean diet (C);
2. C + Vitamin E (200 mg/kg of α-tocopheryl acetate) (C + Vit. E);
3. C + 30 g/kg of fermented grape skin (C + 30 FS);
4. C + 60 g/kg of fermented grape skin (C + 60 FS);
5. C + 30 g/kg of unfermented grape skin (C + 30 UFS);
6. C + 60 g/kg of unfermented grape skin (C + 60 UFS);

At the end of the experimental period, birds were weighed and feed consumption was recorded for feed efficiency computation.

Table 6.5. Ingredients and nutrient composition of experimental diets (g/kg as fed)

Ingredients	Control	Control + Vit. E*	Control + FS[1]30	Control + FS60	Control + UFS[2]30	Control + UFS60
Corn (8.1% CP)	422.90	422.90	411.13	399.35	410.38	397.85
Soybean (48% CP)	375.00	375.00	366.78	358.55	368.75	362.50
Sunflower oil	100.00	100.00	100.00	100.00	100.00	100.00
Salt	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate	17.90	17.90	17.90	17.90	17.90	17.90
Calcium carbonate	14.20	14.20	14.20	14.20	14.20	14.20
Vitamin-mineral premix[3]	5.00	5.00	5.00	5.00	5.00	5.00
DL-Methionine	2.00	2.00	2.00	2.00	2.00	2.00
Straw	50.00	50.00	40.000	30.00	38.75	27.50
Grape skin	0.00	0.00	30.00	60.00	30.00	60.00
Celite[4]	10.00	10.00	10.00	10.00	10.00	10.00
Analyzed composition						
Total polyphenols	2.08	1.90	1.98	2.27	2.42	3.13
Condensed tannins (mg/100g)	0.021	0.017	0.052	0.096	0.074	0.12
Crude protein	207.00	207.00	206.00	205.00	205.00	204.00
Calculated composition						
AME[5] (Kcal/Kg)	3131	3131	3089	3048	3093	3056
Ether extract	122.00	122.00	125.00	127.00	124.00	126.00
Crude fiber	45.00	45.00	44.00	44.00	44.00	43.00
Lysine	12.00	12.00	12.00	12.00	12.00	12.00
Meth+ Cys	9.00	9.00	9.00	9.00	9.00	9.00
Calcium	10.00	10.00	11.00	11.00	11.00	11.00
Available P	5.00	5.00	4.00	4.00	5.00	4.00

[1]FS = fermented grape skin
[2]UFS = unfermented grape skin
[3] Vitamin-mineral mix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B12, 12.5 _g; riboflavin, 5.5 mg; Ca panthothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; delquin, 125 mg; DL-Met, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; and NaCl, 2,500 mg.
[4]Celite Corp, Lompoc, CA.
[5] AME = apparent metabolisable energy; calculated values (FEDNA Tables, 2003).
*Vitamin E: 200 mg/k

III Experiment

A total of two hundred 1-day-old male broiler Cobb chicks were obtained from a commercial hatchery. The birds were housed in electrically heated starter battery brooders in an environmentally controlled room with 23 h of constant overhead fluorescent lighting during three weeks. The chicks were allocated to 40 pens, each pen containing five chicks, to receive 8 dietary treatments during 21 days with five replicates per treatment. Diets in mash form and water were provided ad libitum. The diets were stored in a dark and cool dry location during the experimental period. Celite (Celite Corp., Lompoc, CA), a source of AIA, was added at 10 g/kg to all diets as an indigestible marker. All diets were formulated to meet or exceed the minimum NRC (1994) requirements for broiler chickens. Experimental procedures were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes.

Ingredients and nutrient composition of diets are shown in Table 6.6. Experimental diets were as follows:

1. Control corn soybean diet (C);
2. C + Vitamin E (200 mg/kg of α-tocopheryl acetate) (C+Vit. E);
3. C + 20 g/kg of grape seed+20 g/kg of grape skin (C+GS50% + SS50%) (40 g/kg);
4. C + 30 g/kg of grape seed+10 g/kg of grape skin (C+GS75% + SS25%) (40 g/kg);
5. C + 10 g/kg of grape seed+30 g/kg of grape skin (C+GS25% + SS75%) (40 g/kg);
6. C + 40 g/kg of grape seed (C+GS);
7. C + 40 g/kg of grape skin (C+SS);
8. C + 40 g/kg of grape pomace (C+GS)

At the end of the experimental period, birds were weighed and feed consumption was recorded for feed efficiency computation

Table 6.6. Ingredients and nutrient composition of experimental diets (g/kg as fed)

Ingredients	C	C + Vit. E*	C+ GS[1]50 +SS[2]50	C+ GS75 +SS25	C+ GS25 +SS75	C + GS	C + SS	C + GP[3]
Corn (8.1% CP)	448.10	448.10	456.5	460.2	451.2	465.20	450.00	452.40
Soybean (48% CP)	373.00	373.00	364.0	362.0	364.0	360.00	362.20	360.80
Sunflower oil	86.00	81.00	83.0	82.0	84.0	81.00	85.00	84.00
Salt	3.00	3.00	3.0	3.0	3.0	3.00	3.00	3.00
Monocalcium phosphate	15.30	15.30	15.2	15.5	15.5	15.50	15.50	15.50
Calcium carbonate	15.60	15.60	15.3	15.3	15.3	15.30	15.30	15.30
Vitamin-mineral premix[4]	5.00	5.00	5.0	5.0	5.0	5.00	5.00	5.00
DL-Methionine	2.00	2.00	2.0	2.0	2.0	2.00	2.00	2.00
Straw	42.00	42.00	6.0	5.0	10.0	3.00	12.00	12.00
Grape seed	0.00	0.00	20.0	30.0	10.0	40.00	0.00	0.00
Grape skin	0.00	0.00	20.0	10.0	30.0	0.00	40.00	0.00
Grape pomace	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.00
Celite[5]	10.00	10.00	10.0	10.0	10.0	10.00	10.00	10.00
Analyzed composition								
Total polyphenols	1.8	1.8	4.2	3.9	4.1	4.1	4.4	3.6
Crude protein	203.1	205.6	207.7	206.6	194.0	208.7	204.7	201.1
Calculated composition								
AME[6] (Kcal/Kg)	3095	3095	3089	3089	3080	3093	3076	3078
Ether extract	109.0	109.0	109.0	108.0	109.0	108.0	110.0	110.0
Crude fiber	46.0	46.0	46.0	46.0	46.0	47.0	46.0	46.0
Lysine	12.1	12.1	12.0	12.0	12.1	12.2	12.2	12.2
Meth+Cys	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Calcium	10.0	10.0	10.0	10.0	10.0	11.0	11.0	11.0
Available P	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5

[1]GS = Grape Seed.
[2] SS = Grape skin.
[3] GP = Grape pomace.
[4] Vitamin-mineral mix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B12, 12.5 _g; riboflavin, 5.5 mg; Ca panthothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; delquin, 125 mg; DL-Met, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; and NaCl, 2,500 mg.
[5]Celite Corp, Lompoc, CA.
[6]AME = apparent metabolisable energy; calculated values (FEDNA Tables, 2003).
*Vitamin E: 200 mg/kg

The diets C, C+Vit. E, C+GS, C+SS and C+GS were also used in the experiment IV.

6.5. Collection of samples and measurements

I, II, III Experiments

At 19 days of age, clean stainless steel collection trays were placed under each cage, and excreta from the birds were collected for 48h. A subsample of excreta was collected in polyethylene bags and freeze-dried (Telstar, Terrasa, Spain) for subsequent determination of total exctractable polyphenols content (TEPC) and tannins content (TC).

At 21 days of age, fifteen birds per treatment were euthanized by carbon dioxide (100%) and desangrated (Figure 6.10. and 6.11.), the ileum was quickly dissected out (Figures 6.12 and 6.13) and the content expressed by gentle manipulation into a plastic container and stored at –20ºC. Digesta were pooled from three birds of each replicate within the same treatment. Ileal contents were freeze-dried and ground (1mm screen) (Figure 6.14) and used to determine the TEPC and tannins.

Carcasses from ten birds per treatment were also immediately trimmed for breast and thigh meat (Figure 6.15), and tissues were individually sampled and used to determine lipid oxidation (5 birds/treatment). For lipid oxidation study, tissues samples were wrapped in transparent oxygen-permeable polyvinyl chloride film (13,500 cm^3/m^2/day), frozen and stored at –20º C until required. After thawed, the progress of lipid oxidation, during storage, in meat samples was determined after 1 and 7 days in a no illuminated refrigerated cabinet at 4º C.

Figure 6.10. Chikens CO_2 euthanization

Figure 6.11. Chikens desangration

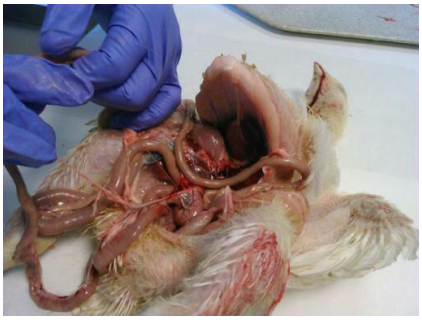


Figure 6.12. Chiken ileum dissection

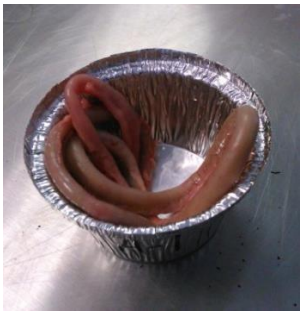


Figure 6.13. Chiken ileum

Figure 6.14. Grounded freeze-dried ileum content

Figure 6.15. Chicken breast and thigh samples

IV Experiment

At 21 days of age, 7 birds per treatment (body weight, 0.824 ± 0.02 kg) were slaughtered and immediately trimmed for breast meat:

- ✓ MC (Control);
- ✓ ME (Control+Vitamin E);
- ✓ MGS (Control + Grape Seed 4%);
- ✓ MSS (Control+ Grape skin 4%);
- ✓ MGP (Control+ Grape pomace 4%).

Samples of raw breast meat were frozen and stored at −20°C until required (no more than 4 days). After thawed in refrigerator during ~12h, meat samples were firstly cut with a knife (Figure 6.16) and then minced (4 mm plate) using a grinder (Mainica, Granollers, Spain) (Figure 6.17). The grinded breast meat was analyzed and then used for the patties preparation.

V Experiment

Fresh chicken thighs (±4 kg) were obtained from a local market of Madrid (Spain) and stored in refrigerator during ~12h. The thigh meat was chosen due to the darker color compared to the breast meat and to compensate for the color that was expected could cause the adding of these by-products. Chicken thighs were firstly boneless, cut and then minced (4 mm plate) using a grinder (Mainica, Granollers, Spain). The grinded meat was then used for patties preparation.

6.6. Preparation of patties

The preparation of chickens patties was different for IV and V experiment, therefore is individually described, as follow.

IV Experiment

Whole eggs, breadcrumbs and salt were obtained from a local market of Madrid (Spain).

Five formulations of patties (Table 6.7.) were prepared:

1. PC (Control);
2. PE (Control+vitamin E);
3. PGS (Control + Grape Seed 4%);
4. PSS (Control+ Grape skin 4%);
5. PGP (Control+ Grape pomace 4%).

The minced meat was first blended in a bowl mixer (Hobart, Model N50, USA) for 60 seconds, and then the salt was added to meat and mixed for an additional 30 seconds. Thereafter, the eggs were beaten and added to the mixture to blend for 20seconds Breadcrumbs were also incorporated into the mixer and mixed for another 60 seconds 60 s to ensure a uniform distribution (Figure 6.18). The same process was repeated for all the samples. From this blend (Figure 6.19), a total of 22 patties (~50 g per patties) for every treatment were formed by using a conventional burger maker (Ministeak burger maker, O.L. Smith Co. Ltd., Italy) (Figure 5.20). The patties were packed in different vacuum high oxygen barrier bags (nylon/polyethylene, 9.3 ml O_2/m_2/24 h at 0ºC, Koch Kansas City, MO) (Figure 5.21), every bag contained 2 patties and were stored in refrigeration at 4º C.

The patties were analyzed on day 0 and after 3, 6 and 9 days of storage.

Table 6.7. Formulation (%) of chicken patties

Sample	Meat	Whole egg	Breadcrumbs	Salt
PC	85.4	6.8	6.8	1
PE	85.4	6.8	6.8	1
PGS	85.4	6.8	6.8	1
PSS	85.4	6.8	6.8	1
PGP	85.4	6.8	6.8	1

Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).

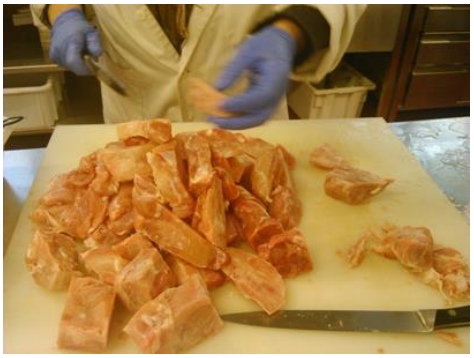


Figure 6.16. Cutting of chicken meat samples with a knife after thawing

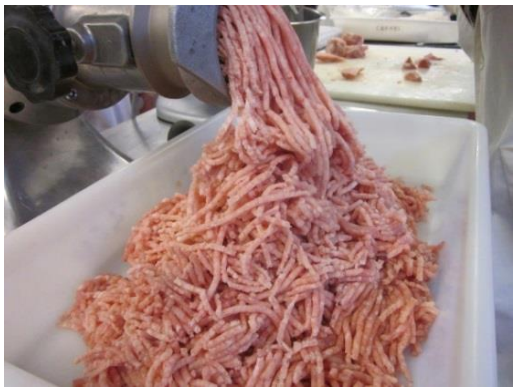


Figure 6.17. Minced meat of chicken

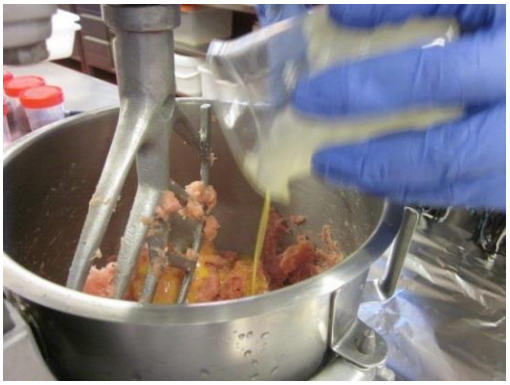


Figure 6.18. Blending of minced meat in a bowl mixer with the other ingredients

Figure 6.19. Blend of chicken meat

Figure 6.20. Burger maker for patties

Figure 6.21. Patties packed in different vacuum high oxygen barrier bag

IV Experiment

Whole eggs, breadcrumbs and salt were obtained from a local market of Madrid (Spain).

Three formulations of patties were prepared (Table 6.8.). The minced meat was firstly blended in a bowl mixer (Hobart, Model N50, USA) during 60 s, and then the salt was added to meat and mixed during 30 s. Thereafter, the eggs were beaten and added to the mixture for blended during 20 s, breadcrumbs were also incorporated into the mixer and mixed during 60 s to ensure a uniform distribution. The same process was follow for the control sample (PC). In the formulations of samples containing the grape by-products was added 2% of GS (PGS) and 2% of SS (PSS) powder in replacement of 2% of breadcrumbs and following the same process (Figure 6.22). From this blend (Figure 6.23), a total of 20 patties (~50 g per hamburger) for every treatment were prepared by using a conventional burger maker (Ministeak burger maker, O.L. Smith Co. Ltd., Italy). The patties were packed in different vacuum high oxygen barrier bags (nylon/polyethylene, 9.3 ml O_2/m_2/24 h at 0ºC, Koch Kansas City, MO), every bag contained 2 patties and were stored in refrigeration at 4º C (Figures 6.24; 6.25; 6.26). The patties were analyzed (Figures 6.27; 6.28) on day 0 and after 3, 6 and 9 days of storage.

Table 6.8. Formulation (%) of chicken patties

Sample	Meat	Whole egg	Breadcrumbs	Salt	GS	SS
PC	85.4	6.8	6.8	1		
PGS	85.4	4.8	6.8	1	2	
PSS	85.4	4.8	6.8	1		2

Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)

Figure 6.22. Blending of minced meat

Figure 6.23. Chicken meat blend

Figure 6.24. Control chicken pattie (left) and grape pattie (right)

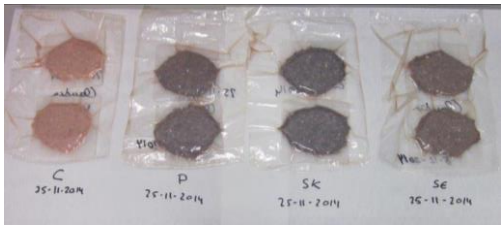


Figure 6.25. Chicken patties packed in different vacuum high oxygen barrier bags

Figure 6.26. Chicken patties appearance

Figure 6.27. Cooking of chicken patties for sensory evaluation

Figure 6.28. Chicken patties appearance after cooking

6.7. Total extractable polyphenols

Ttal extractable polyphenols content in GS, SS and GP grape ingredients, diets, ileal digesta and excreta, meat, raw patties and cooked patties was determined by a double aqueous-organic extraction according to the method of Chamorro et al. (2012).

The samples were shaken in darkness at room temperature with methanol/water (50:50 v/v, pH = 2) 50 mL/g sample in orbital shaker (Orbital KS 250 basic Ika Labortechink) for 1 h, and acetone–water (70:30 v/v, 50 mL/g sample, 60 min,

constant shaking). After centrifugation (15 min, 25°C, 3000g) supernatants were combined and used for determination of polyphenols content. Extractable polyphenols were determined following the Folin–Ciocalteau procedure (Montreau, 1972) using gallic acid (GA) as standard. After reacting for 1 h in dark, absorbance was measured at 750 nm against a blank using an ultraviolet visible spectrophotometer Shimadzu UV-1800 (Shimadzu Inc., Kyoto, Japan) (Figures 6.29; 6.30). Results were expressed as gallic acid equivalents (g GAE/100g of sample in the I, II and III experiments, and mg GAE/100g of sample in the IV and V experiments).

Figure 6.29. Ultraviolet visible spectrophotometer Shimadzu UV-1800 (Shimadzu Inc., Kyoto, Japan)

Figure 6.30. Samples after 1h of Folin–Ciocalteau reaction in dark

6.8. Tannins content

The acidic butanol technique (Waterman and Mole, 1994) was used to quantify the tannins content in GS, SS and GP grape ingredients, diets, ileal digesta and excreta.

A stock solution of 0.07% (w/v) FeSO4.7 H_2O dissolved in 95:5 (v/v) 1-butanol/HCl was prepared. In a test tube, 7 ml of the stock solution and 50 mg of sample were mixed and heated for 50 min at 95ºC. The mixtures were cooled in an ice bath, and after centrifugation the absorbance was measured at 550 nm using an ultraviolet-visible spectrophotometer Hitachi U-2000 (Hitachi, Ltd). The TC was expressed as cyanidin-3-O-glucoside equivalent after the preparation of a standard curve of cyanidin ranging from 0 to 333 mg/l.

6.9. Plasma ROMs and α-tocopherol determination

Plasma was prepared from blood obtained by cardiac puncture (Figure 6.31). for subsequent determination of vitamin E and ROMs (Reactive Oxygen Metabolites) concentration. The blood samples were allowed to clot in polypropylene tubes for 2 h at room temperature. The tubes were centrifuged at 1.500 x g for 10 min, and the supernatant was removed and stored at -20ºC until assayed.

Plasma α-Tocopherol content was determined by the method of Butriss & Diplock (1984). Briefly, 400 µL of plasma was extracted with hexane in three phases, evaporated and diluted with 1 mL of hexane.

The α-tocopherol was measured by normal-phase HPLC using a Hypersil Si 100 (5µm) column and a mobile phase of hexane-isopropanol (98:2 vol/vol) and detected by fluorescence using HPLC system and a mobile phase of hexane-isopropanol (98:2 vol/vol).

The ROMs were spectrophotometrically determined with colorimetric method proposed by Diacron at a wavelength of 505 nm, using a specific commercial kit (Cesarone et al., 1999).

Figure 6.31. Chicken cardiac puncture for blood sampling

6.10. α-tocopherol determination

The content of α-tocopherol in muscle tissue was determined in 100 mg of freeze-dried sample following the method of Buttriss and Diplock (1984), which includes saponification with saturated KOH in the presence of pyrogallol. The α-tocopherol was then extracted with hexane, measured by normal phase and detected by fluorescence in a HPLC system (Hewlett-Packard 1100, Agilent Technologies GmbH, Waldbronn, Germany), using a Zorbax Rx-SIL (Narrow-Bore 2.1 x 150 mm, 5 μm, Agilent Technologies) column.

6.11. TBARS determination

The extent of lipid oxidation was determined in thigh and breast meat and in patties by measuring the thiobarbituric acid reacting substances (TBARS), with some modifications in the method according to the matrix used.

TBARS in chickens meat were determined using the procedure described by Botsoglou et al. (1994). Five g of ground meat were homogenized with 10 mL of 5 % trichloroacetic acid (TCA) in an Ultraturrax at 21,280 x g for 1 min. Butylated hydroxytoluene (BHT) was added prior to homogenization at a level of 125 μg/mg fat. The blended sample was filtered through Wathman number 2V filter (Whatman International Ltd, Maidstone England) and 2.5 mL of the filtrate were mixed with 1.5 mL of 0.8% thiobarbituric acid (TBA) in distilled water in capped test tubes. Tubes were vortex, incubated at 70ºC for 30 min and absorbance was determined at 532 nm using an ultraviolet-visible spectrophotometer Hitachi U-2000 (Hitachi, Ltd). Results were expressed as ng of malondialdehyde (MDA) per gram of muscle after the preparation of a standard curve of 1,1,3,3 - tetraethoxy propane (TEP).

In chickens patties lipid oxidation was determined according to the procedure described by López-López et al. (2010). Briefly, the procedure was as follows: 5 g of each sample was homogenized in 35 ml of a solution of TCA 7.5% and Ethylenediaminetetraacetic acid (EDTA) 0.1% for 30 s at high speed in an omnimixer blender (Omni International, Waterbury, Ct, USA). The blended sample was centrifuged (Solvall BA, RTB6000B, Dupont USA) at 3000 g for 2 min and filtered through a Whatman 2 filter into 50 mL Erlenmeyer flasks. The filtrate (5 mL) was mixed with 5 mL of 0.02 M TBA in distilled water, in capped test tubes. Tubes were

heated for 35 min at 90°C in water. Pink formation was measured spectrophotometrically using a Shimadzu UV-1800 spectrophotometer (Shimadzu Inc., Kyoto, Japan) at 532 nm against a blank containing 5 mL of distilled water and 5 mL of 0.02 M TBA solution (Figure 6.32). A calibration curve was plotted with TEP to measure the MDA. TBARS determinations were performed three times. Values were expressed as ng of MDA per g of sample in the I, II and III experiments and mg of malondialdehyde per kilogram of sample in the IV and V experiments.

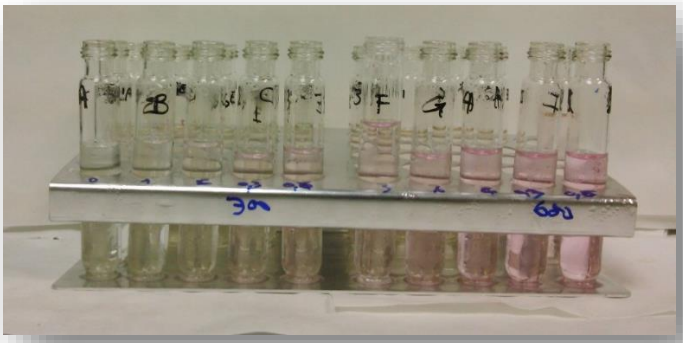


Figure 6.32. The pink formation of the filtrate mixed with TBA in capped test tube

6.12. Microbiological analysis

Microbiological analysis were performed in the ileum content, meat and patties, with some modifications in the method according to matrix used.

Digesta (0.1 to 0.2g) from the ileum and ceca were collected aseptically in pre weighed 20- mL sterilized plastic tubes. The samples were weighed and diluted in peptone water to an initial 10–1 dilution. Microbial populations were determined by serial dilution (10–1 to 10–7) of samples in PBS before inoculation onto Petri dishes of sterile agar. *Lactobacillus* was grown on de Man, Rogosa, and Sharpe agar (Difco Laboratories, Detroit, MI). *Escherichia coli* was grown on Coli ID agar (bioMerieux Espana S.A.). The agar used to grow *Clostridium* was sulfite polymyxin sulfadiazine (Difco Laboratories). The plates were incubated at 37°C anaerobically (73% N, 20% CO_2, 7% H_2) for *Clostridium* and *Lactobacillus*, aerobically for *E. coli*. Plates were counted between 24 and 48 h after inoculation. Colony-forming units were defined as being distinct colonies measuring at least 1 mm in diameter.

For meat and patties microbiological analysis, samples were prepared in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain) taking 10 g of sample (in replicate) in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of peptone water (0.1%) and 0.85% NaCl. After 1 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on the following media: Plate Count Agar (PCA, Oxoid) for the total viable count (TVC) (30 °C, 72 h), determined according to AFNOR (Association Francaise de Normalisation) standard NF V 08-051 (1999); De Man, Rogosa, Sharp Agar (MRS) (Oxoid) for lactic acid bacteria (LAB) (30 °C, 72 h) according to ISO standard 15214 (1998); Violet Red Bile Glucose Agar (VRBG) (Oxoid) for *Enterobacteriaceae* (37 °C, 24 h) according to AFNOR standard NF V 08-054 (1999); and Coli ID agar (Biomerieux, Marcy l'Etoile, France) for enumeration of b-glucuronidase positive coliforms (37 °C, 48 h) according to ISO standard 16649-2 (2001). All microbial counts were converted to logarithms of colony-forming units per gram (log CFU/g).

6.13. pH determination

The pH was determined using a pH meter (827pH Lab Methrom, Herisau, Switzerland) on 10 g of sample that was homogenates in 100 ml of distilled water. Three measurements were performed per sample (Figure 6.33).

Figure 6.33. pH determination in an homogeneizate chicken patties sample

6.14. Color determination

Color, CIE-LAB tristimulus values, lightness (L*), redness (a*) and yellowness (b*) was measured on the surface of raw patties using a CM-3500d Chroma Meter (Konica Minolta Business Technologies, Tokyo, Japan) (Figure 6.34). Before use, the colorimeter was standardized using the white calibration plate (C: Y=93.6, x=0.3130, y=0.3193). Ten determinations were taken for sample.

Figure 6.34. CIE-LAB color measurement with a CR-400 Chroma Meter

6.15. Texture determination

Kramer shear force (KSF) was performed using a miniature Kramer (HDP/MK05) cell. A mini 5-bladed head was used to perform a shearing test. Kramer shear tests were carried out on sections of 2 cm per patties formulation at room temperature. A 5 kg load cell was used. The force was exerted to a compression distance of 20 mm at 8 mm/s crosshead speed using a TA-XT plus Texture Analyzer (Texture Technologies Corp. Scarsdale, NY). KSF values were calculated as the maximum force per g of sample (N/g). Measurements were carried out five times.

6.16. Sensory evaluation test

The patties were assessed by a seventeen (IV experiment) and thirteen (V experiment) non-trained panel members selected in preliminary sessions from the staff of CSIC (Figure 6.36). The patties were cooked in an electric pan (Plactronic, Selecta, J.P. Selecta, S.A. Barcelona, España) 1.5 min per side at 210±4ºC, and with a spoon of extra virgin olive oil (Figure 6.35). Then, the products were cut into pieces of uniform size and served warm to the panelists with a code to identify the samples. The volunteers were asked to evaluate the following sensory attributes measured on unstructured scales (10 cm) with descriptors at either end. These were: flavor, color and overall acceptability (0: I do not like at all, 10: I like very much); hardness (0: very soft, 10: very hard); juiciness (0: very dry, 10: very juicy). The sensory analyses were performed at 6 day (IV experiment) a 2 (V experiment) of the storage.

Figure 6.35. Cooking of chicken patties in an electric pan

Figure 6.36. Sensory evaluation from non-trained panel members of CSIC

6.17. Statistical analysis

I, II and III Experiments

Apparent ileal digestibility (AID) of crude protein and total polyphenols was determined by using the AIA content and calculated by the following formula:

100% − [100% × (AIA concentration in feed/AIA concentration in ileal content or excreta) × (CP and TP concentration in ileal content or excreta / CP and TP concentration in feed)].

Data were subjected to a one-way analysis of variance (ANOVA) by using the general linear model procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC). When the effect was declared significant ($p<0.05$), treatment means were compared using a Duncan's multiple-range test. Orthogonal contrasts were used to test differences between the combined means of several groups. Pen served as experimental unit for performance, ileal and excreta contents and digestibilities and microbial counts, whereas the experimental unit used for TBARS determination was the bird.

IV and V Experiments

Results were expressed by means standard error of three or more separate determinations. Comparison of means was performed by one-way and two-way analysis of variance (ANOVA). Tukey HSD test was used to determine the differences in the mean values ($P<0.05$). Data were analyzed using SPSS V.13.0 software (SPSS Institute Inc., Cary, NC).

CHAPTER 7. I EXPERIMENT

Effect of grape skin, grape seed, grape pomace and vitamin E on digestibility of polyphenols and antioxidant activity in chickens

7.1. Aim

The aim of this study was to evaluate the effect of a dietary addition of SS, GS, and GP (SS+GS) on the performance parameters, ileal and excreta content of total polyphenols and tannins, protein and extractable polyphenols digestibility, plasma vitamin E and oxidative stability of animal products in terms of vitamin E content and MDA level (TBARS).

7.2. Results

7.2.1. Growth performance

The effect of feeding diets containing α-T (α-Tocopherol), GS, SS and GP on growth performance in chickens is reported in Table 7.1. Daily weight gain and feed conversion ratio were significantly ($P<0.01$) lower in SS group, compared with those birds fed the different treatments. No effect of dietary treatments was observed on feed intake.

Table 7.1. Performance of broiler chicks (1 to 21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E

Dietary treatments	Daily weight gain (g/d)	Daily feed intake (g/d)	Feed conversion ratio
Control	35.8[a]	50.4	1.41[b]
Control + Vit. E	38.2[a]	52.6	1.38[b]
Control + 15 GS	35.5[a]	49.9	1.40[b]
Control + 30 GS	34.3[a]	48.6	1.42[b]
Control + 110 SS	29.3[b]	47.8	1.63[a]
Control + 37.5 GP	35.5[a]	49.9	1.41[b]
		SEM[1]	
	1.22	1.84	0.019
		P-value of Contrasts[2]	
Control *vs* GS+SS+GP	ns	ns	*
Control *vs* Vit. E	ns	ns	ns
Vit. E *vs* GS+SS+GP	**	ns	***
Control *vs* GS	ns	ns	ns
Control *vs* SS	**	ns	***
Control *vs* GP	ns	ns	ns

Different letters in the same column (a, b) indicate significant differences (p<0.05).
[1]SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate).
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01
*** P<0.001

7.2.2. Ileal and excreta content of total extractable polyphenols and tannins

The total ileal and excreta polyphenol content of birds fed diets containing α-T, GS, SS and GP is shown in Table 7.2. As expected, ileal and excreta content were not affected by dietary α-T supplementation. Birds fed GS showed a higher (P<0.05) ileal polyphenol content than those fed control diets. With respect to excreta polyphenol content GS, SS and GP showed a higher content, compared to the control group. In general, chickens fed grape by-products showed higher values of condensed tannins in excreta content, compared to those fed vitamin E and control diets. Significant (P<0.001) higher values of ileal and excreta condensed tannins were found particularly in chickens fed SS diet. GS groups showed higher (P<0.05) values of tannins in the ileum while GP group in the exreta (P<0.001).

Table 7.2. Ileal and excreta total polyphenols and condensed tannins content of broilers chicks (21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E.

Dietary treatments	Total polyphenols (g GA/100g)		Condensed tannins (mg cyanidin/100g)	
	Ileal	Excreta	Ileal	Excreta
Control	0.284^{bc}	0.372^{de}	0.032^{c}	0.023^{cd}
Control + Vit. E	0.281^{bc}	0.336^{e}	0.032^{c}	0.016^{d}
Control + 15 GS	0.366^{a}	0.408^{cd}	0.050^{bc}	0.029^{cd}
Control + 30 GS	0.310^{b}	0.461^{bc}	0.060^{b}	0.045^{c}
Control + 110 SS	0.280^{bc}	0.551^{a}	0.092^{a}	0.119^{a}
Control + 37.5 GP	0.258^{c}	0.488^{b}	0.047^{bc}	0.072^{b}
	SEM[1]			
	0.012	0.020	0.008	0.008
	P-value of Contrasts[2]			
Control *vs* GS+SS+GP	ns	***	*	***
Control *vs* Vitamin E	ns	ns	ns	ns
Vitamin E *vs* GS+SS+GP	ns	***	**	***
Control *vs* 15GS+30GS	*	*	*	ns
Control *vs* SS	ns	***	***	***
Control *vs* GP	ns	***	ns	***

Different letters in the same column (a, b, c, d, e) indicate significant differences ($p<0.05$).
[1]SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate for ileon and five birds per replicate for excreta).
[2]ns: no significant effect ($P > 0.05$).
* P<0.05
** P<0.01
*** P<0.001

7.2.3. Protein and polyphenols digestibility

Ileal digestibility of protein was lower ($P<0.05$) in birds fed SS diets than in those fed control, α-T, GS and GP diets, as shown in Table 7.3. Ileal digestibility of polyphenols was increased in birds fed the higher concentration of GS, SS and GP diets. No effect was observed in excreta digestibility of polyphenols.

Table 7.3. Ileal digestibility of protein and ileal and excreta digestibility of total polyphenols of broilers chicks (21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E.

Dietary treatments	Ileal protein digestibility (%)	Ileal total polyphenols digestibility (%)	Excreta total polyphenols digestibility (%)
Control	87.3[a]	60.3[c]	59.3
Control + Vitamin E	89.2[a]	66.2[b]	56.0
Control + 15 GS	87.6[a]	60.0[c]	65.2
Control + 30 GS	87.2[a]	76.2[a]	70.3
Control + 110 SS	84.1[b]	75.3[a]	56.4
Control + 37.5 GP	87.6[a]	77.5[a]	55.3
		SEM[1]	
	0.801	1.58	5.17
		P-value of Contrasts[2]	
Control *vs* GS+SS+GP	ns	***	ns
Control *vs* Vitamin E	ns	*	ns
Vitamin E *vs* GS+SS+GP	*	**	ns
Control *vs* 15GS+30GS	ns	**	ns
Control *vs* SS	*	***	ns
Control *vs* GP	ns	***	ns

Different letters in the same column (a, b, c) indicate significant differences (p<0.05).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01
*** P<0.001

7.2.4. Plasma α and γ- tocopherol content

The effect of dietary treatments on the composition of plasma α and γ-tocopherol is presented in Table 7.4. A significant increase in plasma α-tocopherol concentration was observed in birds fed α-T (P<0.001) and SS (P<0.05) and GP (P<0.001) diets compared with those fed the control diet. This effect was higher (P<0.001) in birds receiving α-T diet than in those fed GS, SS and GP diets. No effect was observed in plasma γ-tocopherol with the different treatments except for the birds fed GP diets which increased (P<0.001) the γ-tocopherol concentration.

Table 7.4. Effect of inclusion of grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E on blood α and ϒ-tocopherol of 21 d broiler chicks.

Dietary treatments	α-T (µg/ml)	ϒ-T (µg/ml)
	21 d	21 d
Control	3.84[b]	0.533[bc]
Control + Vitamin E	36.1[a]	0.662[b]
Control + 15 GS	4.13[b]	0.464[c]
Control + 30 GS	4.69[b]	0.563[bc]
Control + 110 SS	5.89[b]	0.501[bc]
Control + 37.5 GP	9.28[b]	0.867[a]
	SEM[1]	
	1.74	0.060
	P-value of Contrasts[2]	
Control *vs* GS+SS+GP	*	ns
Control *vs* Vitamin E	***	ns
Vitamin E *vs* GS+SS+GP	***	ns
Control *vs* GS	ns	ns
Control *vs* SS	*	ns
Control *vs* GP	***	***

Different letters in the same column (a, b, c) indicate significant differences (p<0.05).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01
*** P<0.001

7.2.5. Meat α and γ- tocopherol and lipid oxidation

The effect of dietary treatments on the composition of α and γ- tocopherol and MDA formation of broiler thigh meat is presented in Table 7.5. A significant increase (P<0.001) in breast meat α and γ-tocopherol was observed in birds fed α-T diet compared with those fed the control diet, GS, SS and GP diets at 1 and 7 days of refrigerated storage. No effect on meat was observed in α and γ- tocopherol GS, SS and GP diets when compared with the birds fed control diet.

The extent of lipid oxidation, measured by MDA formation in thigh meat was significantly lower in the supplemented α-T diet than the control, GS and SS groups after 1 and 7 days of refrigerated storage (Figure 7.1.). There were no differences in MDA concentration among the chicks fed GS and SS diets compared with those fed control diet. The inclusion of GP significantly reduced MDA values after 1 day (P<0.05) and 7 days (P<0.001) of refrigerated storage compared with samples obtained from birds fed the control diet.

Table 7.5. Effect of refrigerated storage on lipid oxidation of breast meat of broiler chicks (21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E.

Dietary treatments	α-T (µg/g)		γ-T (µg/g)		MDA (ng/g)	
	1 d	7 d	1 d	7 d	1 d	7 d
Control	9.89^{bc}	1.64^{c}	2.24^{b}	0.414^{a}	7.21^{a}	47.0^{a}
Control + Vitamin E	71.1^{a}	10.6^{a}	4.02^{a}	0.537^{a}	5.85^{a}	8.45^{b}
Control + 15 GS	12.5^{b}	2.05^{c}	2.13^{b}	0.373^{a}	7.07^{a}	41.6^{a}
Control + 30 GS	8.24^{bc}	1.46^{c}	2.10^{b}	0.336^{a}	7.04^{a}	42.5^{a}
Control + 110 SS	4.69^{c}	3.50^{bc}	1.43^{c}	0.449^{a}	6.67^{a}	40.3^{a}
Control + 37.5 GP	10.7^{b}	4.56^{bc}	2.16^{b}	0.744^{a}	6.37^{a}	14.5^{b}
			SEM[1]			
	1.94	0.534	0.206	0.070	0.265	5.31
			P-value of Contrasts[2]			
Control *vs* GS+SS+GP	ns	*	ns	ns	ns	ns
Control *vs* Vitamin E	***	***	***	ns	***	***
Vitamin E *vs* GS+SS+GP	***	***	***	ns	**	***
Control *vs* 15GS+30GS	ns	ns	ns	ns	ns	ns
Control *vs* SS	ns	*	**	ns	ns	ns
Control *vs* GP	ns	***	ns	**	*	***

Different letters in the same column (a, b, c) indicate significant differences (p<0.05).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01
*** P<0.001

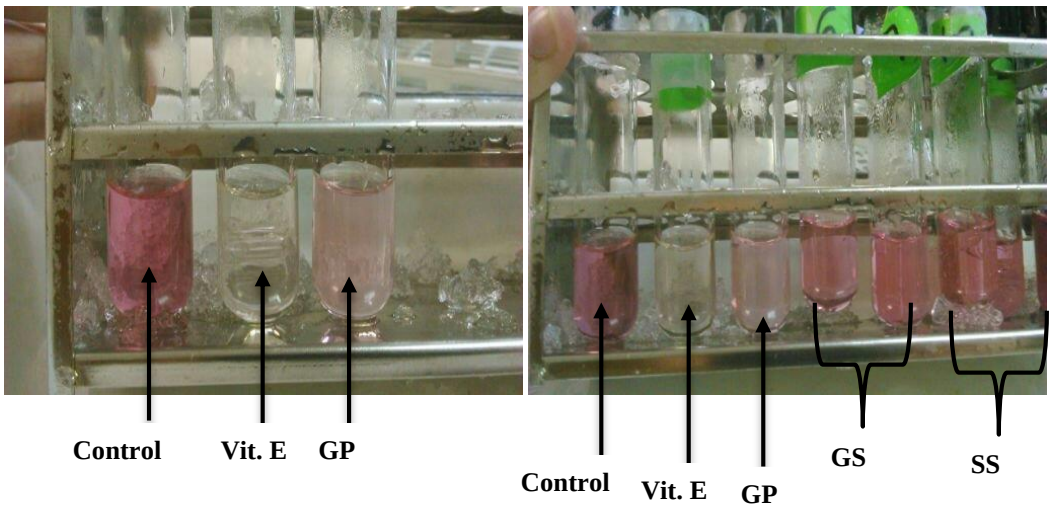


Figure 7.1. TBARS reactive substances of some samples of thigh chicken meat after 7d of refrigerate storage

7.3. Discussion

7.3.1. Growth performance, protein and polyphenol utilization

Previous experiment in our laboratory (Goñi et al., 2007; Brenes et al., 2008; Chamorro et al., 2015) have shown an increase in the antioxidant activity of broiler diet, excreta, and meat as a result of the dietary administration of grape pomace. However, there is no information in relation to the separated effect of the principal components in grape pomace, grape seed and skin. In the present study, the inclusion of GS and GP did not change the daily body weight gain compared with birds fed control and α-T diet. The present study demonstrated that the inclusion of GS and GP in chicken diets did not change the growth performance. Similar results have been obtained in our laboratory by the addition GP (Goñi et al., 2007; Brenes et al., 2008). However, the addition of SS in chicken diets reduced the performance of the birds. The content of total polyphenol and fiber in the higher concentration of GS and SS in diets are similar. The differences could be to the different structures of polyphenols in these grape by-products and the higher composition of condensed tannins in this diet (0.406 mg/100g) in comparison to the other diets (approximately 0.230 mg/100g) Grape skin also differ from seeds ones primarily by the presence of prodelphinidins but also by the higher mean degree of polymerization and lower contents of galloylated derivatives (De Freitas et al., 2000; Travaglia et al., 2011). In addition, although grape seeds contain procyanidin, skin procyanidin are less strongly linked with the structures of the skin than the seed (Amrani-Joutei et al., 1994). Few data are available in the literature in relation to the use of grape by-products, particularly skin, in chicken feeding.

Polyphenols are known to form complexes with protein due to the interaction of their reactive reactive hydroxyl group with the carbonyl group of protein. In our experiment, the digestibility of protein was not affected except in those birds fed SS. This lack of effect could be attributed to the low content of polyphenols in the experimental diets to cause detrimental effect. In the case of birds fed SS could be justified to the high content of tannins in the diet and, consequently in the intestine, and to the chemical properties (higher polarity) of these polyphenols (Travaglia et al., 2011) present in SS in comparison to the other grape by-products.

To better understand the biological effects in target tissues of polyphenols it is important to know their bioavailability, it means how much of the ingested quantity of the polyphenols is able to reach the systemic circulation and exert its beneficial effects in target tissues. Bioavailability includes GI (gastro intestinal) digestion, absorption, metabolism, tissue distribution, and bioactivity. Consequently, it must be demonstrated that the component analyzed is efficiently digested and assimilated and then, once absorbed, exerts a positive effect in chickens tissues.

Over the last decade, several studies have addressed the metabolism of proanthocyanidin. Although initial studies emphasized fairly poor intestinal absorption that was limited to dimers (Donovan et al., 2002; Tsang et al., 2005), later observations indicate that once intact proanthocyanidins reach the colon are widely transformed by the intestinal microbiota into small phenolic acids (Appeldoorn et al., 2009; Stoupi et al., 2010). In the current experiment, birds fed GS, GP and SS diets showed a higher intestinal (ileal and excreta) total polyphenols ad tannins content than those fed control diets. The highest intestinal concentration of polyphenols and tannins were obtained in chicks fed diets containing GP and SS. Recent studies by Monagas et al. (2010) have estimated that the amount of non-absorbable polyphenols reaching the colon is very high and microbe-derived phenolic metabolites excreted in urine represent the largest proportion of polyphenol intake. The lack of specific urinary excretion system in birds explain why the amount of phenolic compounds in excreta was higher than those obtained at ileal content with the exception of birds fed GS diets.

In the current experiment, the ileal and excreta digestibility of total polyphenols reached values in a range of 55 to 77 % in those birds fed the different grape by-products. Brenes et al. (2008) in chickens and Goñi and Serrano (2005) in rats also reported similar or superior digestibilities based on total polyphenol determination using GP.

7.3.2. Plasma and meat α and γ- tocopherol concentration and lipid oxidation

Nutritional interest in polyphenolic compounds has increased greatly in light of their antioxidant capacity (Scalbert and Williamson, 2000). Previous studies showed an increase in the antioxidant activity of broiler diet, excreta, and meat as a result of the dietary administration of GP (Goñi et al., 2007; Brenes et al., 2008;

Sayago-Ayerdi et al., 2009a) that was accompanied by an increase in the concentration of vitamin E in the liver. Chamorro et al. (2015) also showed that dietary addition of GP (10%) reached similar protective effect observed with the addition of vitamin E. In this study was investigated if similar results could be obtained with the addition of GS, SS and GP in a lower concentration (3.75%). As expected, plasma and meat α- and γ-tocopherol concentration were significantly increased by the addition of α-T in the diet. An increase in meat α- tocopherol concentration was also reported by Cortinas et al. (2004) and Voljc et al. (2011) with dietary addition of 200 IU of vitamin E. However, only dietary inclusion of SS and GP increased the concentration of plasma and meat α-tocopherol in comparison to the birds fed the control diet but these results were not equal than those obtained by the addition of Vitamin E. The reason of this lack of effect could be justified, in the case of GP, by the concentration used in our experiment (37.5 g/kg) respect to the concentration used (10%) by Chamorro et al. (2015). In the case of SS and GS due to the different chemical composition of polyphenolic compounds present in these by-products. Particularly this lack of effect might indicate a potential prooxidant effect. It is known that polyphenol compounds can display both antioxidant and prooxidant effects (Decker et al., 1997) depending on several factors (chelating potential, solubility, bioavailability and stability in tissues).

As expected, lipid oxidation (MDA values) was increased in raw thigh chicken samples during storage time. The dietary inclusion of α-T and GP significantly delayed lipid oxidation and reduced the potential risk induced by lipid oxidation products. These results are similar to those reported in our laboratory by Goñi et al. (2007), Brenes et al. (2008) and Chamorro et al. (2015). The inclusion of GP and GSE in chicken diets significantly improved oxidative stability (TBARS) and radical scavenging capacity (ABTS) in raw breast meat and cooked chicken patties (Sayago-Ayerdi et al. 2009 a, b; Selani et al. 2011). Similar results have been reported by Brannan (2008) and Lau and King (2003) in chicken thigh meat during refrigerated storage and by Mielnik et al. (2006) in turkey meat. Sahin et al. (2010) and Liu et al. (2014) reported that the inclusion of resveratrol in quail diets enhanced the antioxidant activity status of birds and eggs and reduced oxidative stress in heat-stressed chickens by increasing serum growth hormone concentrations and modulating the expression of heat shock genes in organs of the immune system. However, higher MDA values were

observed by the inclusion of GS and SS in chicken diet. There is no available information about the supplemental effect of GS and SS on meat lipid oxidation in chicken.

On the other hand, although the mechanism of the antioxidant effect of dietary GP polyphenols could not be deduced from the present study due to the lack of correlation between the reduction of the susceptibility of lipid oxidation of meat by the addition of GP and the increase of meat α-tocopherol, it is possible that the greater antioxidant status of the thigh meat is probably due to and indirect antioxidant effect. This effect could be mediated by a direct antioxidant activity of the tannins in the gastrointestinal tract, such as a removal or chelation of pro-oxidant compounds and a reduction of lipid peroxidation, which would result in an overall improvement of the animal's antioxidant status (Kerem et al., 2006, Halliwell et al., 2005). Furthermore, it is known that dietary condensed tannins strongly modify lipid metabolism and interfere also with gene (Kresty et al., 2011 and Crescenti et al., 2005). In particular, Sgorlon et al. (2006) and Na et al. (2008) found that supplementing sheep with grape skin extract and tea polyphenols which is rich in polyphenols and condensed tannins, increased the expression in plasma of the superoxide dismutase enzyme, which is involved in the elimination or inactivation of reactive oxygen species and in the endogenous antioxidant defence system.

7.4. Conclusion

In conclusion, the results presented in this study showed that the addition of GS (up to 30.0 g/kg) and GP (37.5 g/kg) did not impair the performance and protein digestibility of chickens except in the case of those birds fed SS (110 g/kg). Our results also confirm thart polyphenols present in these by-products were absorbed at sufficient levels to contribute and modulate the antioxidant activity. However, only dietary inclusion of SS and GP increased the concentration of plasma and meat α-tocopherol in comparison to the birds fed the control diet but these results were not equal than those obtained by the addition of Vitamin E.

CHAPTER 8. II EXPERIMENT

Effect of dietary fermented and unfermented grape skin and vitamin E on digestibility of polyphenols and antioxidant and antimicrobial activity in chickens.

8.1. Aim

The aim of this study was to evaluate the effect of a dietary addition of fermented (FS) and unfermented (UFS) grape skin on the performance parameters, ileal and excreta content of total polyphenols and tannins, protein and exctractable polyphenols digestibility, microbiology of ileal content and oxidative stability of animal products.

8.2. Results

8.2.1. Growth performance

Chicken growth performance is summarized in Table 8.1. Performance was not affected by dietary treatment except in the case of birds receiving the higher concentration (60g/kg) of FS and UFS in the diet, which showed decreased ($p<0.05$) daily weight gain, compared to the control and vitamin E groups and higher ($p<0.05$) feed conversion ratio (FCR). A statistical ($P<0.05$) increase of FCR value was also detected in chicken fed the UFS 30, while dietary feed intake of all groups did not change.

Table 8.1. Performance of broiler chicks (1 to 21 d) fed diets containing fermented (FS) and unfermented (UFS) grape skin and vitamin E

	Daily weight gain (g/d)	Daily feed intake (g/d)	Feed conversion ratio
Control	38.6^{a}	51.2	1.33^{b}
Control + Vit. E	40.2^{a}	54.4	1.35^{b}
Control + FS30	38.3^{ab}	53.3	1.40^{ab}
Control + FS60	34.3^{b}	51.6	1.51^{a}
Control + UFS30	37.1^{ab}	55.2	1.49^{a}
Control + UFS60	34.5^{b}	51.8	1.50^{a}
SEM[1]	1.28	1.23	0.038
P-value[2]	*	ns	**

Different letters in the same column (a, b) indicate significant differences ($p<0.05$).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate).
[2]ns: no significant effect ($P > 0.05$).
* $P<0.05$
** $P<0.01$

8.2.2. Ileal and excreta content of total extractable polyphenols and tannins

The total ileal and excreta polyphenol and tannins content is reported in Table 8.2.

As expected, a higher (P<0.05) ileal total phenolic and tannin content was found in the groups receiving grape skin in the diet, compared to the control group. In particular, birds receiving unfermented grape skin in the diet showed a higher (P<0.001) total phenolic and tannin content, compared to the groups fed fermented grape skin. These results are related with the higher composition of total polyphenols present in UFS reported in Table 8.1 but not respect to the tannin content.

Table 8.2. Ileal and excreta total polyphenols and condensed tannins content of broilers chicks (21 d) fed diets containing fermented (FS) and unfermented (UFS) grape skin and vitamin E

Dietary treatments	Total polyphenols (g GA/100g)		Condensed tannins (mg cyanidin/100g)	
	Ileal	Excreta	Ileal	Excreta
Control	0.426^{c}	0.351^{b}	0.025^{f}	0.033^{d}
Control + Vitamin E	0.442^{c}	0.330^{b}	0.028^{ef}	0.036^{d}
Control + FS30	0.430^{c}	0.328^{b}	0.039^{d}	0.047^{c}
Control + FS60	0.418^{c}	0.347^{b}	0.064^{b}	0.071^{b}
Control + UFS30	0.480^{b}	0.330^{b}	0.050^{c}	0.065^{b}
Control + UFS60	0.522^{a}	0.405^{a}	0.080^{a}	0.114^{a}
	SEM[1]			
	0.012	0.011	0.001	0.002
	P-value of Contrasts[2]			
Control *vs* FS + UFS	*	ns	***	***
Vitamin E *vs* FS + UFS	ns	ns	***	***
FS *vs* UFS	***	*	***	***
FS30 + UFS30 *vs* FS60+ UFS60	ns	***	***	***

Different letters in the same column (a, b, c, d, e, f) indicate significant differences (p<0.05).
[1]SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate).
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01
*** P<0.001

8.2.3. Protein and polyphenols digestibility

Ileal protein digestibility and ileal and excreta polyphenols digestibility are reported in Table 8.3.

Ileal protein digestibility was not affected by grape skin inclusion in chicken diets, except in the case of birds fed the highest concentration (60 g/kg) of UFS which showed lower values (P<0.05), compared to the control group. Ileal digestibility of total polyphenols was not affected by dietary treatment, however, excreta digestibility of total polyphenols was significantly increased in birds fed FS and UFS compared to those birds fed the control diet. In addition, birds fed UFS showed a higher (P<0.001) excreta total phenolic digestibility compared to those fed FS.

Table 8.3. Ileal digestibility of protein and ileal and excreta digestibility of total polyphenols of broilers chicks (21 d) fed diets containing fermented (FS) and unfermented (UFS) grape skin and vitamin E

Dietary treatments	Ileal protein digestibility (%)	Ileal total polyphenols digestibility (%)	Excreta total polyphenols digestibility (%)
Control	80.3^{a}	40.9^{b}	51.2^{c}
Control + Vitamin E	78.7^{a}	39.9^{b}	63.4^{ab}
Control + FS30	77.6^{ab}	37.5^{b}	57.4^{bc}
Control + FS60	78.3^{a}	41.7^{b}	55.7^{bc}
Control + UFS30	79.6^{a}	51.6^{a}	68.8^{a}
Control + UFS60	74.7^{b}	37.3^{b}	62.6^{ab}
		SEM[1]	
	1.10	2.31	2.30
		P-value of Contrasts[2]	
Control *vs* FS + UFS	ns	ns	**
Vitamin E *vs* FS + UFS	ns	ns	ns
FS *vs* UFS	ns	ns	***
FS30+UFS30*vs*FS60+UFS60	ns	ns	ns

Different letters in the same column (a, b, c) indicate significant differences (p<0.05).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01
*** P<0.001

8.2.4. Microbiological counts

The effect of inclusion of FS and UFS in chicken diets on microbiological count of different bacterial species is reported in Table 8.4. In the current study no differences were detected on colony-forming units of anaerobic (*Lactobacillus* and *Clostridium*) and aerobic (*E. coli*) bacterial species by the addition of FS and UFS in chicken diet.

Table 8.4. Effect of inclusion of fermented (FS) and unfermented (UFS) grape skin and vitamin E on colony-forming units of anaerobic (Lactobacillus and Clostridium) and aerobic (E. coli) bacteria species per gram of ileal content of 21 d fed broilers chicks

Dietary treatments	*Lactic acid bacteria* (log cfu/g)	*Escherichia coli* (log cfu/g)	*Clostridium* (log cfu/g)
Control	7.72	6.75	6.54
Control + Vitamin E	6.51	6.22	6.08
Control + FS30	7.42	7.10	6.73
Control + FS60	7.96	5.60	6.39
Control + UFS30	8.35	6.96	8.03
Control + UFS60	8.53	7.99	7.26
SEM[1]	0.632	0.486	0.653
P-value[2]	ns	ns	ns

[1]SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).

8.2.5. Meat lipid oxidation

The extent of lipid oxidation, as measured by MDA formation in thigh meat is reported in Table 8.5.

The extent of lipid oxidation, MDA formation, in thigh meat after 1 and 7 days of refrigerated storage was significantly lower (P<0.05) in birds fed diets supplemented with α-T than the control group. Data also indicated that the inclusion of both concentration of fermented and unfermented grape skin in chicken diets did not reduced MDA values in thigh samples after 1d and 7 d of refrigerated storage compared with samples obtained from birds fed the control diet. On the other hand, the inclusion of 30 g/kg and 60 g/kg of FS in the diets seems to act tendencially better than unfermented grape skin. As expected, lipid oxidation (MDA concentration) increased (P<0.001) with storage time, reflecting the reduction of meat capacity to resist against lipid oxidation during storage time.

Table 8.5 Effect of refrigerated storage on lipid oxidation of thigh meat of broiler chicks (21 d) fed diets containing fermented (FS) and unfermented (UFS) grape skin and vitamin E

	MDA (ng/g meat)	
Dietary treatments	1 d	7 d
Control	3.96[a]	18.8[ab]
Control + Vitamin E	2.52[b]	4.20[c]
Control + FS30	3.39[a]	13.9[b]
Control + FS60	3.83[a]	13.3[b]
Control + UFS30	4.95[a]	21.4[a]
Control + UFS60	4.05[a]	16.6[ab]
	SEM[1]	
	0.509	3.40
	P-value of Contrasts[2]	
Control *vs* FS + UFS	ns	ns
Vitamin E *vs* FS + UFS	**	**
FS *vs* UFS	ns	ns
FS30+UFS30 *vs* FS60+ UFS60	ns	ns

Different letters in the same column (a, b, c) indicate significant differences (p<0.05).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01

8.3. Discussion

8.3.1. Growth performance, protein and polyphenols utilization

Few studies have been carried out on the use of grape by-products such as grape seed extracts or grape pomace in poultry and few data are available on their effect on growth performance (Brenes et al., 2010; Viveros et al., 2011; Dorri et al, 2012 a, b; Iqbal 2014). Previous results published by Goñi et al. (2007) did not report growth depression in birds fed diet supplemented with grape pomace concentrate up to 30 g/Kg. Similarly, Brenes et al. (2008, 2010) and Viveros et al. (2011) did not report growth depression in broilers when fed diet supplemented with grape pomace, grape seed extract and polyphenol-rich grape products, respectively. However, less attention has been directed to the effect of grape skin supplementation in animal nutrition. The present study showed that the inclusion of FS and UFS up to 60 g/kg reduced the performance of the chickens.

Grape skin contains a significant amount of insoluble dietary fiber (above 97%) and bound condensed tannins (Deng et al. 2011). In general, the skin contains the

highest amounts of tannins in the grape berry and these tannins differ from the other grape fractions by having a higher polymerization degree (DP) (Souquet et al. 1996; Torres and Bobet 2001). The average polymerization degree (mDP) for skin tannins is ~28, with 80 being the maximum DP detected (Yilmaz and Toledo, 2004). In the fermentation process the pomace cell structure is modified with the release of polysaccharides and some polyphenols. For that reason the type of and amount of polyphenols is different when using fermented versus unfermented skin. The chemical analysis reported in Table 7.1 showed that the concentration of total polyphenols in UFS contained a higher amount of total polyphenols and a lower concentration of dietary fiber, protein and condensed tannins in comparison to FS analysis. Available data on the effect of grape pomace before and after oenological fermentation has only been reported by Vergara-Salinas et al. (2013). An increase in the release of numerous pomace derived compounds including polysaccharides, mannoproteins and polyphenols were generated by the fermentation process. In fermented pomace extracts a higher procyanidin dimers and trimers and a higher polymerization degree were found than those obtained in unfermented extracts.

The structure and molecular weight of polyphenols play an important role in protein–polyphenol interactions. It has been shown that high molecular weight polyphenols (tannins) are able to bond more strongly or preferentially to proteins (Frazier et al., 2010), before their breakdown by pancreatic enzymes, due to mainly non-covalent hydrophobic interactions which may subsequently be stabilized by hydrogen bonding (Yuksel et al., 2010). These interactions might influence biological activities of proteins, the availability of certain amino acids or even the digestibility of proteins. In the present study, the inclusion of the highest level of unfermented grape skin negatively affected protein digestibility of birds probably due to the interaction of polyphenols with proteins. Similarly, Chamorro et al. (2013) observed that the inclusion of grape seed extract in chickens diet at high level (5g/kg), in parallel with the worsening in growth performance, reduced apparent ileal digestibility of CP and that of some essential (arginine, histidine, phenylalanine) and non-essential (cystine, glutamic acid and proline) amino acids. However, Goñi et al. (2007) reported that apparent ileal digestibility of protein and nonessential amino acids were not affected.

The lack of effect on protein digestibility could be due to the low content of polyphenols and tannins in the experimental diets to cause detrimental effect.

There are many references in the literature to the composition and antioxidant properties of grape polyphenols (Gonzalez-Paramas et al., 2004; Yilmaz and Toledo, 2004), but there have been very few studies on the digestibility and intestinal degradation of polyphenols and other major grape constituents.

Monomeric and some oligomeric polyphenols have been found to be directly absorbed at the small intestine with no prior chemical modification while oligomeric or polymeric forms are not absorbed in their native forms and must be hydrolyzed by the intestinal microbiota (Gonthier et al., 2003). Consequently, polyphenols are metabolized by gut bacteria that produce new phenolic compounds in situ, which could have better bioavailability and higher biological activity than their parent compounds, and be involved in both body systemic and local action (Requena et al. 2010)

In the current experiment, total extractables polyphenols content in chickens fed grape skin apparently were not absorbed and utilized in the small intestine, considering that no differences were observed in their digestibility in the ileum while were quantified high polyphenols values in the ileum content. On the other hand, the total polyphenols determined in excreta in this experiment were reduced due to the degradation of polyphenols by the intestinal microbiota. For that reason, excreta digestibility was higher in that birds receiving both grape skin up to 69 % in comparison to control birds (51 %). These differences were higher in birds fed UF than in those fed FS justified by the different composition of total polyphenols in these skins. In previous studies in our laboratory, similar digestibilities were found in chickens fed grape pomace (Goñi et al., 2007; Brenes et al., 2010; Brenes et al., 2008). No references have been published with the use of skin in chicken diets.

8.3.2. Microbiological counts

Several in vitro studies (Papadopoulou et al., 2005; Özkan et al., 2004; Rodríguez-Vaquero et al., 2007a, b; Gañan et al., 2009; Silván et al., 2013) have shown that flavonoids present in grape by-products have the capacity to inhibit the growth of certain organisms. However, evidence related to the in vivo effects of grape polyphenols on the intestinal microbiota is scarce. Studies conducted in rats (Dolara et

al. 2005; Larrosa et al., 2009; Pozuelo et al., 2012) reported increases in the colonic populations of *Bacteroides*, *Lactobacillus* and *Bifidobacterium* that were associated with the dietary inclusion of grape seed polyphenols. In chickens, Viveros et al. (2011) reported that the inclusion of grape seed extract exerted an antimicrobial effect on *Clostridium* in the ileum, while in the caecum it was associated with an increase in populations like *Lactobacillus* and *Enterococcus*. The different composition of polyphenols present in the skin used might justify the lack of effect on the modification of intestinal microbiota in the current experiment.

8.3.3. Meat lipid oxidation

Lipid oxidation is one of the primary processes of quality deterioration in meat. Nutritional interest in polyphenolic compounds has increased greatly in light of their antioxidant activity (Scalbert and Williamson, 2000).

In this study, the extent of lipid oxidation was lower in birds fed diets supplemented with α-T than the control group. Dietary α-tocopheryl acetate supplementation has been proved to protect fatty acids (Botsoglou et al., 2005; Bou et al., 2006; Galobart et al., 2001; Jensen et al., 1998; Lauridsen et al., 1997; Mielche and Bertelsen, 1994; Wood and Enser, 1997) and cholesterol (Galobart et al., 2002; Grau et al., 2001; Morrissey et al., 1998) from oxidation in eggs and in both raw and cooked poultry meat. Coetzee and Hoffman (2001) showed that vitamin E supplementation of broiler feed increases the oxidative stability of broiler carcasses under frozen and refrigerated storage. This high efficiency is because the radical scavenger α-tocopherol is efficiently incorporated into cell membranes where the oxidation is initiated (Jensen et al., 1998; Lauridsen et al., 1997).

It has also been shown that the addition of polyphenolic grape extract in animal diets, especially oligomeric flavanols, enhances oxidative stability in chicken and turkey meat (Lau and King, 2003; Rababah et al., 2006). Previous studies carried out by Goñi et al. (2007), Brenes et al. (2008) and Sayago-Ayerdi, et al. (2009a) reported a protective effect of grape polyphenols, similar to those of vitamin E, with dietary addition of up to 6% of grape pomace in terms of enhanced oxidative stability in chicken meat products (TBARS, thigh and breast) during the refrigeration process.

However, in the present study dietary FS and UFS did not reach similar protective effect observed with the addition of αT, in delaying the lipid oxidation of meat.

These results might indicate a potential pro-oxidant effect of the polyphenols present in grape skin. It is well known that polyphenol compounds cannot only be considered purely as antioxidants, because under certain reaction conditions, they can also display pro-oxidant activity (Surai 2014) depending on several factors such as metal chelating potential, solubility characteristics, bioavailability and stability in tissues (Decker, 1997; Perron & Brumaghim, 2009).

In some studies grape extracts are not always effective in decreasing lipid peroxidation in biological tissues. For example, grape seed extract (GSE) supplementation has limited protective effect in liver tissue of diabetic rats (Belviranli et al., 2012). It is interesting to note that in a study of Vossen et al. (2011), there was no effect of plant extracts on lipid peroxidation index (TBARS) in broiler plasma.

8.4. Conclusion

In conclusion, the results presented in this study showed that increasing concentration of fermented and unfermented grape skin up to 60 g/kg had adverse effect on growth performance and protein digestibility. The UF and UFS grape skin supplementation was not equally as effective in antioxidant potential as vitamin E. Dietary grape skin addition prove extensive polyphenol metabolism but the polyphenol content of unfermented diet and the modifications generated by the fermentation process in this by-product did not improve the antioxidant and microbiological activities in chickens.

CHAPTER 9. III EXPERIMENT:

Effect grape seed, skin and pomace, and their combination on growth performance, polyphenol digestibility and antioxidant activity in chicken diets.

9.1. Aim

The aim of this study was to evaluate the effect of a dietary addition of GS, SS (individually and in combination), and GP on the performance parameters, ileal and excreta content of total polyphenols and tannins, protein and exctractable polyphenols digestibility, plasma vitamin E, and oxidative stability of animal products.

9.2. Results

9.2.1. Growth performance

The effect of feeding diets containing α-T, GS, SS, GP and their combination on growth performance in chickens is reported in Table 9.1. Daily weight gain, feed intake and the conversion of feed into body weight, expressed as feed conversion ratio (feed intake/weight gain) were not affected by dietary treatments.

Table 9.1. Performance of broiler chicks (1 to 21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E.

Dietary treatments	Daily weight gain (g/d)	Daily feed intake (g/d)	Feed conversion ratio
Control	37.9	55.6	1.46
Control + Vitamin E	36.1	55.0	1.52
Control + GS50 + SS50	37.0	56.2	1.52
Control + GS75 +SS25	36.8	52.8	1.44
Control + GS25 + SS75	35.8	57.2	1.59
Control + GS	38.6	56.1	1.46
Control + SS	36.9	55.2	1.50
Control + GP	37.7	52.9	1.41
SEM[1]	1.06	1.81	0.05
P-value[2]	ns	ns	ns

[1]SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate).
[2]ns: no significant effect (P > 0.05).

9.2.2. Ileal and excreta content of total exctractable polyphenols and tannins

The total ileal and excreta polyphenol content of birds fed diets containing α-T, GS, SS, GP and their combination is shown in Table 9.2. As expected, ileal and excreta content were not affected by dietary α-T supplementation.

Birds fed grape by-products showed a higher ileal and excreta polyphenol content than those fed control diets, except for the GP group that did not show differences with control group in ileal total polyphenols content. The groups receiving GS, SS, GP and their combinations showed also higher ileal condensed tannins content, compared to the control group, while excreta condensed tannins content was not influenced by dietary treatment.

Table 9.2. Ileal and excreta total polyphenols and condensed tannins content of broilers chicks (21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E.

Dietary treatments	**Total polyphenols (g GA/100g)**		**Condensed tannins (mg cyanidin g/100g)**	
	Ileal	Excreta	Ileal	Excreta
Control	0.504^{c}	0.518^{d}	0.055^{b}	0.062
Control + Vitamin E	0.543^{bc}	0.546^{d}	0.053^{b}	0.067
Control + GS50 + SS50	0.680^{a}	0.594^{bc}	0.140^{a}	0.065
Control + GS75 + SS25	0.675^{a}	0.612^{ab}	0.121^{a}	0.079
Control + GS25 + SS75	0.686^{a}	0.672^{a}	0.131^{a}	0.075
Control + GS	0.636^{b}	0.642^{ab}	0.100^{a}	0.069
Control + SS	0.689^{a}	0.669^{a}	0.117^{a}	0.071
Control + GP	0.581^{abc}	0.631^{ab}	0.100^{a}	0.065
	SEM[1]			
	0.037	0.021	0.013	0.006
	P-value of Contrasts[2]			
Control *vs* GS + SS + GP	***	***	***	ns
Control *vs* GS + SS	***	***	***	ns
Control *vs* GS	*	***	*	ns
Control *vs* SS	**	***	**	ns
Control *vs* GP	ns	***	*	ns

Different letters in the same column (a, b, c, d) indicate significant differences ($p<0.05$).
[1]SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate for ileon and five birds per replicate for excreta).
[2]ns: no significant effect ($P > 0.05$).
* P<0.05
** P<0.01
*** P<0.001

9.2.3. Protein and polyphenols digestibility

Ileal and excreta protein and polyphenols digestibility is reported in Table 9.3.

Ileal digestibility of protein was lower in birds fed grape by-product diets than in those receiving the control diet. Particlualry lower (P<0.001) were the ileal protein digestibility values of birds fed grape seed and skin combinations (50:50) compared with those of the control group. No effect on protein digestibility was observed in chickens fed GS, SS and GP separatey, compared with the control group.

Ileal digestibility of polyphenols was clearly higher in chickens receiving grape by-products as dietary supplement, compared with the control and vitamin E groups. In particular, ileal polyphenols digestibility significantly increased (P<0.001) in birds fed GS, SS and GP separately, compared with the control group, while the combination of GS and SS did not affect this parameter.

The excreta total polyphenols digestibility was significantly higher (P<0.001) in all treated groups compared with the control and vitamin E groups.

Table 9.3. Ileal digestibility of protein and ileal and excreta digestibility of total polyphenols of broilers chicks (21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E.

Dietary treatments	Ileal protein digestibility (%)	Ileal total polyphenols digestibility (%)	Excreta total polyphenols digestibility (%)
Control	81.3^{a}	33.4^{c}	47.2^{b}
Control + Vitamin E	78.6^{ab}	28.2^{c}	37.8^{b}
Control + GS50 + SS50	64.2^{c}	25.7^{c}	64.9^{a}
Control + GS75 + SS25	75.6^{ab}	50.0^{b}	65.5^{a}
Control + GS25 + SS75	72.0^{b}	32.6^{c}	65.3^{a}
Control + GS	77.1^{ab}	60.1^{a}	67.6^{a}
Control + SS	81.5^{a}	61.3^{a}	65.9^{a}
Control + GP	81.6^{a}	63.9^{a}	70.2^{a}
		SEM[1]	
	2.04	3.30	2.15
		P-value of Contrasts[2]	
Control *vs* GS + SS + GP	*	***	***
Vitamin E vs GS + SS + GP	ns	***	***
Control *vs* GS + SS	***	ns	***
Control *vs* GS	ns	***	***
Control *vs* SS	ns	***	***
Control *vs* GP	ns	***	***

Different letters in the same column (a, b, c) indicate significant differences (p<0.05).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).
* P<0.05
*** P<0.001

9.2.4. Plasma ROMs and α-tocopherol

The effect of dietary treatments on the composition of plasma ROMs and α-tocopherol is presented in Table 9.4. The inclusion of grape skin, seed and pomace in chick diets increased plasma ROMs values, while the control group showed the lowest value. Plasma α-tocopherol concentration was clearly higher in the group fed vitamin E in comparison to those birds fed control and grape by-products Data also showed a significant effect of dietary treatment with SS ($P<0.05$), GP ($P<0.01$) and combination of GS and GP on plasma α-tocopherol, while the birds fed grape seed did not show any difference in the concentration of α-tocopherol compared with the control group.

Table 9.4. Effect of inclusion of grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E on blood reactive oxygen metabolites (ROMs) and α-tocopherol of 21 d broiler chicks.

Dietary treatments	**ROMs (U/Carr)**	**α-T (µg/ml)**
	21 d	21 d
Control	25.1^{c}	7.06^{b}
Control + Vitamin E	25.9^{bc}	37.4^{a}
Control + GS50 + SS50	26.5^{abc}	6.96^{b}
Control + GS75 + SS25	26.4^{ab}	9.21^{b}
Control + GS25 + SS75	27.0^{ab}	9.48^{b}
Control + GS	26.8^{ab}	7.88^{b}
Control + SS	26.7^{ab}	9.13^{b}
Control + GP	28.0^{a}	9.60^{b}
	SEM[1]	
	0.439	1.12
	P-value of Contrasts[2]	
Control *vs* GS + SS + GP	**	**
Control *vs* GS + SS	*	***
Control *vs* GS	*	ns
Control *vs* SS	*	*
Control *vs* GP	***	**

Different letters in the same column (a, b, c) indicate significant differences ($p<0.05$).
[1] SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect ($P > 0.05$).
* $P<0.05$
** $P<0.01$
*** $P<0.001$

9.2.5. Meat α-tocopherol and lipid oxidation

The extent of lipid oxidation, as measured by MDA formation, in thigh meat, was not significantly affected by dietary treatment (Table 9.5) except in the case of those birds fed Vitamin E that was increased. In fact, birds fed grape by-products did not show any difference in TBARS values, compared with the birds receiving the control diet, neither after 1 day nor after 7 days of refrigerate storage. With respect to meat α-tocopherol concentration, the group supplemented with α-T showed higher levels of this vitamin, compared with the other groups, both after 1 day and 7 days of refrigerate storage.

Higher levels of meat α-tocopherol were found in chickens fed grape by-products after 1 day of refrigerate storage, while after 7 days a significant dietary effect was found only in birds fed the separated GS, SS and GP diets, in comparison to those birds fed the control diet.

Table 9.5. Effect of refrigerated storage on lipid oxidation of thigh meat of broiler chicks (21 d) fed diets containing grape seed (GS), grape skin (SS), grape pomace (GP) and vitamin E.

Dietary treatments	α-T (µg/g)		MDA (ng/g meat)	
	1 d	7 d	1 d	7 d
Control	10.7^{d}	0.67^{b}	4.12^{a}	40.9^{b}
Control + Vitamin E	72.7^{a}	36.2^{a}	2.19^{b}	17.6^{c}
Control + GS50 + SS50	15.7^{cd}	1.69^{b}	3.12^{a}	33.5^{b}
Control + GS75 + SS25	14.1^{cd}	1.25^{b}	4.85^{a}	55.1^{a}
Control + GS25 + SS75	14.8^{cd}	0.96^{b}	3.54^{a}	34.0^{b}
Control + GS	16.6^{cd}	3.38^{b}	4.24^{a}	40.6^{b}
Control + SS	17.2^{c}	2.77^{b}	3.22^{a}	31.2^{b}
Control + GP	17.3^{c}	2.50^{b}	3.79^{a}	32.1^{b}
	SEM[1]			
	1.21	0.588	0.410	6.29
	P-value of Contrasts[2]			
Control *vs* GS + SS + GP	***	ns	ns	ns
Control *vs* GS + SS	**	ns	ns	ns
Control *vs* GS	**	**	ns	ns
Control *vs* SS	***	*	ns	ns
Control *vs* GP	***	**	ns	ns

Different letters in the same column (a, b, c) indicate significant differences (p<0.05).
[1]SEM, standard error of means; each value represents the mean of five replicates (three birds per replicate
[2]ns: no significant effect (P > 0.05).
* P<0.05
** P<0.01
*** P<0.001

9.3. Discussion

9.3.1. Growth performance, protein and polyphenols utilization

The results of the present study showed that the inclusion of grape seed, skin and pomace and combination of them in chicken diets did not affect neither the daily weight gain nor feed intake and feed conversion ratio of birds. Similar results have been obtained in previous studies conducted in our laboratory with broilers fed on diets containing grape pomace at levels of 5, 15 and 30 g/kg (Goñi et al., 2007), grape pomace concentrate at levels of 15, 30 and 60 g/kg (Brenes et al., 2008; Viveros et al., 2011) or 3.6 g/kg of grape seed extract (Brenes et al., 2010). The data of the present experiment also agree with the first study of this thesis in which, the inclusion of GS (15 and 30 g/kg) and GP (37.5 g/kg) in chicken diets did not change their growth performance. On the other hand, in the first two experiments of this thesis, the addition of SS (110 g/kg and 60g/kg) reduced the performance of the birds probably due to the different structures of polyphenols in grape skin from polyphenols in grape seed, while in the present work seem that the incorporation of grape skin at level of 40g/kg is adequate for chickens diet. The fact that grape by-products inclusion did not affect these parameters also agrees with recent results reported by Francesch and Cartañà (2015), who supplemented 5% of grape seed in the diet of the Penedes chicken to 5 weeks of age. Similarly, Iqbal et al. (2014) found that grape pomace polyphenols did not depress the growth performance of broilers when incorporated in diet up to 75mg/kg in place of vitamin E and Rotava et al. (2009), who included 2.35% of grape seed in broiler diets to 21 d of age.

Some authors (Hughes et al., 2005; Lau and King, 2003) observed growth depression in chickens fed 3% and 5.18% of grape seed extract, which according to Goñi et al. (2007) and Brenes et al. (2010) may have been be provoked by the higher concentration of polyphenols. Thus, differences also in the dietary ingredients, type and dosage of bioactive compounds used in the studies may explain the discrepancies.

Polyphenols are part of the composition of many plants and are considered anti-nutritional factors of great importance. They are highly chemically active and may react reversibly or irreversibly with proteins, impairing the digestibility and bioavailability of essential amino acids. Protein digestibility is a nutritional parameter that evaluates the use of a protein source. This is influenced by several factors, for

example, phenolic compounds (Antunes et al., 1995) that have the ability to form complexes, as well as to precipitate proteins (Bressani et al., 1991). In the present study protein digestibility was not impaired by grape seed, skin and pomace supplemented separately. According also with the first experiment of this thesis, the lack of effect could be attributed to the low content of polyphenols in the experimental diets to cause detrimental effect. On the other hand, the different combinations of grape seed and skin decreased protein digestibility of chickens probably due to the different profile of polyphenols generated by these associations and the lower total polyphenol digestibility in the birds fed these by-products in comparison to the other groups (Table 9.3). Anyway, this effect on protein digestibility did not negatively affect chickens growth performance.

As expected, in the current experiment birds fed grape by-products diets showed a higher intestinal (ileal and excreta) total polyphenols content than those fed control and vitamin E diets. Higher tannins values were quantified only at ileal level in the chicks receiving grape by-products, compared to the control group. Like the first experiment of this thesis, chicks fed diets containing GP did not show any difference with the control group in the ileal content of polyphenols while the ileal polyphenols digestibility reached the highest values in this group. Considering these results, total extractable polyphenols apparently were absorbed and utilized in the small intestine of chickens fed GS, SS and GP individually more efficiently than chickens fed grape seed and skin combinations. In fact, in these latter no differences were observed in the polyphenols digestibility in the ileum while high polyphenols values in the ileum content were quantified.

The total polyphenols determined in chickens excreta in this experiment were quite similar to those of the the ileal content. Excreta digestibility of polyphenols was higher in birds receiving grape by-products in their diets in comparison to control and vitamin E diets, reaching values in a range of 65 to 70% and reflecting the higher polyphenols content quantified in the excreta. The results from intestinal polyphenols digestibility suggests that polyphenols, or their metabolites, could be bioefficient in some tissues.

Similar digestibilities have been found in the previous experiments of this thesis and in previous studies carried out in our laboratory in chickens fed grape

pomace (Goñi et al., 2007; Brenes et al., 2010; Brenes et al., 2008). The nutritional effects of polyphenols would be a consequence of the absorbed monomers and aromatic acid, the interaction of unabsorbed polyphenols with components of the intestinal tract, or both. As reducing agents, they may be active in the gastrointestinal tract and modify the intestinal environment (Scalbert and Williamson, 2000). Goñi and Serrano (2005) reported that intestinal bacteria showed a high capacity to degrade extractable polyphenols in rats. Deprez et al. (2000) and Ward et al. (2004) also showed that major polyphenolic constituents of grape seed (polymeric proanthocyanidins) were degraded by human colonic microflora into smaller compounds including phenolic acids that could be absorbed and metabolized.

9.3.2. Plasma ROMs and α-tocopherol

ROMs (mainly hydroperoxides, ROOH) represent the primary products of an intermediate step of oxidative damage and are generated by the peroxidation of biomolecules such as lipids, proteins, and nucleic acids, in the early phase of the oxidative cascade (Alberti et al. 2000, Halliwell and Gutteridge 2007). ROMs are more stable than ROS (Reactive Oxygen Spieces) and therefore they can be detected and quantified and have been used as a marker of early oxidative damage (i.e., damage of biomolecules early in the oxidation cascade) in several studies (Costantini and Dell'Omo 2006; Bonisoli-Alquati et al. 2010; Costantini and Bonadonna 2010; Monaghan et al. 2009). Besides being the product of oxidation of biomolecules, ROMs are themselves pro-oxidants and can therefore further propagate the oxidation chain reaction (Halliwell and Gutteridge 2007) and impair animal performance (Miller et al., 1993). Some studies of birds have found that the level of ROMs increases as a consequence of immune challenge and are related positively to oxygen consumption (Costantini and Dell'Omo 2006, van de Crommenacker et al. 2010). The dROMs test has already successfully used for measuring total oxidative stress (Celi 2011), as it measures the blood concentration of hydroperoxides, in whole blood and serum of livestock animals (bovine, porcine, equine) by several authors (Trotti et al., 2001; Rekitt et al., 2002; Andresen et al., 2003; Sauerwein et al., 2005). However, scarce information is present in literature about the use of this test in chickens.

The observed range of ROMs in this study is consistent with that reported by Celi et al. (2013) in broiler chickens. These authors did not observe changes in plasma ROMs concentration of birds after Se supplementation in agreement with the findings of Chauhan et al. (2012) who was not able to observe changes in ROM concentrations between sheep whose diets were supplemented with either recommended or physiological concentrations of Se and Vitamin E.

Contrarily to what was expected, in the present study low values of plasma ROMs in the control sample and high values in chickens fed grape by-products were observed. Therefore, ROMs concentrations do not reflect dietary changes of grape by-products concentrations. Regenhard et al. (2014) affirmed that the dROM test cannot be validly used with chicken whole blood or serum. In fact, these authors in a study on the evaluation of the applicability of dROMs test for assessment of oxidative stress in poultry, concluded that the presence of serum components, as uric acid, exclusively present in blood serum of poultry species and not in mammals could interfere with the dROMs test prohibiting its valid application.

With regard to α-tocopherol concentration in chicken plasma, the present study shows that vitamin E supplementation increased the concentration of this vitamin in the plasma of birds compared to the rest of the groups. According to Jakobsen et al. (1995) immediately after the intake of tocopherols, chicken plasma reflects the tocopherol composition of the diet. Also grape by-products supplementation increased the concentration of this vitamin in chick plasma, compared to the control group in accordance with the first experiment of this thesis. However, in the current study, plasma α-tocopherol content was not increased in birds fed 4% GS diets, as was observed with the other treatments and in comparison to the control diet. Vitamin E, due to its hydrophobic nature, is transported in plasma by low- and very low-density lipoproteins before being incorporated into the lipid bilayers (Khalil, 2002). In contrast, polyphenols could be present at the aqueous‑lipid interface (Manach et al., 2004) where, close to vitamin E, they could favor the recycling of the α-tocopheroxyl radical, as shown by Zhou et al. (2005) for green tea polyphenols *in vitro* and by Mukai et al. (2005) for catechins in micellar solutions. A second potentially relevant property of polyphenols would be their ability to spare α-tocopherol by limiting the initiation

of the propagation phase, as observed *in vitro* in liver microsomes of rats given PUFA and vitamin E associated with plant flavonoid-rich diets (Fremont et al. 1998).

Probably, the flavonoids present in grape skin and pomace by-products directly scavenge free radicals and may also regenerate α-T from its radical form. Studies conducted by Frank et al. (2006, 2003) showed that dietary supplementation with flavonoids quercetin, catechin and epicatechin resulted in a substantial increase in α-T concentrations in blood plasma and liver tissue of male Sprague-Dawley rats. It was suggested that, owing to their one-electron reduction potentials, polyphenols may spare endogenous antioxidants similar to the recycling of vitamin E by ascorbic acid (Buettner, 1993). The lipophilic vitamin E is thought to take part in an antioxidant network in which vitamin E radicals are recycled at the lipid-water interface by the hydrophilic antioxidant ascorbic acid, which, in turn, is regenerated from its ascorbyl radical by thiol or polyphenol antioxidants, which eventually are recycled through the conversion of NAD (P) $H+H^+$ to NAD $(P)^+$ (Laranjinha 2001; Packer et al., 2001).

9.3.3. Meat α-tocopherol and lipid oxidation

One of the main factors limiting the quality and acceptability of meat and meat products is lipid oxidation, as it can impact the nutritional value, shelf-life and sensory quality (flavor and color). Lipid oxidation in foods is one of the major degradation processes that are responsible for food quality loss. Plant extracts exhibit high antioxidant and antimicrobial activities and are used in animal feeding for improving health status of the birds, but also for increasing shelf-life of food products by retarding microbial spoilage and oxidative processes (Friedman et al., 2002; Rababah et al., 2004). Wine processing by-products (grape pomace and grape seed extract), rich in a wide range of polyphenols, have been used in broiler chicken diets and, as discussed in the previous experiments (Goñi et al., 2007; Brenes et al., 2008; Sayago-Ayerdi et al., 2009a; Chamorro et al., 2015). The results showed that the addition of grape by-products to chickens diet lowered the susceptibility of meat to undergo lipid oxidation. In our study we investigated if GS, SS and GP fed up to 4% have the same effect observed in previous experiment, and to know the participation of the different components of grape pomace were implicated in this effect.

α-Tocopherol is the predominant form of vitamin E in animal plasma and the major antioxidant localized in muscle lipids, thus its level is an important contributor to the resistance of lipids to oxidation. Higher dietary intake of α-tocopherol is accompanied by its higher accumulation in muscles. In fact, in the present experiment a clear increase of α-tocopherol concentration was observed in the thigh meat of chickens fed vitamin E. We expected that also the dietary supplementation of the antioxidant compounds contained in grape by-products was able to increase vitamin E concentrations in chickens plasma and muscle. As previously discussed and similarly to the first experiment, in this study only the dietary inclusion of SS and GP increased the concentration of plasma α-tocopherol in comparison to the birds fed the control diet while meat α-tocopherol was increased by dietary GS, SS and GP and their combinations after 1 day of storage, while after 7 days of storage only the GS, SS and GP supplemented separately were effective.

The anomalous effect of GS that did not affect plasma concentration of α-tocopherol but significantly elevated α-T levels in the muscle show that different phenolic compounds behave differently as regards their effect on body tocopherol. A similar conclusion was reported by Kamal-Eldin et al. (2000) who found that curcumin did not affect plasma or liver concentrations of α-T or γ-T but significantly elevated α-T levels in the lungs and that ferulic acid lacked effect on α-T and γ-T levels in plasma, liver and lung.

Regarding meat lipid oxidation, the results from this study showed that storage time increased MDA values of chicken thigh meat. Meat from chickens fed 4% of GS, SS (individually or in combination) and GP supplemented diets has similar TBARS values as meat from birds fed control diet and significantly higher than meat from chicks fed α-tocopherol-enriched diets. Protective effects of grape polyphenols were previously reported in our laboratory (Goñi et al., 2007; Brenes et al., 2008) with dietary addition of up to 6% of GP. However, in this study polyphenols intake from a diet containing lower concentration of grape by-products (4%), in general did not improve the oxidative status of chickens thigh muscle. Unlike the first experiment of this thesis, dietary inclusion of GP did not delayed lipid oxidation. It is also known that vinification processes, extraction methods, grape variety, agronomic and environmental factors affect the proportions of various polyphenols present in grape

by-products, thus these different results could be explained by the use of a different commercial GP that probably had a different polyphenolic profile from the GP used in the first experiment.

The results from both experimental trials showed that dietary grape seed and grape skin supplemented at different concentrations had no effect on meat lipid oxidation in chickens. This may suggest that dietary phenolics from these two different grape fractions and their metabolites are not accumulated in muscles, or are accumulated in trace amounts only. However, we were no able to find information about the supplemental effect of GS and SS on chicken meat quality.

9.4. Conclusions

In conclusion, the inclusion of grape by-products added individually or in combination up to 4% did not impair the performance of the birds. Our findings also suggest that an important fraction of polyphenols are digested along the intestinal tract of birds and did not affect the protein digestibility. Although no differences were observed in the MDA values among the control and the grape by-products treatments, however birds fed all the grape by-product diets except GS (at 1 day of thigh refrigerated storage) and particularly GS, SS and GP diets (at 7 days) increased plasma and meat α-tocopherol concentration.

CHAPTER 10. IV EXPERIMENT

Antioxidant and antimicrobial effect of dietary grape by-product on chicken breast meat and patties

10.1. Aim

The aim of this study was to evaluate the effect of a dietary addition of GS, SS, and GP on proximate composition, microbiological stability, lipid oxidation, sensory attributes, instrumental color, pH, total extractable polyphenols content and texture of chicken meat and patties.

10.2. Results and discussion

10.2.1. Characterization of chicken meat

The study of proximate composition (protein, moisture, fat and ash), total extractable polyphenols content and microbiological count in the meat obtained from the chicken diet formulated with the grape skin, seed and pomace is an important aspect to consider.

Mainly because it was a first control of the effect of this diet in the chicken raw meat and this meat will be the raw material for the elaboration of the patties. Therefore this meat will influence in the properties of the patties. Moreover, the assessment of these parameters will help to better understand the patties physicochemical, microbiological and sensorial characteristics during storage. This meat also will be the main source of polyphenols which apart of the role on the patties lipid oxidation and microbial growth it is expected that will provide a functional final product rich in polyphenols with positive effects on consumer health.

10.2.1.1. Proximate composition of chicken meat

Table 10.1. shows the effect of the dietary supplementation with grape by-products and vitamin E on proximate composition of chicken breast meat. These values are within the ranges observed also by other authors (Jo et al., 2009). Fat and protein did not show significant differences among chicken samples (Table 10.1.).

Only in the case of moisture and ash small significant differences were observed, although less relevant. Meat from chickens supplemented with vitamin E,

grape skin and grape pomace showed statistically (P<0.05) lower moisture values compared to the control (MC). With respect to ash content, meat from diets containing grape by products exhibited lower ash values (P<0.05) compared to the meat from diets with vitamin E (ME) and control. Also Shirzadegan and Falahpour (2014) found a linear decrease in crude ash of chicken thigh meat fed with a diet with a medicinal herbal extract mixture derived from, green tea, cinnamon, garlic, and chicory that are sources of natural antioxidants or functional materials.

10.2.1.2. Total exctractable polyphenols content of chicken meat

Dietary supplementation with grape seed and grape skin resulted in a statistical (P<0.05) higher meat total phenolic content, compared to the control and MGP breast meat, as showed in Table 10.1. In disagreement with the expected results, ME showed the highest (P<0.05) polyphenols value (58.63 mg GAE/100g) while the MGP sample had the same polyphenols content of the control meat. Jo et al. (2009) observed a higher polyphenols content in chicken thigh meat of treated groups (P<0.05) compared to the control. However, other authors (Yong et al., 2013) have not found significant difference in the total phenolic content of breast chicken meat fed with diet formulated (integrated) with different percentage (0.25% and 0.5%) of wild grape powder even with the control sample which presented similar levels of polyphenols than the other two samples. Similar results were also observed by Jung et al. (2010) in breast meat from broilers fed with a dietary mixture of gallic acid and linoleic acid, who also did not find differences among the groups. On the other hand, in our experiment it was observed that the important part of the polyphenols from the diets was less compared to the levels found in the raw meat. Other authors (Manach et al., 2004) also observed that many types of polyphenols can lose a part of their antioxidant capability in vivo.

10.2.1.3. Microbiological counts of chicken meat

The microbiological counts in breast chicken are shown in Table 10.1. The levels of TVC were between 4.68 and 5.37 Log cfu/g. In general there were no significant differences in the TCV and LAB count, except for the meat from chickens fed diet enriched with grape seed (MGS) which showed a higher (P<0.05) level of TVC and LAB (5.37 and 4.28 Log cfu/g, respectively). These results are in agreement with the findings of Jung et al., (2010) in breast meat of broilers fed a mixture of gallic

acid and linoleic acid (MGL), who observed higher levels of TVC in the meat sample of chickens receiving the higher level of MGL.

The results of *Enterobacteriaceae* showed no statistical differences among all groups, with values between 2.51 and 3.23 Log cfu/g that are considered hygienically acceptable for raw meat. The higher levels of *Enterobacteriaceae* were found in the samples MGS and ME, these meats also presented significant ($P<0.05$) high levels of coliforms bacteria. The microbial count in this stage was mainly caused by the handling of the breasts during the boning, peeling, and chopping to prepare the final meat material for the patties.

Table 10.1. Proximate analysis (%), microbiological counts (Log cfu/g) and total extractable polyphenols content (TEPs) (mg/100g GAE) of different chicken breast meat.

Parameters	MC	ME	MGS	MSS	MGP
Proximate composition					
Moisture	75.35±0.04^{a}	74.77±0.02^{b}	74.85±0.08^{ab}	74.38±0.13^{b}	74.61±0.00^{b}
Protein	22.32±0.09^{a}	22.83±0.11^{a}	21.86±0.32^{a}	22.46±0.24^{a}	22.53±0.10^{a}
Fat	1.71±0.18^{a}	1.54±0.07^{a}	1.97±0.20^{a}	1.59±0.11^{a}	1.74±0.05^{a}
Ash	1.29±0.02^{ab}	1.36±0.03^{a}	1.23±0.02^{b}	1.25±0.02^{b}	1.24±0.03^{b}
Microbiological count					
TVC	5.10±0.02^{ab}	4.68±0.03^{b}	5.37±0.10^{a}	5.04±0.23^{ab}	5.15±0.11^{ab}
LAB	3.83±0.03^{b}	3.72±0.09^{b}	4.28±0.06^{a}	3.72±0.03^{b}	3.63±0.04^{b}
Enterobacterias	2.57±0.02^{a}	3.14±0.13^{a}	3.23±0.07^{a}	3.07±0.70^{a}	2.51±0.24^{a}
Coliformes	2.40±0.05^{b}	3.15±0.11^{a}	3.32±0.09^{a}	2.54±0.11^{b}	2.48±0.01^{b}
TEPs	47.76±0.08^{c}	58.63±0.31^{a}	53.33±0.31^{b}	56.03±0.00^{ab}	46.46±0.08^{c}

Sample denomination: MC (Control); ME (Control+vitamin E); MGS (Control + Grape Seed 4%); MSS (Control+ Grape skin 4%); MGP (Control+ Grape pomace 4%).
Means ± SD. Different letters in the same row (a, b, c) indicate significant differences ($p<0.05$).

10.2.2. Physico-chemical composition of chicken patties

10.2.2.1. Proximate composition of chicken patties

The proximate composition of chicken patties is reported in Table 10.2. These results are related to the components used in the preparation. The results were similar of that found in the other study on chicken patties with similar ingredients in the formulation, except in the case of protein which was higher in this study among 21.27-20.36 %. These differences could be attributed to the higher proportion of protein from

meat chicken breasts used in these patties (Table 10.1). In general, the difference found in the patties composition for the different samples was not relevant.

Table 10.2. Proximate analysis (%) of different chicken patties

Parameters	PC	PE	PGS	PSS	PGP
Moisture	69.63±0.82^{a}	67.98±0.48^{bc}	69.17±0.32^{ab}	67.85±0.15^{c}	68.68±0.04^{abc}
Protein	20.43±0.22^{b}	21.27±0.28^{a}	20.36±0.18^{b}	21.25±0.20^{a}	20.80±0.08^{ab}
Fat	2.22±0.06^{b}	2.53±0.08^{a}	2.53±0.02^{a}	2.21±0.04^{b}	2.34±0.08^{ab}
Ash	2.01±0.02^{ab}	2.05±0.01^{a}	1.98±0.02^{b}	2.04±0.03^{ab}	2.00±0.03^{ab}

Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).
Means ± SD. Different letters in the same row (a, b, c) indicate significant differences ($p<0.05$).

10.2.2.2. pH of chicken patties

The results of the pH in the patties during storage time are presented in the Table 10.3. The initial (day0) pH value of patties ranged from 6.00 to 6.14, similar levels was found also by other author in pork patties reformulated with natural extracts (Lorenzo et al., 2014; Naveena et al., 2008; Devatkal et al.2011). The patties with the meat from the chicken fed with grape by products and vitamin E presented lower ($P<0.05$) pH values than the control sample, during the experiment. The lower pH in the samples with grape could be a consequence of the properties and the nature of the active compounds present in the meat of chicken fed with diet rich in grape by products. This behavior was also observed by other authors in pork patties elaborated with natural extracts (tea, grape, chesnut and seaweed) (Lorenzo et al. 2014). Lower pH value were observed in the thigh muscle of broiler fed diets supplemented with a medicinal herbal extract mixture (green tea, cinnamon, garlic and chicory) (Shirzadegan et al., 2014) and dietary Chinese medicine by-products (Park and Yoo (1999) compared to control muscle.

During the chilled storage of the patties, a slight decreased of the pH values in all the samples was observed (Table 10.3). A less sharp decline was observed in the V experiment of this thesis. This decrease was mainly produced by the growth of lactic acid bacteria, and the lactic acid production (Karabagias et al. 2011; Triki et al., 2013).

Table 10.3. pH levels of different chicken patties during refrigerated storage

Sample	Storage (days) at 4°C			
	0	3	6	9
PC	6.14±0.02^{a1}	6.11±0.01^{a2}	6.07±0.01^{a3}	6.06±0.00^{a3}
PE	6.06±0.00^{b1}	6.00±0.00^{c2}	6.00±0.01^{d2}	5.99±0.02^{cd2}
PGS	6.05±0.01^{b1}	6.03±0.01^{b2}	6.02±0.00^{c3}	6.02±0.01^{b3}
PSS	6.05±0.00^{b1}	6.04±0.00^{b1}	6.04±0.01^{b1}	6.01±0.01^{bc2}
PGP	6.00±0.00^{c1}	6.00±0.01^{c1}	5.97±0.00^{e2}	5.98±0.01^{d2}

Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).
Means ± SD. Different letters in the same column (a, b, c, d, e) and number in the same row (1, 2, 3) indicate significant differences ($p<0.05$).

10.2.2.3. Total exctractable polyphenols content of chicken patties

Table 10.4 shows the total extractable polyphenols content in raw chicken patties during refrigeration storage. The initial levels of polyphenols in these samples were among 36.24-31.26 mg /100 g GAE. The lower levels of polyphenols were observed in the patties PE, PSS and PGP compared with control and PGS, while no significant differences were observed between these two last samples. The results were in disagreement with the data of the total phenolic content of meat (Table 10.1) used for the patties formulation where, in fact, were observed higher polyphenols levels in the ME, MGS and MSS samples. In relation with these results other authors also have not found clear differences in the polyphenols levels in the meat products with extracts rich in polyphenols and the control sample. In part this may be due the non-specificity of the Folin Ciocalteu analysis and the possible interference of many compound in the patties (sugars, aromatic amines, sulfur dioxide, ascorbic acid, organic acids, inorganic substances, Fe(II) etc.) with this reagent, thus overestimating the content of phenolic compound.

During the chilled storage a significant ($P<0.05$) increase of total phenolic content was observed in all samples including the control diet (higher in the control) until day 6 and while a significant decrease was observed at the end of the storage period (Table 10.4.). These results are in relation with the results of the V experiment of this thesis, on chicken patties formulated with grape seed and grape skin. Increase of polyphenols during chilled storage has been observed by other authors (Devatkal et al., 2011; Naveena et al., 2008). This behavior was attributed to the increases of the activity of an enzyme which accelerates the buildup of phenolics (Padda and Picha,

2008). In fact, these results are in relation with the polyphenols content found in the cooked chicken patties (analysis in the patties at day 6 of storage) were the higher levels of polyphenols were found in the control (61.49 mg/100g GAE) and in the PGS (60.47 mg/100g GAE) similar to the PSS (59.28 mg/100g GAE) and the lower in PE (52.08 mg/100g GAE) and in PGP (56.41 mg/100g GAE). The higher levels of polyphenols in the cooked samples could be due to the cooking loss during the heat treatment. Other authors have also reported significant increase of the total phenolic content in cooked chicken patties (Naveena et al., 2008)

Table 10.4. Total extractable pholyphenols content (mg/100g GAE) of different chicken patties during refrigerated storage.

Sample	Storage (days) at 4 °C			
	0	3	6	9
PC	34.23 ± 0.69^{ab4}	42.29 ± 0.46^{a3}	55.00 ± 0.54^{a1}	48.73 ± 0.08^{a2}
PE	31.26 ± 0.00^{b3}	$47.11\pm1.30^{a2,3}$	48.89 ± 0.92^{b1}	43.75 ± 0.23^{b2}
PGS	36.24 ± 0.15^{a3}	47.54 ± 0.38^{a2}	51.27 ± 0.76^{b1}	46.46 ± 0.38^{ab2}
PSS	31.53 ± 0.38^{b2}	42.24 ± 1.91^{a1}	46.30 ± 1.07^{b1}	42.29 ± 1.38^{b1}
PGP	32.72 ± 0.23^{ab3}	42.94 ± 0.92^{a2}	49.92 ± 0.08^{b1}	43.65 ± 0.38^{b2}

Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).
Means ± SD. Different letters in the same column (a, b) and number in the same row (1, 2, 3) indicate significant differences ($p<0.05$).

10.2.2.4. Microbiological count of chichen patties

Microbiological studies of chicken patties are shown in Table 10.5. The bacterial contamination in these products was a result of the quality of raw meat (Table 10.1) and the other non-meat ingredients plus the handling and process of chicken patties.

The initial levels of TVC and LAB were around 4 Log cfu/g and 3.5 Log cfu/g respectively with no significant difference among all samples. These results are in relation with the raw chicken meat used for the formulation of these patties (Table 10.1) and it indicate a good manufacturing practices of the patties (APHA-American public Health Association, 1984; Dawson et al., 1995).

The level of *Enterobacteriacea*e and *Coliforms* were very low <3 Log cfu/g observing initially the lowest levels for both organisms in the control samples (PC),

although at the end of storage no significant differences were noted among the samples and the levels remained very similar to those observed at the beginning of the storage.

The levels of TVC and LAB slowly increased during chilled storage, the main increase was observed at the end of storage, but no significant differences among the samples were observed except in the case of TVC and the LAB in the samples PE and PSS, that presented the lower levels (Table 10.5). The levels of TVC and the LAB were associated to the anaerobic storage conditions and to the pH in these samples (Table 10.3). A clear effect of the grape by-products and vitamin E in chicken diet was not observed in the growth of microorganism in this study. Other authors also did not observe a clear relation between the addition of grape extract in pork patties and the microbial count (Lorenzo et al., 2014). However antimicrobial effect of grape extracts were shown *in vitro* studies (Ahn et al., 2004).

These results could be explained by the increased effectiveness of plants extracts and fruits rich in polyphenols against gram-positive bacteria than gram-negative as showed by other authors (Rodriguez-Carpena et al., 2011; Kossah et al., 2011). However, it is necessary to consider that the different antimicrobial activity of extracts could be due to the nature of the substances present in the extracts and their mechanism of action on the tested microorganism (Rodriguez-Carpena et al., 2011). Other authors have found higher antimicrobial activity of plant extract with less total polyphenols content (Bystrom et al., 2009).

Table 10.5. Microbiological counts (Log cfu/g) in different chicken patties during refrigerated storage

Parameters	Sample	Storage (days) at 4 °C			
		0	3	6	9
Total viable count (TVC)	PC	4.26 ± 0.00^{a1}	$3.91\pm0.12^{a1,2}$	3.86 ± 0.03^{c2}	4.42 ± 0.39^{a1}
	PE	4.31 ± 0.01^{a2}	4.86 ± 1.44^{a2}	3.83 ± 0.03^{c2}	5.27 ± 0.02^{a1}
	PGS	4.39 ± 0.07^{a1}	4.10 ± 0.02^{a1}	4.02 ± 0.03^{b1}	4.67 ± 0.62^{a1}
	PSS	4.34 ± 0.03^{a2}	3.92 ± 0.11^{a2}	4.06 ± 0.03^{ab2}	5.09 ± 0.07^{a1}
	PGP	4.36 ± 0.51^{a2}	4.16 ± 0.02^{a2}	4.18 ± 0.04^{a2}	4.55 ± 0.35^{a2}
***Lactic acid bacteria* (LAB)**	PC	3.48 ± 0.26^{a3}	3.75 ± 0.04^{a2}	3.85 ± 0.03^{a2}	4.22 ± 0.02^{a1}
	PE	3.47 ± 0.12^{a3}	3.84 ± 0.09^{a2}	4.01 ± 0.04^{a2}	4.06 ± 0.03^{b1}
	PGS	3.62 ± 0.21^{a1}	3.86 ± 0.02^{a1}	3.93 ± 0.07^{a1}	4.13 ± 0.02^{ab1}
	PSS	3.49 ± 0.15^{a1}	3.86 ± 0.03^{a1}	3.98 ± 0.02^{a1}	4.04 ± 0.06^{b1}
	PGP	$3.69\pm0.12^{a1,2}$	3.36 ± 0.51^{a2}	$3.88\pm0.04^{a1,2}$	4.20 ± 0.04^{a1}
Enterobacteriaceae	PC	1.39 ± 0.55^{b1}	1.85 ± 0.00^{b21}	2.26 ± 0.00^{c1}	2.60 ± 0.56^{a1}
	PE	2.13 ± 0.18^{ab1}	2.04 ± 0.00^{b1}	1.95 ± 0.00^{e1}	2.17 ± 0.24^{a1}
	PGS	2.20 ± 0.00^{ab1}	2.04 ± 0.00^{b1}	2.20 ± 0.00^{d1}	2.37 ± 0.23^{a1}
	PSS	2.62 ± 0.11^{a1}	2.63 ± 0.21^{a1}	2.59 ± 0.00^{b1}	2.77 ± 0.01^{a1}
	PGP	2.86 ± 0.02^{a3}	2.91 ± 0.01^{a2}	2.99 ± 0.01^{a1}	2.84 ± 0.01^{a3}
Coliforms	PC	1.90 ± 0.00^{c1}	1.90 ± 0.00^{d1}	2.40 ± 0.00^{c1}	2.45 ± 0.64^{a1}
	PE	2.11 ± 0.00^{c1}	2.23 ± 0.00^{c1}	2.00 ± 0.00^{d1}	2.22 ± 0.31^{a1}
	PGS	2.04 ± 0.00^{c1}	2.00 ± 0.00^{e2}	2.04 ± 0.00^{d1}	2.37 ± 0.24^{a1}
	PSS	$2.67\pm0.09^{b1,2}$	2.61 ± 0.00^{b2}	2.57 ± 0.04^{b2}	2.82 ± 0.01^{a1}
	PGP	3.05 ± 0.14^{a1}	3.03 ± 0.11^{a1}	2.86 ± 0.02^{a1}	2.84 ± 0.04^{a1}

Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).
Means ± SD. Different letters in the same column (a, b, c, d, e) and number in the same row (1, 2, 3, 4) indicate significant differences ($p<0.05$).

10.2.2.5. Lipid oxidation of chicken patties

The TBA-reactive substances levels of chicken patties during refrigerated storage are presented Table 10.6. The initial levels of TBARS were very low in all the samples (0.23-0.56 mg MDA/Kg sample). Significantly ($P<0.05$) lower TBARS values were detected for chicken patties of PE, PSS and PGP groups compared to the control group, while PGS group showed similar values to the control patties. These results indicated that feeding chicken with 4% of grape pomace and grape skin could be effective against TBARS formation as vitamin E. Surprisingly, this effect was not found for seed contrary to what was observed in the V experiment, in which, however, the patties were elaborated directly by adding grape seed powder. The level of

polyphenols of this latter was higher compared to the grape seed used in the present experiment. In this trial we have to take into account that the grape by-products that was integrated in the chicken diets and, for this reason, the final effect in the patties could be very different.

On the other hand, the antioxidant effect of the chicken diet integrated with vitamin E clearly demonstrated to be very effective in protecting fatty acids and decreasing lipid oxidation in eggs and in both raw and cooked poultry meat (Brenes et al., 2010; Brenes et al., 2008; Decker and Xu, 1998; Jensen et al., 1998; Botsoglou et al., 2005; Galobart et al., 2001; Lauridsen et al., 1997; Mielche and Bertelsen, 1994; Wood and Enser, 1997; Galobart et al., 2002; Grau et al., 2001; Morrissey et al., 1998). This high efficiency is due to the radical scavenger α-tocopherol which is incorporated into cell membranes where the oxidation is initiated (Jensen et al., 1998; Lauridsen et al., 1997). The antioxidant effect of grape by-products has also been demonstrated by several authors (Brenes et al., 2008; Brenes et al., 2010; Smet et al., 2008; Iqbal et al., 2014; Goñi et al., 2007; Sáyago-Ayerdi et al., 2009 a, b; Chamorro et al., 2015) associated mainly to the phenolic content and their ability to scavenge free radicals, to form complexes with metal ions and to prevent or reduce the development of singlet oxygen (Rice-Evans and Miller 1996; Yilmaz & Toledo 2006; Surai 2014). The inclusion of GP and GSE in chicken diets significantly improved oxidative stability (TBARS) and radical scavenging capacity (ABTS) in raw breast meat and cooked chicken patties (Sayago-Ayerdi et al. 2009 a; Selani et al. 2011). Similar results have been reported by Brannan (2008) and Lau and King (2003) in chicken thigh meat during refrigerated storage and by Mielnik et al. (2006) in turkey meat.

However, the relationship between phenolic compounds and antioxidant capacity was inconsistent among the results from different studies, which indicated that, besides the concentration, the antioxidant capacities of phenolic compounds were affected by other factors (Radovanovic et al, 2009; Di Majo et al., 2008). A study of Falchi et al. (2006) observed that although total phenolic index was lower in grape flesh than in grape skin because anthocyanins were absent in the flesh, they possessed equal amounts of reactivity to hydroxyl radicals. In another study, the results also showed that the anti-radical activity was due to the flavanols, rather than anthocyanins (Arnous et al., 2002). Anthocyanins contribute more to the antioxidant capacity of

fruits (90%) than flavonols, flavan-3-ols, and phenolic acids (10%) (Jakobek et al., 2009). On this matter, grape skins have been highlighted for their antioxidant activity because of their anthocyanins and proanthocyanidins content (Laurent et al., 2007; Ricardo da Silva et al., 1991). Moreover, the antioxidant activity of a sample could be a synergic effect among several compositions, rather than a single compound (Maier et al., 2009; Monagas et al., 2005).

On the other hand the main polyphenol detected in chicken meat was gallic acid and isoflavones since are the well-absorbed polyphenols whereas the least are proanthocyanidins and anthocyanins (Williamson and Manach, 2005). This could explain the effectiveness of grape skin and grape pomace supplementation in reducing lipid oxidation of the storage patties formulated with chicken meat from these two groups.

Table 10.6. Lipid oxidation as changes in thiobarbituric acid-reactive substances (TBARS mg MDA/kg sample) values of different row chicken patties during refrigerated storage.

Sample	Storage (days) at 4°C			
	0	3	6	9
PC	0.56 ± 0.05^{a1}	0.35 ± 0.01^{a2}	0.38 ± 0.05^{a2}	0.40 ± 0.01^{a2}
PE	0.23 ± 0.01^{b1}	0.23 ± 0.02^{c1}	0.20 ± 0.04^{c1}	0.24 ± 0.01^{c1}
PGS	0.47 ± 0.00^{a1}	0.39 ± 0.01^{a2}	0.35 ± 0.00^{ab3}	0.40 ± 0.00^{a2}
PSS	0.29 ± 0.01^{b1}	0.27 ± 0.02^{b1}	0.26 ± 0.03^{bc1}	0.30 ± 0.01^{b1}
PGP	0.31 ± 0.06^{b1}	0.28 ± 0.02^{b1}	0.28 ± 0.07^{abc1}	0.27 ± 0.02^{bc1}

Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).
Means ± SD. Different letters in the same column (a, b, c) and number in the same row (1, 2) indicate significant differences ($p<0.05$).

10.2.2.6. Color parameters of chicken patties

Color is one of the most important factors and visual cue involved in the consumer perception of acceptable meat quality and in the decision to purchase it (Mancini & Hunt, 2005; Faustman et al., 1990). Poultry meat color is affected by several factors such as age, sex, strain, diet, intramuscular fat, meat moisture content, preslaughter conditions and processing variables (Santiago et al., 2005; Young et al., 2001; Ekiz et al., 2010; Santos et al., 2007; Muchenje et al., 2008, 2009a, b).

The effect of grape by-products supplementation on the color stability (Lightness L*, redness a*, yellowness b*) of chicken patties is shown in the Figures 10.1, 10.2. and 10.3.

The initial higher ($p<0.05$) levels of Lightness L* were found in the control and PGS samples with levels of 49.60-49.82 respectively. These two samples also presented the higher ($p<0.05$) initial levels of polyphenols (Table 10.4).

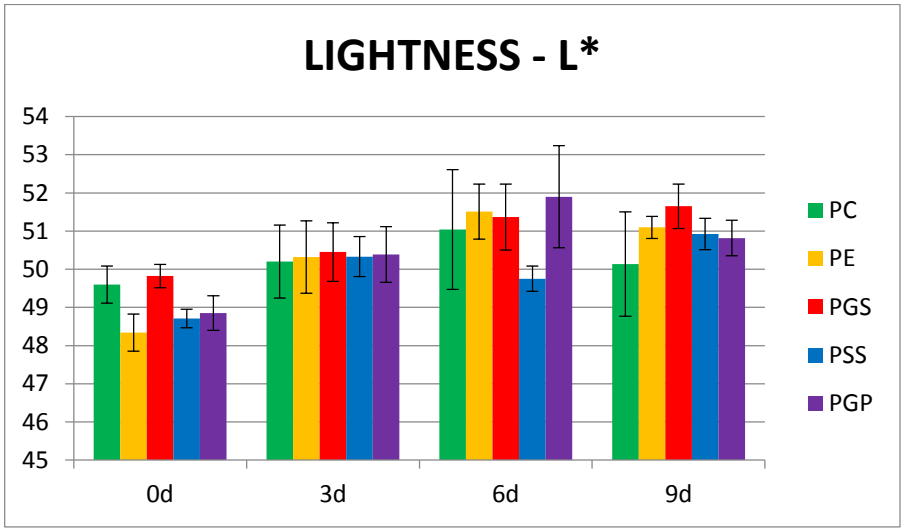
L* levels in this study were lower than those observed by other authors in chicken patties formulated with fruit extract rich in polyphenols and BHT (Naveena et al., 2008), but similar to other authors (Sáyago-Ayerdi et al., 2009 a). During the storage time was observed a slight increase of the parameter with some differences between the parameter but without a clear relation with the polyphenols content of the patties.

Other authors have observed differences in color parameters of patties containing grape extracts or grape powder but when these are added directly into their formulation (Sáyago-Ayerdi et al., 2009 b; Hernandez-Hernandez et al., 2009, Naveena et al., 2008, Lorenzo et al., 2014).

The initial yellowness values (12.86-12.41) did not present significant differences among the patties (Figure 10.3.). In general, during storage period hardly any changes were observed in the levels of b*.

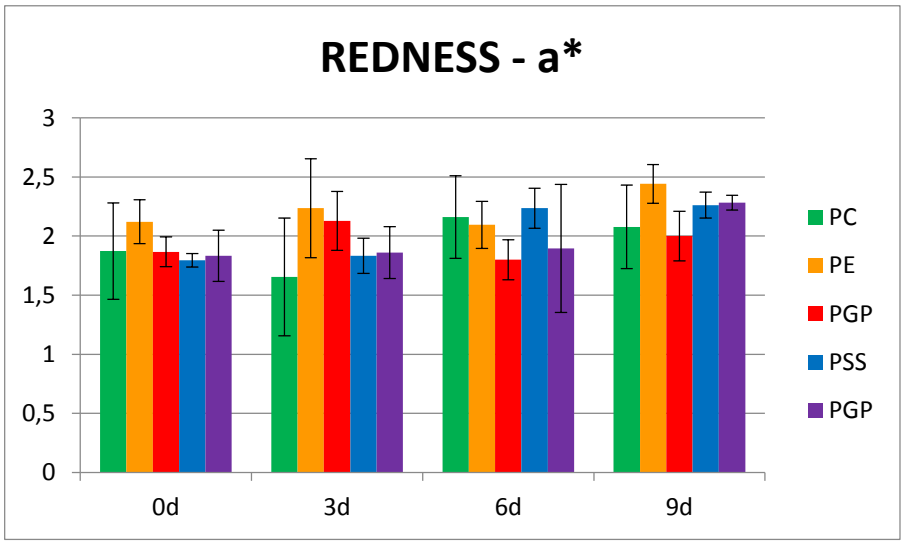
The initial higher levels of redness values (a*) were observed in the PE sample (2.12) staying this trend also at the end of storage. During storage, an increase in this value (a*) for all samples was observed. Alpha-tocopherol is widely used to reduce lipid oxidation and drip losses and to maintain color stability (López-Bote et al., 2001). Vitamin E limits the myoglobin oxidation and in consequence the gradually oxidized into metamyoglobin which presented a dull brown-color while the oxymyoglobin keep a red color more typical of meat (Erener et al., 2011). In connection with these results, in our study the patties made with the meat of chickens fed vitamin E also presented the lower oxidation levels (Table 10.6.). Several studies reported that the L* value is also under the influence of meat pH (Perlo et al., 2010; Mothershaw et al., 2009; Barbut, 1997; Saláková et al., 2009). However, unlike other authors (Muchenje et al. 2008) our study did not observe this relation. Other studies on dietary supplementation with oregano essential oil showed modification in the meat color, probably by modifying pigment distribution in animal tissues (Simitzis et al., 2008).

Figure 10.1. Lightness (L*) of different chicken patties during refrigerated storage



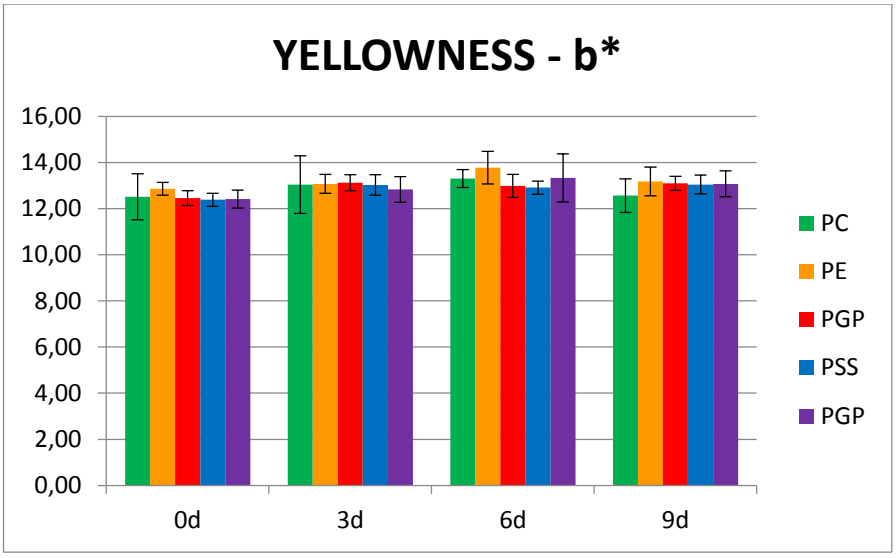
Sample denomination: PC (patties Control); PE (patties Vitamin E); PGP (patties Grape Pomace); PGS (patties Grape Seed); PSS (patties Grape skin)

Figure 10.2. Redness (a*) of different chicken patties during refrigerated storage



Sample denomination: PC (patties Control); PE (patties Vitamin E); PGP (patties Grape Pomace); PGS (patties Grape Seed); PSS (patties Grape skin)

Figure 10.3. Yellowness (b*) of different chicken patties during refrigerated storage



Sample denomination: PC (patties Control); PE (patties Vitamin E); PGP (patties Grape Pomace); PGS (patties Grape Seed); PSS (patties Grape skin)

10.2.2.7. Texture of chicken patties

The effect of grape by products supplementation on the Kramer shear force (KSF) of chicken patties is shown in Table 10.7. The initial levels of KSF were among 2.13 and 2.66 N/g and no significant differences were found among the treatments and control samples (PC). These results indicated that grape by-products and vitamin E supplementation in animals did not significantly affect the textural characteristics of the meat products elaborated. Similarly, the inclusion of 5% of grape seed substituting for maize in the Penedes chicken diet did not affect organoleptic texture attributes (Francesch and Cartañà, 2015). Significant decreases in KSF values were observed during storage except for PGP samples (Table 10.7). In pork patties formulated with a combination of phyto-extracts (sea buckthorn and grape seed extracts) hardness didn't follow any particular trend during storage (Kumar et al., 2015).

Table 10.7. Kramer shear force (KSF) (N/g) of different chicken patties during refrigerated storage.

Sample	Storage (days) at 4 °C			
	0	3	6	9
PC	2.66±0.56^{a1}	2.36±0.45^{a1}	2.54±0.54^{a1}	2.13±0.34^{a2}
PE	2.36±0.31^{a1}	2.26±0.33^{a1}	2.51±0.23^{a1}	1.53±0.30^{b2}
PGS	2.20±0.35^{a1}	2.18±0.45^{a1}	2.44±0.34^{a1}	1.47±0.28^{b2}
PSS	2.66±0.56^{a1}	1.88±0.13^{a1,2}	2.14±0.43^{a1,2}	1.63±0.53^{b2}
PGP	2.13±0.15^{a1}	2.43±0.27^{a1}	2.29±0.08^{a1}	2.24±0.54^{a1}

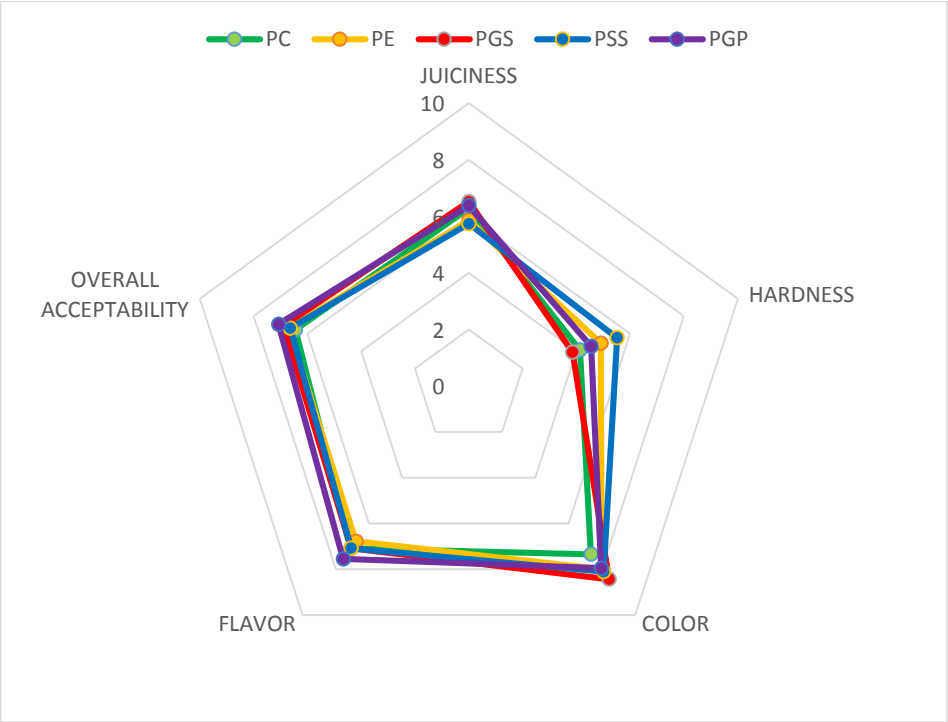
Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).

10.2.2.8. Sensory evaluation test of chicken patties

Sensory attributes of different chicken hamburgers are shown in Figure 10.4. In general the sensorial parameters did not present significant differences among patties and the punctuations for the studied parameters were very high except in the case of the hardness. In addition, the overall acceptability parameter was very well punctuated for all the patties, with no significant differences among them, therefore all the products were acceptable for the panellist. These results are in relation with the other parameters studied in this experiment, with little differences between the control and the other samples. Only a slight relation was observed in the case of samples PE and PSS which presented the lowest levels of polyphenols (Table 10.4.) and also these samples were lower punctuated in the juiciness parameters. Other authors have also not found differences in the color appearance, flavor and overall acceptability of goat patties formulated with extract of fruit (Devatkal et al., 2010) and cooked chicken patties (Naveena et al., 2008).

However, other authors observed the effect of addition of vitamin E and grape by products on sensorial attributes (mainly color and flavor) of patties when they are directly added and of extract from different plant or fruit (Sáyago-Ayerdi et al.2009, Leheska et a., 2006). However, when supplementing chicken diets with these products the effect in the color and other sensorial parameters was removed.

Figure 10.4. Sensory evaluation of different cooked chicken patties



Sample denomination: PC (Control); PE (Control+vitamin E); PGS (Control + Grape Seed 4%); PSS (Control+ Grape skin 4%); PGP (Control+ Grape pomace 4%).

10.3. Conclusions

A clear relation among the polyphenolic content of the chicken diet, raw meat and patties was not observed. Grape by product (pomace, seed and skin) supplementation of chickens diet results in improved oxidative stability of meat patties elaborated with the breast meat from these chickens. However, it was not possible to correlate this activity with the polyphenols content of patties, among which not clearly differences were found. Therefore, it should be taken into account that not only the levels of total polyphenols in the diet and in the raw meat are important aspects, but also necessary to know the absorption and transference of this polyphenols in the raw meat and also their polyphenolic profile.

In addition, no clear effect was observed in the microbial growth and in the technological properties (texture and color). All the patties were well evaluated for their sensory attributes, with a higher punctuation in the general acceptability.

CHAPTER 11. V EXPERIMENT

Effect of grape seed and skin addition on physicochemical properties of chicken thigh patties during refrigerated storage

11.1. Aim

The aim of this study was to evaluate the effect of GS and SS addition during chicken meat processing, on proximate composition, lipid oxidation, sensory attributes, instrumental color, pH, and total extractable polyphenols content of chicken meat and patties.

11.2. Results and discussion

11.2.1. Characterization of tested products

11.2.1.1. Proximate composition of tested products

The proximate composition of SS and GS is showed in Table 11.1. The level of protein was similar (P<0.05) in both ingredients. The main significant difference was observed in the polyphenolic content. GS showed approximately the 15% of total polyphenols, 5 times more than polyphenol content of the SS sample. This content is associated to the level of crude fiber present in this sample, higher levels of polyphenols are associated to the higher levels of fiber (GS). Other authors have also noted this relationship (Saura-Calixto, 2011, Sayago-Ayerdi et al., 2009b).

Table 11.1. Proximate composition (%) and total extractable polyphenols (mg GA/100g) of grape skin (SS) and grape seed (GS)

Parameters	GS	SS
Protein	13.41±0.06^{a}	13.81±0.05^{a}
Fiber	16.93±0.26^{a}	12.21±0.38^{b}
Total extractable polyphenols	15792.32±16^{a}	3273.84±12^{b}

Means ± SD. Different letters in the same row (a, b) indicate significant differences (p<0.05).

11.2.2. Characterization of chicken meat

11.2.2.1. Proximate compostition of chicken meat

The proximate composition of the thighs was the following: moisture: 73.95%, protein: 18.43%, fat: 6.63% and ash: 0.97%).

11.2.3. Physico-chemical composition of chicken patties

11.2.3.1. Proximate composition of chicken patties

The chemical composition of chicken patties is showed in Table 11.2. Moisture, fat and protein did not show significant differences among chicken patties. The addition of grape seed and skin resulted in a higher ($P<0.05$) ash content compared with the control sample, due to the presence of grape by-products powder in the treatments. Similar results were found in ground chicken thigh meat treated with grape seed extract (Brannan et al. 2008).

Table 11.2. Proximate analysis (%) of different chicken patties

Parameters	PC	PGS	PSS
Moisture	68.80±0.09[a]	69.99±0.27[a]	69.08±0.02[a]
Protein	18.30±0.33[a]	18.72±0.06[a]	18.44±0.02[a]
Fat	6.45±0.45[a]	6.16±0.34[a]	6.25±0.18[a]
Ash	1.90±0.04[b]	2.04±0.01[a]	2.03±0.08[a]

Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)
Means ± SD. Different letters in the same row (a, b, c,) indicate significant differences ($p<0.05$).

11.2.3.2. pH of chicken patties

The pH of all samples is showed in Table 11.3. The initial levels of pH were higher than 6.20 in all samples, the lower levels were observed in the PSS. These results are similar to those of other authors in chicken patties (Calliaria et al., 2015) and in cooked chicken patties elaborated with extract of kinnow rind, pomegranate rind and pomegranate seed powder (Devatkal et al., 2011).

During storage time, a significant decrease in the pH was observed, with lower levels in samples containing GS and SS. Other authors (Lorenzo et al., 2014) also found that the addition of grape seed extract lead to lower pH values in porcine patties. This pH reduction was associated with the microbial growth mainly lactic acid bacteria

(Karabagias et al. 2011, Triki et al., 2013) and the content of polyphenols in these grape by-products. Grape seed containing gallic acid, protocatechuic acid and galloylated proanthocyanidins was the most acid compounds (Lorenzo et al. 2014). This could be explain the lower levels of pH in the sample PGS at the end of storage.

Table 11.3. pH levels of different chicken patties during refrigerated storage

Sample	Storage (days) at 4 °C			
	0	3	6	9
PC	6.64 ± 0.01^{a1}	6.39 ± 0.06^{a2}	5.30 ± 0.05^{a3}	4.99 ± 0.03^{a4}
PGS	6.52 ± 0.01^{b1}	6.26 ± 0.01^{a2}	5.12 ± 0.01^{b3}	4.78 ± 0.05^{b4}
PSS	6.20 ± 0.01^{c1}	6.05 ± 0.01^{b2}	5.14 ± 0.01^{b3}	4.83 ± 0.02^{c4}

Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)
Means ± SD. Different letters in the same column (a, b, c,) and numbers in the same row (1,2,3) indicate significant differences ($p<0.05$).

11.2.3.3. Total exctractable polyphenols content of chicken patties

The initial levels of polyphenols content were higher ($P<0.05$) in the patties with grape seed by-products (PGS) while the lower were in the control sample (Table 11.4). These results are in relation with the content of polyphenols of the GS (Table 11.1) and similar to the results of Zhao et al (1999) that estimated approximately 60–70% of grape polyphenols in grape seeds (Zhao et al., 1999) and about 5% in the skin (Thorngate and Singleton 1994). Even if the polyphenols content of GS was 5 times higher than polyphenols content of SS, no significant differences were observed between the patties formulated with these by-products. The present result could be probably due to the addition of these by-products in the form of powder, since other authors (Devatkal et al., 2010) observed that the differences in the polyphenols content of patties are the same of the by-products used in their formulation, when added in the form of extracts.

The polyphenols content observed both in the control patties and in those formulated with polyphenols-rich grape by-products was similar to those found by other authors in patties enriched with extract of fruits (Devatkal et al., 2010) and in the thigh meat of broilers fed the wild grape (Yong et al., 2013).

However, the polyphenols content in the patties of the present study was lower than those observed by other authors in low-salt meat pork emulsion containing seaweed (López-López et al., 2010) and walnut (Ayo et al., 2007)

During storage time, an increase in the levels of polyphenolic compounds in all the samples was observed, keeping the higher levels in the sample PGS followed by the PSS. A similar behavior of total phenolic content increase during chilled storage was observed by Devatkal et al. (2011) in chicken patties elaborated with extract of kinnow rind, pomegranate rind and pomegranate seed powder, and Naveena et al. (2008) in cooked chicken patties treated with pomegranate juice and rind extract. This behavior could be explained because of the total phenolics are synthesized by phenylpropanoid pathway involving an enzyme phenylalanine ammonia-lyase (Devatkal et al. 2011). Padda and Picha (2008) also reported that chilling temperature increases the activity of this enzyme and accelerates the build up of phenolics.

The levels of polyphenols in the cooked patties at the second day of storage were higher ($P<0.05$) in the sample with grape seed (HGS) (73.91 mgGAE/100g) compared with the HSS (52.79 mgGAE/100g) and the control (43.59 mgGAE/100g) samples. This was in relation with the polyphenolic content of raw patties (Table 11.4). As expected, the levels of polyphenols were higher in cooked patties compared to the raw patties because of the loss of moisture after cooking. Maintaining the polyphenols after cooking, the patties demonstrate that they could be a healthy product able to improve the health of consumer.

Table 11.4. Total extractable polyphenols content (mgGAE/100g) of different chicken patties during refrigerated storage

Sample	Storage (days) at 4 °C			
	0	3	6	9
PC	38.79 ± 0.6^{c3}	39.78 ± 0.6^{b3}	42.46 ± 0.5^{c2}	64.26 ± 1.4^{c1}
PGS	57.55 ± 0.6^{a3}	59.71 ± 1.3^{a3}	95.55 ± 0.6^{a2}	114.81 ± 1.0^{a1}
PSS	40.01 ± 1.0^{c3}	41.12 ± 0.4^{b3}	57.70 ± 1.3^{b2}	84.18 ± 0.2^{b1}

Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)
Means ± SD. Different letters in the same column (a, b, c) and numbers in the same row (1,2,3) indicate significant differences ($p<0.05$).

11.2.3.4. Lipid oxidation of chicken patties

The initial TBARS values of patties were very low (0.57-0.96) (Table 11.5). In general, during chilled storage the lower ($P<0.05$) values were observed in the patties treated with the grape by-products, mainly in the PGS followed by PSS. This is in relation with the polyphenols content of grape seed and grape skin used. The lower TBARS values correspond to the higher polyphenols content in the treated patties (Table 11.5). The antioxidant effect of grape polyphenols in chickens has been described by many authors (Goñi et al., 2007; Brenes et al., 2008; Brenes et al., 2010; Chamorro et al., 2014). Flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Yilmaz and Toledo, 2004). Grape skin and seed are a rich source of flavonoids including monomeric phenolic compounds, such as (+)-catechins, (-)-epicatechin, and (-)-epicatechin-3-O-gallate and dimeric, trimeric, and tetrameric procyanidins. Moreover, several authors also have observed a delay in the lipid oxidation when added grape seed extract to chicken meat (Brannan 2008), ground dark turkey meat (Lau and King 2003) beef (Bañon et al., 2007; Brannan and Mah, 2007; Rojas and Brewer, 2008), pork (Brannan and Mah, 2007; Carpenter et al., 2007; Rojas and Brewer, 2008; Lorenzo et al., 2014) and fish (Pazos et al, 2005; Sánchez-Alonso and Borderías, 2008; Sánchez-Alonso et al., 2008).

The increased effectiveness of GS versus SS in the reduction of lipid oxidation could be due not only to the different concentrations of phenolic compounds present in these by-products, but also to their different phenolic profile. Yilmaz and Toledo (2004) found lower concentrations of gallic acid, monomeric catechin and epicatechin in winery by-product grape skin than in seed due to the possible release of polyphenolics present in grape skin into the wine during winemaking process. Besides, an higher antioxidant capacity of grape seed extract compared with grape skin extract was found, and dimeric, trimeric, oligomeric or polymeric procyanidins account for most of their superior antioxidant capacity in addition to the monomers (Yilmaz and Toledo, 2004).

Other authors also found a lower oxidative stability of grape skin in dehydrated chicken meat that was mechanically deboned, compared with other natural antioxidants (coffee, rosemary and green tea) and similar to the control (Nissen et al.

2000). According to the authors, this low effectiveness of grape skin extract is because of a lack of any significant protection of vitamin E, in addition to its polyphenolic content and profile. While, the antioxidant mechanism of grape procyanidin might be explained by its capacity to repair the oxidized α-tocopherol and to delay the ascorbic acid depletion of muscle tissues (Iglesias et al. 2012). Goñi et al. (2007) reported a better oxidative stability of meat samples from chickens receiving a diet supplemented with GP that was also accompanied by an increase in the concentration of vitamin E in the liver that may prove beneficial for the enhancement of vitamin E status and for the reduction in lipid oxidation of the tissues.

Table 11.5. Lipid oxidation as changes in thiobarbituric acid-reactive substances (TBARS mg MDA/ kg sample) values of different chicken patties during refrigerated storage.

Sample	Storage (days) at 4 °C			
	0	3	6	9
PC	0.96 ± 0.12^{a1}	0.39 ± 0.03^{a2}	0.67 ± 0.01^{a2}	0.70 ± 0.00^{a2}
PGS	0.57 ± 0.06^{b1}	0.21 ± 0.04^{b2}	0.39 ± 0.01^{c2}	0.46 ± 0.01^{c2}
PSS	0.64 ± 0.04^{b1}	0.40 ± 0.03^{a1}	0.49 ± 0.01^{b2}	0.57 ± 0.01^{b2}

Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)
Means ± SD. Different letters in the same column (a, b, c,) and numbers in the same row (1, 2) indicate significant differences ($p<0.05$).

11.2.3.5. Color parameters of chicken patties

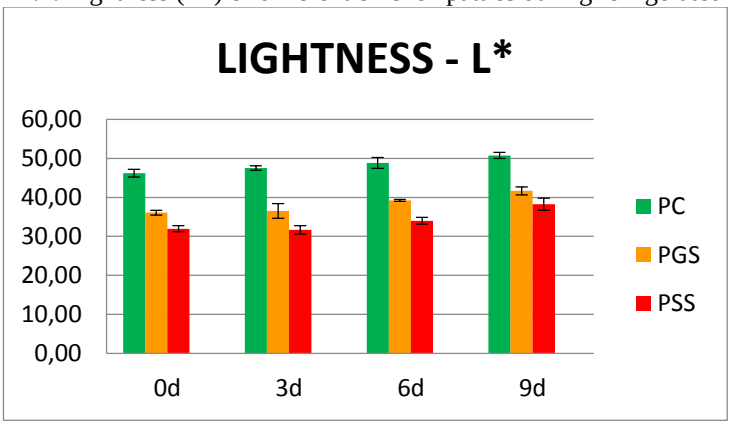
Color changes are an important factor influencing the quality and acceptability of meat and meat products and present a close relationship with the decision to purchase from the consumer (Carpenter et al. 2007). The results of the color parameters (L*, a* and b*) are showed in Figures 11.1, 11.2 and 11.3. In meat products, color can be influenced by many factor associated with the formulation of products such as level of protein (myoglobin content) and fat and by the addition of water and non-meat ingredients (Pietrasik & Janz, 2009). In this study, as said above, no significant differences were observed in protein, moisture and fat content among the meat products (Table 10.2), therefore the significant changes in the color parameters were attributed to the grape residues used in the patties formulation. The addition of GS and SS in chicken meat caused significantly less lightness (L*), redness (a*), and yellowness (b*) of patties, with the lowest values in those formulated with

SS. A similar behavior was observed by Sáyago-Ayerdi et al. (2009b) in chicken humburgers prepared with grape dietary fiber (grape pomace). During the storage period, in general, was observed an increase with a similar trend, in all the color parameters (except b*) for the three samples, with lower color values of the samples treated with grape by-products, compared to the control patties. This color reduction in PGS and PSS samples could due to the dark red color of grape ingredients. The darkening of samples with the addition of grape extract was also reported in cooked chicken meat treated with grape seed and peel extracts (Selani et al., 2011) and in grounded chicken thigh and breast treated with grape seed extract (Brannan, 2009).

Even if grape skin and seed used in the present trial are characterized by a strong red color, the reduction of redness values (a*) in the patties containing these grape by-products could be due to the considerable amounts of bioactive components present in these samples as explained by Cofrades et al. (2011) who observed a decreased redness in restructured poultry steaks incorporated with sea Spaghetti seaweed, in addition to a decreased lightness.

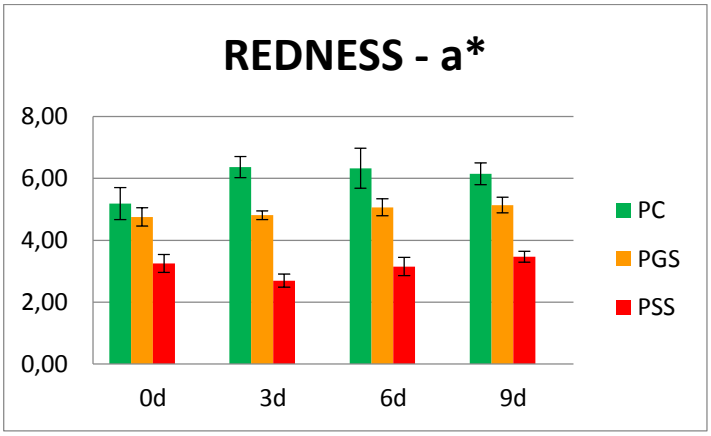
On the other hand, other authors (Selani et al. 2011; Sasse et al., 2009) did not observe significant changes in the L*, a* and b* color parameters when grape seed and peel extracts were added to chicken or pork meat. However, these authors have used grape by-products extracts while, in the present experiment grape by-products powders were directly added into chicken meats. GS and SS powers could have interfered with the components of patties (proteins, myoglobin, water or fat) that influence the color of meat product (Pietrasik & Janz, 2009).

Figure 11.1. Lightness (L*) of different chicken patties during refrigerated storage



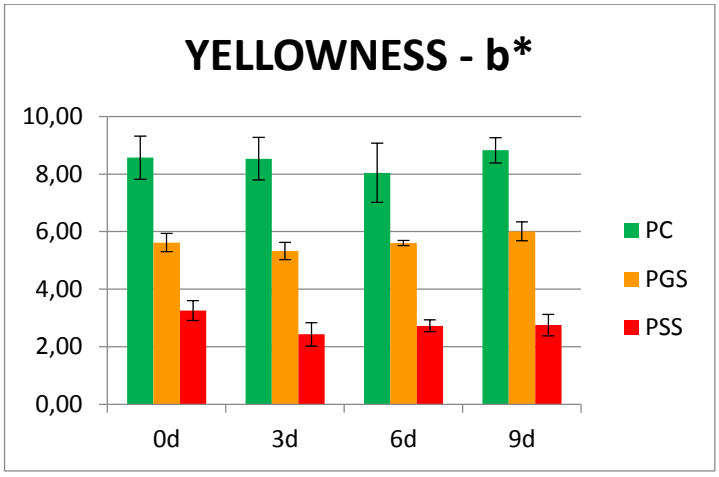
Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)

Figure 11.2. Redness (a*) of different chicken patties during refrigerated storage



Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)

Figure 11.3. Yellowness (b*) of different chicken patties during refrigerated storage



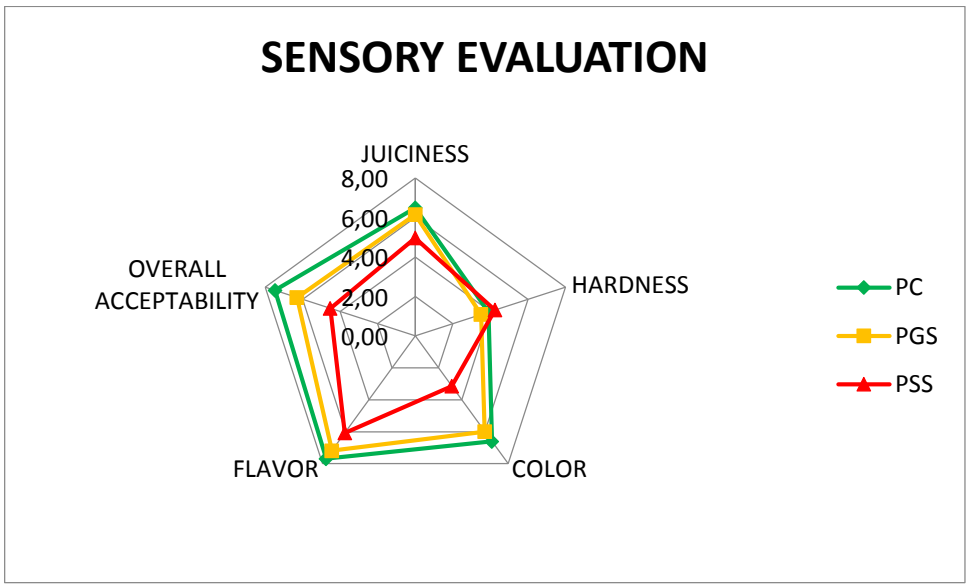
Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)

11.2.3.6. Sensory evaluation of chicken patties

Sensory attributes of different chicken patties are shown in Figure 11.4. The grape by-products addition significantly affected the color and overall acceptability parameters of the patties. In general the low punctuation was observed for the patties containing grape skin (PSS), and very similar punctuation was observed in the control sample and in patties with grape seed (PGS). Unlike the color, no significant differences were found for the other parameters, among the three experimental samples. Besides, all the samples present an acceptable punctuation by the panelist, in

fact the juiciness, flavor, color and general acceptability scores were greater than 5 and hardness acceptability was similar ($P<0.05$) to the control. Therefore, the panel did not evaluate negatively the addition of grape seed powder into the patties. The present results are in relation to Sáyago-Ayerdi et al., (2009b) who did not observed similar flavor tenders and odor in chicken hamburgers with grape pomace, associate this effect to the dietary fiber content in grape pomace.

Figure 11.4. Sensory evaluation test of different cooked chicken patties



PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape skin)

11.3. Conclusions

The use of grape by products skin and seed in the formulation of patties has demonstrated its effectiveness in reducing the lipid oxidation of these products. Grape seed was considered more effective in retarding lipid oxidation than the grape skin. Even the patties elaborate with grape seed was evaluated more similar than the control sample. The development of these patties could also provide positive effects on human health due to their polyphenolic content. Therefore, these results are important for both the meat industry and the agro-food industry since could collaborate in the using of grape waste as natural antioxidants in the food industry and could also represent a significant step towards the maintaining of environmental balance.

CHAPTER 12

GENERAL CONCLUSIONS

Nowadays there is a high request of research on alternative feeding systems that are able to improve poultry performance and welfare, and to warrant high-quality and safe meat products. This thesis provides a valuable contribution to scientific community which aims to find a natural and cheaper but functionally equivalent product with antioxidant activity that could partially replace vitamin E. Furthermore, the study afford new data regarding the separated effect of the principal components in grape pomace, ie grape seed and skin in chickens diet, which are still poorly studied.

Performance parameters of chickens were not affected by vitamin E supplementation. The inclusion of different concentrations of grape seed (15, 30 and 40 g/kg), grape skin (40 mg/kg) and grape pomace (40 mg/kg) in broiler diets did not impair neither daily weight gain nor fee intake and feed conversion ratio of chickens. Also, the different combinations of grape seed and grape skin (50:50, 75:25 and 25:75), in the third experiment, included at a concentration of 4% in chickens diet had no effect on performance parameters. On the other hand, the inclusion of high levels of grape skin (11%) and the inclusion of fermented and unfermented skin diets (60 mg/kg) reduced the performance of chickens, by decreasing their daily weight gain and increasing feed conversion ratio.

The optimum dose of inclusion of polyphenols in animal diets is difficult to define due to the different composition of phenolic compounds present in these by-products. However, our findings suggest that an optimal concentration to include in chicken diets, without having any negative effect on the growth performance, is about 4% both for grape pomace and for its components skin and seed.

Ileal and excreta total polyphenol content were increased by the inclusion of grape seed (15, 30 and 40 g/kg), unfermented and fermented skin (30 and 60 g/kg, respectively) and the different combinations of grape seed and skin (experiment III) in broiler diets. Ileal tannins content was increased in all treated groups except in the case of birds fed grape pomace (3.75%). Excreta tannins content was not affected in birds fed different concentrations of grape seed and the different combinations of grape seed

and skin. Birds fed skin (fermented and unfermented skin diets) and grape pomace showed highest content of excreta tannins content.

The inclusion of grape pomace (40 mg/kg), skin (30 and 40 g/kg), seed (15, 30 and 40 g/kg) and combinations of seed and skin in chicken diets had no adverse effect on protein digestibility. However, the inclusion of unfermented skin (60 g/kg) and skin diets (110 g/kg) showed lower values.

Ileal total polyphenol digestibility were increased in chickens fed grape pomace (40 mg/kg), grape seed (30 and 40 g/kg) and skin (40 and 110 mg/kg). However, the ileal digestibility of total polyphenols in birds fed fermented and unfermented skin (60 mg/kg), grape seed (15 mg/kg) and the different combinations of grape seed and skin diets was not affected.

Excreta total polyphenols digestibility was not affected by grape seed (15 and 30 mg/kg), skin (110 g/kg) and grape pomace (40 g/kg) supplementated diets. However, an increment was observed in chickens fed fermented and unfermented grape skin (with the highest values for unfermented skin (60 g/kg), 40 g/kg of grape seed, skin and grape pomace, respectively, and the combinations of grape seed and skin diets.

No differences were detected on colony-forming units of anaerobic (*Lactobacillus* and *Clostridium*) and aerobic (*E. coli*) bacterial species by the addition of fermented and unfermented (30 and 60 g/kg) diets in chicken ileal content.

Plasma and meat α-tocopherol concentration were increased in birds fed α-tocopherol, grape pomace (up to 40 g/kg), grape seed (40 g/kg) and skin (40 and 110 g/kg). This effect was higher in birds receiving α-tocopherol diets than in those fed the different grape by-products.

The extend of lipid oxidation measured as malondialdehyde formation in breast meat after 1 and 7 days of refrigerated storage was lower in birds fed diets supplemented with α-tocopherol and grape pomace (37.5%). This grape by-product delay the lipid oxidation reaching a similar protective effect observed with dietary α-tocopherol. However, the inclusion of the different grape by-products (grape seed, skin

and grape pomace) did not enhance the oxidative stability in thigh meat during the refrigerated process.

The different fat content of breast and thigh meat could partially explain the different behavior of GS and SS in reducing the lipid oxidation. The higher fat content of chickens leg meat, compared to the breast meat, could make it more susceptible to lipid oxidation thus making ineffective the antioxidant action of such products at the concentration tested in this study. Therefore, these findings suggest that are probably necessary higher concentrations of these by-products than those used in this thesis trials, in order to delay lipid oxidation in thigh meat.

Dietary addition of 40 g/kg of grape seed, skin and grape pomace, respectively, and direct addition of 20 g/kg of grape seed and skin, respectively, had no effect on the proximate compositions of chicken patties, but decreased their pH values. The addition of 20 g/kg of grape seed and 20 g/kg of grape skin, respectively in thigh chicken patties decreased lightness, yellowness and redness values in these samples, while breast meat obtained from chicken fed grape seed, skin and grape pomace diets showed no clear effect in color parameters and even in texture of chicken breast patties.

Thigh chicken patties formulated with the direct addition of grape seed and skin showed higher total phenolic content, while no clear differences were found in the breast patties formulated with meat of chicken supplemented with 4% GS, SS and GP. However, an increase of total phenolic content in all patties during chilled storage was observed. The phenolic content in these samples was even keep after the cooked treated of the patties. The development of these patties could provide positive effects on human health due to their polyphenolic content.

The inclusion of grape pomace and skin in the chicken diet up to 40 g/kg, and direct addition of 20 g/kg of grape seed and 20 g/kg of skin improved the oxidative stability of breast and thigh chicken patties, respectively. These results provide an evidence of a protective effect of dietary grape pomace and skin against lipid oxidation.

In general, the inclusion of 4% GS, SS and GP had no effect on the microbial growth of chicken patties, except in the case of lactic acid bacteria in the samples PE and PSS that presented the lowest levels.

Our results are important for both meat industry and the agro-food industry since could collaborate in using grape waste as natural antioxidant in the food industry and could also represent a significant step towards the maintaining of environmental balance and also an economic revaluation of the waste raw material.

The beneficial effects of grape by-products are thought to derive mainly from the bioactivities of their polyphenols. However, their potential as feed ingredients or additives in animal production remains largely unexploited and further experimental investigations are needed in this direction.

The metabolism of polyphenols by the microbiota, the bioactivity of microbe-derived metabolites and their presence in different organs all need to be investigated. Advances in knowledge of the interaction between these bioactive compounds and the intestinal microbiota should also be a subject of increasing interest. The identification of polyphenol-metabolizing bacteria and their possible use as a probiotic could be a good strategy for increasing the bioavailability and potential bioactivity of these compounds.

REFERENCES

Addis P.B. (1986). Occurrence of lipid oxidation products in foods. *Food and Chemical Toxicology*, 24(10/11), 1021-1030.

Ahmed S.T., Hossain M.E., Kim G.M. Hwang,J.A., Ji H., Yang C.J. (2013). Effects of resveratrol and essential oils on growth performance, immunity and microbial shedding in challenged piglets. Asian-Australas. *Journal of Animal Science*, 26, 683–690.

Ahn D.U., Nam, K. C., and Lee, E J. (2009). Lipid oxidation and flavor. Chapter 12, *Applied Muscle Biology and Meat Science*, 227-246.

Ahn J., Grun I.U., Fernando L.N. (2002). Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science*, 67, 1364–1369.

Ahn J., Grün I.U., Mustapha A. (2004). Antimicrobial and Antioxidant Activities of Natural Extracts in Vitro and in Ground Beef. *Journal Of Food Protection*, 67, 1, 148-55.

Ahn J., Grun I.U., Mustapha A. (2007). Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiology*, 24, 7–14.

Alberti A., Bolognini L., Macciantelli D., Caratelli M. (2000). The radical cation of N,N-diethyl-para-phenylendiamine: a possible indicator of oxidative stress in biological samples. *Research on Chemical Intermediates*, 26, 253–267.

Ali K., Maltese F., Choi Y., Verpoorte R. (2010). Metabolic constituents of grapevine and grape-derived products. *Phytochemistry Reviews*, *9*, 357–378.

Allard J., Kurian R., Aghdassi E., Muggli R., Royall D. (1997). Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans. *Lipids*, 32, 535–541.

Allen C.D., Fletcher D.L., Northcutt J.K., Rusell S.M. (1998). The relationship of broiler breast color to meat quality and shelf life. *Poultry Science*, 77, 361- 366

Allen C.D., Russell S.M., Fletcher D.L. (1997). The relationship of broiler breast meat color and pH to shelf-life and odor development. *Poultry Science*, 76, 1042-1046.

Amico V., Chillemi R., Mangiafico S., Spatafora C., Tringali C. (2008). Polyphenol-enriched fractions from Sicilian grape pomace: HPLC–DAD analysis and antioxidant activity. *Bioresource Technology, 99,* 5960–5966.

Amico V., Napoli E.M., Renda A., Ruberto G., Spatafora C., Tringali C. (2004). Constituents of grape pomace from the Sicilian cultivar "*Nerello Mascalese*". *Food Chem*istry, *88*, 599–607.

Amrani-Joutei K., Glories I., Mercier M. (1994). Localisation des tannins dans la pellicule de baie de raisin. *Vitis*, 33, 133-138.

Anadon H.L.S. (2002). Biological, Nutritional, and Processing Factors Affecting Breast Meat Quality of Broilers. Doctor of Philosophy Thesis in Animal and Poultry Sciences. Virginia Polytechnic Institute and State University. Blacksburg, Virginia. USA.

Ananga A., Georgiev V., Tsolova V. (2013). Manipulation and engineering of metabolic and biosynthetic pathway of plant polyphenols. *Current Pharmaceutical Design,19,* 6186–6206.

Anastasiadi M., Pratsinis H., Kletsas D., Skaltsounis A.L., Haroutounian A. (2012). Grape stem extracts: Polyphenolic content and assessment of their *in vitro* antioxidant properties. *Food Science and Technology, 48,* 316–322.

Andersen H. A., Oksbjerg N., Young J. F., Therkildsen M. (2005). Feeding and meat quality – a future approach. *Meat Science,* 70, 543–554.

Andersen O.M., Markham K.R. (2006). Flavonoids: Chemistry, biochemistry and applications. CRC Press, Boca Raton, USA.

Antunes P.L., Bilhalva A.B., Elias M.C., Soares G.J.D. (1995). Valor nutricional de feijão (Phaseolus vulgaris L.), cultivares rico 23, carioca, pirata-1 e rosinha-G2. *Revista Brasileira de Agrociência*, 1, 12–18.

AOAC (2005). Association of Official Analytical Chemists. Official methods of analysis (18th ed.) Association of Official Analytical Chemistry. Gaithersburg, MD, USA:

AOAC (1995). Association of Official Analytical Chemists. Official methods of analysis (16th edition). Association of Official Analytical Chemists International, Arlington, VA, USA.

Apajalahti J., Kettunen A., Graham H. (2004) Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World's Poultry Science Journal*, 60, 223–232.

APHA (1984). Compendium of methods for the microbiological examination of foods. 2nd edn, (ed. ML Speck). American Public Health Association, Washington DC.

Apostolou A., Stagos D., Galitsiou E., Spyrou A., Haroutounian S., Portesis N., Trizoglou I., Hayes A.W., Tsatsakis A.M., Kouretas D. (2013). Assessment of polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of *Vitis vinifera* stem extracts. *Food and Chemical Toxicology*, *61*, 60–68.

Appeldorn M.M., Vincken J.P., Aura A.M., Hollman P.C., Gruppen H. (2009). Procyanidins dimers are metabolized by human microbiota with 2-(3,4-cihydroxyphenil)acetic acid and 5-(3,4-dihydroxyphenil)-γ- valerolactone as the major metabolites. *Journal of Agricultural and Food Chemistry*, 57, 1084-1092.

Arnous A., Makris D.P., Kefalas P. (2002).Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *Journal of Food Composition and Analysis*, 15, 655-665.

Arora A., Nair M.G., Strasburg G.M. (1998). Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. *Free Radical Biology and Medicine*, 24, 1355–1363.

Arvanitoyannis, I. S., Ladas, D. & Mavromatis, A. (2006). Potential uses and applications of treated wine waste: a review. *International Journal of Food Science and Technology*, 41, 475–487.

Ashmore C.R., Parker W., Doerr L. (1972). Respiration of mitochondria isolated from dark cutting beef postmortem changes. *Journal of Animal Science*, 34, 46-48.

Aurousseau B. (2002). Les radicaux libres dans l'organisme des animaux d'elevage consequences sur la reproduction, la physiology et la qualite de leurs produits. *INRA Productions Animals*, 15, 67–82.

Avila-Ramos F., Pro-Martínez A., Sosa-Montes E., Cuca-García J.M., Becerril-Pérez C.M., Figueroa Velasco J.L., Narciso-Gaytán C. (2012). Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. *Poultry Science*, 91, 505–511

Ayo J., Carballo J., Serrano J., Olmedilla-Alonso B., Ruiz-Capillas C., JiménezColmenero F. (2007). Effect of total replacement of pork backfat with walnut on the nutritional profile of frankfurters. *Meat Science*, 77, 173–181.

Banon S., Díaz P., Rodríguez M., Garrido M.D., Price A. (2007). Ascorbate, green tea, and grape seed extracts increase the shelf life of low sulphite beef patties. *Meat Science*, 77, 626–633

Barbut S. (1997). Problem of pale soft exudative meat in broiler chickens. *British Poultry Science*, 38, 355-358.

Barbut S. (2002). Poultry Products Processing (An Industry Guide). CRC Press LLC. Florida. USA.

Bard J., Townsend W.E. (1978). Cured meats. The science of meat and meat products. Ed. Price J.F., Schweigert B.S. Food and Nutrition Press, Inc., Connecticut, 452-483.

Barnes E.M. (1979). The intestinal microflora of poultry and game birds during life and after storage. *Journal of Applied Bacteriolog*y, 46, 407–419.

Barros-Velazquez J. (2015). Antimicrobial Food Packaging, first edition.

Belviranli M., Gokbel H., Okudan N., Buyukbas S. (2012). Oxidative stress and anti-oxidant status in diabetic rat liver: effect of plant polyphenols. *Archives of Physiology and Biochemistry*, 118, 237–243.

Bengoechea M.L., Sancho A.I., Bartolomé B., Estrella I., Gómez-Cordovés C., Hernández T. (1997). Phenolic composition of industrially manufactured Purées and concentrates from peach and apple fruits. *Journal of Agricultural and Food Chemistry*, *45*, 4071.

Benouaret R., Goujon E., Goupil P. (2014). Grape marc extract causes early perception events, defence reactions and hypersensitive response in cultured tobacco cells. *Plant Physiology and Biochemistry*, 77, 84-89.

Bhaisare D.B., Thyagarajan D., Churchil R.R., Punniamurthy N. (2014). Bacterial Pathogens in Chicken Meat: Review. *International Journal of Life Sciences Research*, 2, (3), 1-7.

Bligh E.G., Dyer W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37 (8), 911−917.

Boling C.S., Graham P.P., Marriott N.G., Kelly R.F. (1990). Effect of butylated hydroxyl anisole and ascorbic acid on storage stability of restructured pork. *Journal of Animal Science*, 68, 108–116.

Bonisoli-Alquati A., Mousseau T.A., Møller A.P., Caprioli M., Saino N. (2010). Increased oxidative stress in barn swallows from the Chernobyl region. *Comparative Biochemistry and Physiology Part A*, 155, 205–210.

Botsoglou N.A., Fletouris D.J., Papageorgiu G.E., Vassilopoulos V.N., Mantis A.J., Trakatellis A.G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry*, 42, 1931–1937.

Botsoglou N.A., Florou-Paneri P., Nikolakakis I., Giannenas I., Dotas V., Botsoglou E.N., Aggelopoulos S. (2005). Effect of dietary saffron (Crocus sativus L.) on the oxidative stability of egg yolk. *British Poultry Science*, 46, 701– 707.

Bou R., Grimpa S., Baucells M.D., Codony R., Guardiola F. (2006). Dose and duration effect of alpha-tocopheryl acetate supplementation on chicken meat fatty acid composition, tocopherol content, and oxidative status. *Journal of Agricultural and Food Chemistry*, 54, 5020–5026.

Boulton R.B., Singleton V.L., Bisson L.F., Kunkee R.E. (1996). *Principles and Practices of Winemaking*. Springer.

Bourre J.M. (2005). Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: What is actually useful? *The Journal of Nutrition Health and Aging*, 9, 232–242.

Bourzeix M.D., Weyland D., Heredia N. (1986). EÄ tude des cate´chins et des procyanidols de la grappe de raisin, du vin et d'autres de´rive´s de la vigne. *Bull. O. I. V., 669-670*, 1171-1254

Brannan R.G, Mah E. (2007) Grape seed extract inhibits lipid oxidation in muscle from different species during refrigerated and frozen storage and oxidation catalyzed by peroxinitrite and iron/ascorbate in a pyrogallol red model system. *Meat Science*, 77, 540-546.

Brannan R.G. (2008) Effect of grape seed extract on physicochemical properties of ground, salted, chicken thigh meat during refrigerated storage at different relative humidity levels. *Journal of Food Science*, 73, C36-C40.

Brannan R.G. (2009). Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. *Meat Science*, 81, 589-595.

Braune A., Gütschow M., Engst W., Blaut M. (2001). Degradation of quercetin and luteolin by Eubacterium ramulus. *Applied and Environmental Microbiology*, 67, 5558–5567.

Bravo L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, 56, 317-333.

Bravo L., Saura-Calixto F. (1998). Characterization of dietary fiber and the in vitro indigestible fraction of grape pomace. *American Journal of Enology and Viticulture*, 49, 135-141.

Brenes A., Viveros A., Chamorro S., Arija I. (2016). Use of polyphenol-rich grape by-products in monogastric nutrition. A review. *Animal Feed Science and Technology*, 211, 1–17.

Brenes A., Viveros A., Goni I., Centeno C., Saura-Calixto F., Arija I. (2010). Effect of grape seed extract on growth performance, protein and polyphenol digestibilities, and antioxidant activity in chickens. *Spanish Journal of Agricultural Research*, 8, 326–333.

Brenes A., Viveros A., Goñi I., Centeno C., Sayago-Ayerdi SG., Arija I. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poulty Science*, 87, 307-316.

Bressani R., Mora D.R., Flores R., Brenes-Gomes R. (1991). Evaluación de los métodos para estabelecer el contenido de polifenoles en frijol crudo y cocido, y efecto que estos provocan en la digestibilidad de la proteina. *Archivos Latinoamericanos de Nutrición*, 41, 569–583.

Brisbin J.T., Gong J., Sharif S. (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. *Animal Health Research Reviews*, 9, 101–110.

Briskey E.J. (1964). Etiological status and associated studies of pale, soft, exudative porcine musculature. *Advances in Food Research*, 13, 89, 178.

Brown J.E., Khodr H., Hider R.C., Rice-Evans C. (1998). Structural dependence of flavonoid interactions with Cu2+ ions: implications for their antioxidant properties. *Biochemical Journal*, 330, 1173–8.

Buettner G.R. (1993.) The pecking order of free radicals and antioxidants: lipid peroxidation, a-tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics*, 300 (2), 535-43

Burdock, G.A.(2005). Fenaroli's Handbook of Flavor Ingredients, 5th ed.; CRC Press: Boca Raton, FL, USA.

Burton G.W., Traber M.G. (1990). Vitamin E: Antioxidant activity, biokinetics, and bioavailability. *Annual Review of Nutrition*, 10, 357–382.

Bustos I., Garcia-Cayuela T., Hernandez-Ledesma B., Pelaez C., Requena T., Martinez-Cuesta M.C. (2012). Effect of flavan-3-ols on the adhesion of potential probiotic lactobacilli to intestinal cells. *Journal of Agricultural and Food Chemistry*, 60, 9082–9088

Buttriss J.L., Diplock A.T. (1984). High-performance liquid chromatography methods for vitamin E in tissues. *Methods in Enzymology*, 105, 131-138.

Bystrom L., Lewis B.A., Brown D.L., Obendorf R.L. (2009). Phenolics, Sugars, Antimicrobial and Free-Radical-Scavenging Activities of Melicoccus bijugatus Jacq. Fruits from the Dominican Republic and Florida. *Plant foods for human nutrition*, 64, 2, 160-166.

Cabanis J. C., Flanzy C. (2003). Lípidos. In: C. Flanzy, Enología fundamentos científicos y tecnológico., Madrid, Spain: Mundi-Prensa, pp. 61-63.

Calliari C.M., de Souza E.L., Castro-Goméz R.J., Honório V.G., Magnani M. (2015). Characterisation and microstructure of reduced-fat chicken patties made with a novel polymer from Agrobacterium radiobacter k84. *Food Chemistry*, 173, 1150-1157.

Camo J., Beltrán J. A., Roncales P. (2008). Extension of the display life of lamb with an antioxidant active packaging. *Meat Science*, 80, 1086–1109.

Campos L.M.A.S., Leimann F.V., Pedrosa R.C., Ferreira S.R.S. (2008). Free radical scavenging of grape pomace extracts from Cabernet Sauvingnon (*Vitis vinifera*). *Bioresource Technology*, *99*, 8413−8413.

Cantos E., Espín J.C., Tomás-Barberán F.A. (2002). Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC–DAD–MS–MS. *Journal of Agricultural and Food Chemistry, 50,* 5691–5696.

Cao G., Russell R.M., Lischner N., Prior R.L. (1998). Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *Journal of Nutrition,* 128, 2383–90.

Carpenter R., O'Grady M.N., O'Callaghan Y.C., O'Brien N.M., Kerry J.P. (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science*, 76, 604-610.

Carreras I., Guerrero L., Guardia M.D., Esteve-García E., Garcia J.A., Regueiro J.A., Sárraga C. (2004). Vitamin E levels, thiobarbituric acid test and sensory evaluation of breast muscles from broilers fed α-tocopheryl acetate and β-carotene supplemented diets. *Journal of the Science of Food and Agriculture*, 84, 313–317

Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. (2007). Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *Journal of Agricultural and Food Chemistry, 55*, 992–1002.

Celi P. (2011). Biomarkers of oxidative stress in ruminant medicine. *Immunopharmacology and Immunotoxicology,* 33, 233–240.

Celi P., Selle P.H., Cowieson A.J. (2013). The effects of dietary supplementation with different organic selenium sources on oxidative stress in broilers. 24th Australian Poultry Science Symposium, 17th – 20th February 2013, 76-79.

Cesarone M.R., Belcaro G., Caratelli M. (1999). A simple test to monitor oxidative stress. *International Angiology*, 18, 127-130.

Chaijan M. (2008). Review: Lipid and myoglobin oxidations in muscle foods. Songklanakarin. *Journal of Science and Technology*, 30, 47-53.

Chamorro S., Goni I., Viveros A., Hervert-Hernández D., Brenes A. (2012a). Changes in polyphenolic content and antioxidant activity after thermal treatments of grape seed extract and grape pomace. *European Food Research and Technology*, 234, 147–155.

Chamorro S., Viveros A., Alvárez I., Vega E., Brenes A. (2012b). Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. *Food Chemistry*, 133, 308–314.

Chamorro S., Viveros A., Centeno C., Romero C., Arija I., Brenes A. (2013). Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma lipids and mineral content in broiler chicks. *Animal*, 7, 555-561.

Chamorro S., Viveros A., Rebolé A., Rica A., Arija B.D., Brenes A.A. (2015). Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. *Food Research International*, 73, 197–203.

Chastain M.F., Huffman D.L., Hsieh W.H., Cordray J.C. (1982). Antioxidants in restructured beef/pork steaks. *Journal of Food Science*, 47, 1779-1782.

Chauhan S., Celi P., Fahri F., Leury B.J., Dunshea F.R. (2012). Inclusion in the diet of Vitamin E and selenium above recommended level improves oxidative status and reduces heat stress in sheep. *Proceedings of the Nutrition Society of Australi*a, 36-39.

Chen G., Xiong Y.L., Wang L., Gomez-Basauri J., Nicastro F. (2008). Effect of Preventox® on the storage stability of raw and precooked pork patties. *Journal of Muscle Foods*, 19, 1–16.

Cherian G., Hayat Z. (2009). Long-term effects of feeding flaxseed on hepatic lipid characteristic and histopathology of laying hens. *Poultry Science*, 88, 2555–2561.

Chesson A., Russel W.R., Provan A.J. (1997). Metabolites of the phenylpropanoid pathway. Common origin, common proprieties? Polyphenols in foods. Proceedings of a European COST concerted action scientific workshop. Aberdeen, Scotland. 17-23.

Choe D.B., Min D.B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, 5, 169-181.

Chouchouli V., Kalogeropoulos N., Konteles S.J., Karvela E., Makris D.P., Karathanos V.T. (2013). Fortification of yoghurts with grape (*Vitis vinifera*) seed extracts. *Food Science and Technology, 53*, 522–529.

Chouliara E., Karatapanis A., Savvaidis I. N., Kontominas M. G. (2007). Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C.*Food Microbiology*, 24, 607–617.

Choy Y.Y., Quifer-Rada P., Holstege D.K., Frese S.A., Christopher C., Calvert C.C., Mills D.A., Lamuela-Raventos R.M., Waterhouse A.L. (2014). Phenolic metabolites and substantial microbiome changes in pig feces by ingesting grape seed proanthocyanidins. *Food & Function*, 5, 2298–2308.

CIE. (1978). International Commission on Illumination, Recommendations on uniform colour spaces, colour difference equations, psychometric colour terms. CIE Publication (No. 15 (E-1.3.1) 1971/(TO-1.3) (Suppl. 15). Bureau Central de la CIE, Paris, France.

Clench M.H., Mathias J.R. (1995). The avian cecum: a review. *Wilson Bull*, 107, 93–121.

Clinquart A., Leroy B., Dottreppe O., Hornick J.l., Dufrasne I., Istasse L. (2000). Les facteurs de production qui influencent la qualité de la viande des bovins BBB. *L'élevage du Blanc Bleu belge – Journée CESAM*, 26 mai 2000, 19p.

Coetzee G.J.M., Hoffman L.C. (2001). Effect of dietary vitamin E on the performance of broilers and quality of broiler meat during refrigerated and frozen storage. *South African Journal of Animal Science*, 31, 158-173.

Cofrades S., Lopez-Lopez I., Ruiz-Capillas C., Triki M., Jimenez-Colmenero F. (2011). Quality characteristics of low-salt restructured poultry with microbial transglutaminase and seaweed. *Meat Science*, 87, 373–380.

Cofrades S., Serrano A., Ayo J., Carballo J., Jimenez-Colmenero F. (2008). Characteristics of meat batters with added native and preheated defatted walnut. *Food Chemistry*, 107, 1506–1514.

Cohn J.S. (2002). Oxidized fat in the diet, postprandial lipaemia and cardiovascular disease. *Current opinion in lipidology*, 13:19–24

Combs G.F. (1981). Assessment of vitamin E status in animals and man. *Proceedings of the Nutrition Society*, 40, 187–194

Contini C., Alvarez R., O'Sullivana M., Dowling D.P., Gargan S.O., Monahan F.J. (2014). Effect of an active packaging with citrus extract on lipid oxidation and sensory quality of cooked turkey meat. *Meat Science*, 96, 1171–1176.

Cook J.D., Reddy M.B., Hurrell R.F. (1995). The effect of red and white wines on nonheme-iron absorption in humans. *American Journal of Clinical Nutrition*, 61, 800-804.

Cornforth D.P., Egbert W.R. (1985). Effect of rotenone and pH on the color of pre-rigor muscle. *Journal of Food Science*, 50, 34-35, 44.

Corrales M., Toepfl S., Butz P., Knorr D., Tauscher B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Innovative Food Science and Emerging Technologies*, 9, 85–91.

Cortinas L., Barroeta A., Galobart J., Jensen S.K. (2004). Distribution of α-tocopherolstereoisomers in liver and thigh of chickens. *British Journal of Nutrition*, 92, 295-301.

Costantini D., Bonadonna F. (2010). Patterns of variation of serum oxidative stress markers in two seabird species. *Polar Research*, 29, 30–35.

Costantini D., Dell'Omo G. (2006). Effects of T-cell-mediated immune response on avian oxidative stress. *Comparative Biochemistry and Physiology* A, 145, 137–142.

Coyle J.T., Puttfracken P. (1993). Oxidative stress, Glutamate, and neurodegenerative disorders. *Science, New Series*. 262 (5134), 689-695.

Crescenti A., Maria del Bas J., Arola-Arnal A., Oms-Oliu G., Arola L., Caimari A. (2015). Grape seed procyanidins administered at physiological doses to rats during pregnancy and lactation promote lipid oxidation and up-regulate AMPK in the muscle of male offspring in adulthood. *Journal of Nutritional Biochemistry*, 26, 912-920.

Crozier A. (2003). Classification and biosynthesis of secondary plant products: an overview in: Goldberg, G. (Eds.), Plants: Diet and health. Chapman and Hall, London, pp. 27-48.

Crozier A., Jaganath I.B., Clifford M.N. (2009). Dietary phenolics: Chemistry, bioavailability and effects on health. *Natural Product Reports*, 26, 1001-1043.

Cueva C., Moreno-Arribas M.V., Martín-Álvarez P.J., Bills,G., Vicente M.F., Basilio A., López Rivas C., Requena T., Rodríguez J.M., Bartolomé B. (2010). Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. *Research in Microbiology*, 161, 372–382.

Cushnie T.P., Lamb A.J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38, 99–107.

Dahiya J.P., Wilkie D.C., van Kessel A.G., Drew M.D. (2006).Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. *Animal Feed Science and Technology*, 129, 60–88.

Dai J., Mumper R.J. (2010). Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules*, 15, 7313-7352.

Dani C., Oliboni L.S., Vanderlinde R., Pra D., Dias J.F., Yoneama M.L., Bonatto D., Salvador M., Henriques J.A.P. (2009). Antioxidant activity and phenolic and mineral content of rose grape juice. Journal of Medicinal Food, 12, 188–192.

Daroch F., Hoeneisen M., Gonzalez C.L., Kawaguchi F., Salgado F., Solar H. (2001). In vitro antibacterial activity of Chilean red wines against Helicobacter pilori. *Microbios*, 104, 79–85.

Davidson H.J.D., Naidu A.S. (2000). "Phyto-phenols," in Natural Food Antimicrobial Systems, ed. A. S. Naidu (Boca Raton, FL: CRC Press), 265–294.

Dawson P.L., Han I.Y., Voller L.M., Clardy C.B., Martinez R.M., Acton J.C. (1995). Film oxygen transmission rate effects in ground chicken meat quality. *Poultry Science*, 74, 1381-1387.

Day A., Stansbie D. (1995). Cardioprotective effect of red wine may be mediated by urate. *Clinical Chemistry*, pp. 41.

De Freitas V.A.P., Glories Y., Monique A. (2000). Development changes of procyanidins in grapes of red Vitis vinifera varieties and their composition in respective wines. *American Journal of Enology and Viticulture*, 51, 397-403.

Decker E. (1997). Phenolics: prooxidants or antioxidants? *Nutrition Reviews*, 55, 396-398.

Decker E.A., Xu Z.M. (1998). Minimizing rancidity in muscle foods. *Food Technology*, 52, 54–59.

Deng Q., Penner M., Zhao Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International*, 44, 2712-2720.

Deprez S., Brezillon C., Rabot S., Philippe C., Mila I., Lapierre C., Scalbert A. (2000). Polymeric proanthocyanidins are catabolized by a human colonic microflora into low molecular weight phenolic compounds. *Journal of Nutrition*, 130, 2733–2738.

Devatkal S.K., Narsaiah K., Borah A. (2010). Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. *Meat Science*, 85, 155–159

Devatkal S.K., Narsaiah K., Borah A. (2011). The effect of salt, extract of kinnow and pomegranate fruit by-products on colour and oxidative stability of raw chicken patties during refrigerated storage. *Journal of Food Science and Technology*, 48, 472–477.

Di Lecce G., Arranz S., Jáuregui O., Tresserra-Rimbau A., Quifer-Rada P., Lamuela-Raventós R.M. (2014). Phenolic profiling of the skin, pulp and seeds of Albariño grapes using hybrid quadrupole time-of-flight and triple-quadrupole mass spectrometry. *Food Chemistry*, *145*, 874–882.

Di Majo D., La Guardia M., Giammanco S., La Neve L., Giammanco M. (2008) The Antioxidant Capacity of Red Wine in Relationship with Its Polyphenolic Constituents. *Food Chemistry*, 111, 45-49.

Dixon R.A. (2004) Phytoestrogens. *Annual Review of Plant Physiology and Plant Molecular Biology*, 55, 225-261.

Dolara P., Luceri C., De Filippo C., Femia A.P., Giovannelli L., Caderni G., Cecchini C., Silvi S., Orpianesi C., Crecí A. (2005). Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutation Research: *Fundamental and Molecular Mechanisms of Mutagenesis*, 591, 237-246.

Donovan J.L., Manach C., Rios L., Morand C., Scalbert A., Rémésy C. (2002). Procyanidins are not available in rats fed a single meal containing a grape seed extract or the procyanidin dimer B3. *British Journal of Nutrition*, 87, 299-306

Dopico-Garcia M.S., Fique A., Guerra L., Afonso J.M., Pereira O., Valentao P., Andrade P.B., Seabra R.M. (2008), Principal components of phenolics to characterize red Vinho Verde grapes: anthocyanins or non-coloured compounds? *Talanta*, 75, 1190–1202.

Dorri S., Tabeidian A.S., Toghyani M., Jahanian R., Behnamnejad F. (2012a). Effect of different levels of grape pomace on blood serum biochemical parameters broiler chicks at 29 and 49 days of age. Proceeding of the 1th International and the

4th national Congress on Recycling of organic waste in agriculture 26-27 April 2012 Isfahan, Iran.

Dorri S., Tabeidian A.S., Toghyani M., Jahanian R., Behnamnejad F. (2012b). Effect of different levels of grape pomace on performance broiler chicks. Proceeding of the 1th International and the 4th national Congress on Recycling of organic waste in agriculture 26-27 April 2012 Isfahan, Iran.

Du M., Ahn D.U., Nam K.C., Sell J.L. (2000). Influence of dictay conjugated linoleic acid on the volatile, color and lipid oxidation of irradiated raw chicken meat. *Meat Science*, 56, 387-395.

Durmic Z., Blache D. (2012). Bioactive plants and plant products: Effects on animal function, health and welfare. *Animal Feed Science and Technology*, 176, 150–162.

Duthie G., Campbell F., Bestwick C., Stephen S., Russell W. (2013). Antioxidant effectiveness of vegetable powders on the lipid and protein oxidative stability of cooked turkey meat patties: Implications for health. *Nutrients*, 5(4), 1241–1252.

Enberg M., Lauridsen C., Jensen S.K., Jacobsen K. (1996). Inclusion of oxidised vegetable oil in broiler diets. Influence on nutrient balance and on the antioxidative status of broilers. *Poultry Science*, 75, 1003-1011.

En-Qin X., Gui-Fang D., Ya-Jun G., Hua-Bin L. (2010). Biological Activities of Polyphenols from Grapes. *International Journal of Molecular Sciences*, 11 (2), 622-646.

Enser M. (1987). What is lipid oxidation. *Food Science and Technology Today*, 1, 151-3.

Erener G., Ocak N., Altop A., Cankaya S., Murat Aksoy H., Ozturk E. (2011). Growth performance, meat quality and caecal coliform bacteria count of broiler chicks fed diet with green tea extract. *Asian-Australasian Journal of Animal Science*, 24, 1128-1135.

Escribano-Bailon M.T., Guerra M.T., Rivas-Gonzalo J.C., Santos-Buelga C. (1995). Proanthocyanidins in skins from different grape varieties. *Zeitschrift fur lebensmittel-untersuchung und-forschung, 200*, 221-224.

Escribano-Bailon M.T., Guitie´rrez-Fernandez Y., Rivas-Gonzalo J.C., Santos-Buelga C. (1992) Characterization of procyanidins of *Vitis* V*inifera* variety Tinta del Pais grape seeds. *Journal of Agricultural and Food Chemistry, 40*, 1794-1799.

Esterbauer H. (1993). Cytotoxicity and genotoxicity of lipid oxidation products. *The American Journal of Clinical Nutrition*, 57, S779–S786.

Esterbauer H., Schaur R.J., Zollner H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine*, 11, 81–128.

Estévez M., Ventana, S., Cava M. (2007). Oxidation of lipids and proteins in frankfurters with different fatty acid compositions and tocopherol and phenolic contents. *Food Chemistry*, 100, 55–63.

EUROSTAT (2014). Statistical Data-base. http://ec.europa.eu/eurostat/statistics-explained/index.php/Meat_production_statistics

Falchi M., Bertelli A., Scalzo R.L., Morassut M., Morelli R., Das S., Cui J.H., Das D.K. (2006). Comparison of cardioprotective abilities between the flesh and skin of grapes. *Journal of Agricultural and Food Chemistry*, 54, 6613–6622.

FAO (2004)- Food and Agriculture Organization of the United Nations. Food safety and quality in europe: aspects concerning in particular quality, nutritional balance, the importance of agricultural land and cultural heritage ("terroirs"). Twenty-fourth fao regional conference for Europe, Montpellier, France, 5-7 may, item 6.

FAO. (2013). Agribusiness Handbook, Grapes wines. Retrieved 2014 November, from http://www.fao.org/

Faostat (2012). Food and Agriculture Organization Corporate Statistical Database. http://faostat.fao.org/site/291/default.aspx

FAOSTAT-FAO (2010). Statistical Data-base, 2010. http://www.fao.org

Faria A., Calhau C., De Freitas V., Mateus N. (2006). Procyanidins as antioxidants and tumor cell growth modulators. *Journal of Agricultural and Food Chemistry*, 54, 2392–2397.

Fasseas M.K., Mountzouris K.C., Tarantilis P.A., Polissiou M., Zervas G. (2007). Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry*, 106, 1188–1194.

Faustman C., Cassens R.G. (1990) The biochemical basis for discoloration in fresh meat: A review. *Journal of Muscle Foods*, 1, 3, 217–243.

Fedna (2003). Tablas de composicion y valor nutritivo de los alimentos para lafabricacio´ n de piensos compuestos. FEDNA, Madrid, Spain.

Feedinfo News Service. (2008). Vitamin E Feedgrade: Market to Stay Tight This Summer. Feedinfo News Service, Labège, France. Accessed June 30. http://www.feedinfo.com/console/Pa-geViewer.aspx?page=925674&public=yes.

Feedinfo News Service. (2009). Exclusive Interview: BASF Discusses the Vitamin E and A Markets. Feedinfo News Service, Labège, France. Accessed Oct. 6. http://www.feedinfo.com/con- sole/PageViewer.aspx?page=1421596&public=yes

Fernandes L., Casal S., Cruz R., Pereira J.A., Ramalhosa E. (2013). Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. *Food Research International*, 50(1), 161-166.

Ferrali M., Signorini C., Caciotti B., Sugherini L., Ciccoli L., Giachetti D. (1997). Protection against oxidative damage of erythrocyte membranes by the flavonoid quercetin and its relation to iron chelating activity. FEBS Letters, 416, 123–129.

Ferrer J., Páez G., Mármol Z., Ramones E., Chandler C., Marín M., Ferrer A. (2001). Agronomic use of biotechnologically processed grape wastes. *Bioresource Technology*, 76, 39–44.

Fiesel A., Gessner D.K., Erika Most E., Eder K. (2014). Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. *BMC Veterinary Research*, 10, 196–206.

Figuerola F., Hurtado M.L., Estévez A.M., Chiffelle I., Asenjo F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry*,91, 395–401

Filimon M.N., Borozan A., Sinitean A., Popescu R., Török-Oance R., Verdeș D. (2010). Bacteriological studies on Timiş and Bega Rivers with a role in apreciating pollution. *Annals of the Romanian Society for Cell Biology*, XV, 2, 217-220.

Fleet G.H. (1999) Microorganisms in food ecosystems. *International Journal of Food Microbiology*, 50 (1-2), 101–117.

Francesch A., Cartañà M. (2015).The effects of grape seed in the diet of the Penedes chicken, on growth and on the chemical composition and sensory profile of meat. *British Poultry Science*, 56, 4, 477–485.

Frank J. (2005). Beyond vitamin E supplementation: an alternative strategy to improve vitamin E. *Journal of Plant Physiology*, 162, 834-843.

Frank J., Budek A., Lundh T., Parker R.S., Swanson J.E., Lourenço C.F., Gago B., Laranjinha J., Vessby B., Kamal-Eldin A. (2006). Dietary flavonoids with a catechol structure increase alpha tocopherol in rats and protect the vitamin from oxidation in vitro. *Journal of Lipid Research*, 47, 2718–2725.

Frank, J., Lundh T., Parker R.S., Swanson J.E., Vessby B., Kamal-Eldin A. (2003). Dietary (1)-catechin and BHTmarkedly increase a-tocopherol concentrations in rats by a tocopherol-v-hydroxylaseindependent mechanism. *Journal of Nutrition*, 133, 3195–3199.

Fratianni F., De Martino L., Melone A., De Feo V., Coppola R., Nazzaro F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science*, 75, 528–535.

Frazier R.A., Deaville E.R., Green R.J., Stringano E., Willoughby I., Plant J., Mueller-Harvey I. (2010). Interactions of tea tannins and condensed tannins with proteins. *Journal of Pharmaceutical and Biomedical Analysis*, 51, 490–495.

Fremont L., Gozzelino M.T., Franchi M.P., Linard A. (1998). Dietary flavonoids reduce lipid peroxidation in rats fed polyunsaturated or monounsaturated fat diets. *Journal of Nutrition*, 128, 1495–1502.

Friedman M., Henika P.R., and Mandrell R.E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and salmonella enterica. *Journal of Food Protection*, 10, 1545-1560.

Fuleki T., JRicardo-Da-Silva .M. (1997). Catechin and procyanidin composition of seeds from grape cultivars grown in Ontário. *Journal of Agricultural and Food Chemistry*, 45 (4), 1156-1160.

Galobart J., Barroeta A.C., Baucells M.D., Guardiola F. (2001). Lipid oxidation in fresh and spray-dried eggs enriched with omega3 and omega6 polyunsaturated fatty acids during storage as affected by dietary vitamin E and canthaxanthin supplementation. *Poultry Science*, 80, 327–337.

Galobart J., Guardiola F., Barroeta A.C., Lopez-Ferrer S., Baucells M.D. (2002). Influence of dietary supplementation with alpha-tocopheryl acetate and canthaxanthin on cholesterol oxidation in omega 3 and omega 6 fatty acid-enriched spray-dried eggs. *Journal of Food Science*, 67, 2460–2466.

Gañan M., Martinez-Rodriguez A.J., Carrascosa A.V. (2009). Antimicrobial activity of phenolic compounds of wine against Campylobacter jejuni. *Food Control*, 20, 739–742.

Gao J., Lin H., Wang X.J., Song Z.G., Jiao H.C. (2010). Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. *Poultry Science*, 89, 318–327.

Gardner HW. (1989). Oxygen radical chemistry of polyunsaturated fatty acids. *Free Radical Biology and Medicine*, 7, 65-86.

Gasaway W.C., White R.G., Holleman D.F. (1976). Digestion of dry matter and absorption of water in the intestine and cecum of rock ptarmigan. *Condor*, 78, 77–84.

Georgiev V., Ananga A., Tsolova V. (2014). Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients*, 6, 391–415.

Ghafoor K., Choi Y.H., Jeon J.Y., Jo I.H. (2009). Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from grape (*Vitis vinifera*) seeds. *Journal of Agricultural and Food Chemistry, 57*, 4988–4994.

Gigaud V., Debut M., Berri C., Le Bihan-Duval E., Travel A., Bordeau T. (2008). Influence des facteurs ante-mortem sur la qualité technologique des filets de poulet de type standard et label. 11èmes Journées des sciences du muscle et technologies des viandes (Clermont-Ferrand, France, 10/04/2006). Viandes et Produits carnés, numéro hors-série, 213-214.

Gill C.O., Newton K.G. (1978). The ecology of bacterial spoilage of fresh meat at chill temperatures. *Meat Science*, 2(3), 207-217.

Gladine C., Morand C., Rock E., Bauchart D., Durand D. (2007). Plant extracts rich in polyphenols (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids from animals fed n-3 PUFA supplemented diets. *Animal Feed Science and Technology*, 136, 281–296.

Gobert M., Martin B., Ferlay A., Chilliard Y., Graulet B., Pradel, Bauchart D., Durand D. (2009). Plant polyphenols associated with vitamin E can reduce plasma lipoperoxidation in dairy cows given n-3 polyunsaturated fatty acids. *Journal of Dairy Science*, 92, 6095–6104.

Goldstein D.L. (1989). Absorption by the cecum of wild birds: is there interspecific variation. *Journal of Experimental Zoology, The Supplement*, 3, 103–110.

Gomez-Plaza E., Minano A., Lopez-Roca J. (2006). Comparison of chromatic properties, stability and antioxidant capacity of anthocyanin-based aqueous extracts from grape pomace obtained from different vinification methods. *Food Chemistry*, 87-94.

Gong J., Si W., Forster R.J., Huang R., Yu H., Yin Y., Yang C., Han Y. (2007). 16S rRNA genebased analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. *FEM Microbiology Ecology*, 59, 147-157.

Goñi I., Serrano J. (2005). The intake of dietary fiber from grape seeds modifies the antioxidant status in rat cecum. *Journal of the Science of Food and Agriculture*, 85, 1877–1881.

Goñi I., Brenes A., Centeno C., Viveros A., Saura-Calixto F., Rebolé A., Arija I., Esteve R. (2007). Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation. *Poultry Science*, 86, 508-516.

Gonthier M.P., Donovan J.L., Texier O., Felgines C., Remesy C., Scalbert A. (2003). Metabolism of dietary procyanidins in rats. *Free Radical Biology and Medicine*, 35, 837–844.

Gonzalez-Castejon M., Rodriguez-Casado A. (2011). Dietary phytochemicals and their potential effects on obesity. A rewiw. *Pharmacological Research*, 64, 438-455.

González-Centeno M.R., Jourdes M., Fermenia A., Simal S., Rosselló C., Teissedre P-L. (2012). Proanthocyanidin composition and antioxidant potential of the stem winemaking byproducts from 10 different grape varieties (Vitis vinifera L.). *Journal of Agricultural and Food Chemistry*, 60, 11850–11858.

González-Centeno M.R., Rosselló C., Simal S., Garau M.C., López F., Femenia A. (2010). Physico-chemical properties of cell wall materials obtained from tem grape varieties and their By-products: Grape pomace and stems. *LWT Food Science and Technology*, 43, 1580–1586.

González-Lamothe R., Mitchell G., Gattuso M., Diarra M.S., Malouin F., Bouarab K. (2009). Plant antimicrobial agents and their effects on plant and human pathogens. *International Journal of Molecular Sciences*, 10, 3400–3419.

Gonzalez-Neves G., Gil G., Ferrer M. (2002). Effect of different vineyard treatments on the phenolic contents in Tannat (Vitis vinifera L.) grapes and their respective wines. *Food Science and Technology International*, 8, 315–321.

Gonzalez-Paramas A.M., Esteban-Ruano S., Santos-Buelga C., Pascual-Teresa S., Rivas-Gonzalo J.C. (2004). Flavanol content and antioxidant activity in winery products. *Journal of Agricultural and Food Chemistry*, 52, 234–238.

Goupil P., Benouaret R., Charrier O., ter Halle A., Richard C., Eyheraguibel B., Thiery D., Ledoigt G. (2012). Grape marc extract acts as elicitor of plant defence responses. *Ecotoxicology*, 21, 1541e1549.

Grashorn M.A. (2007): Functionality of poultry meat. *Journal of Applied Poultry Research*, 16, 99-106.

Grashorn M.A. (2010). Use of phytobiotics in broiler nutrition – an alternative to infeed antibiotics? *Animal Feed Science and Technology*, 19, 338–347.

Grau A, Codony R, Grimpa S, Baucells MD, Guardiola F (2001). Cholesterol oxidation in frozen dark chicken meat: influence of dietary fat source, and alpha-tocopherol and ascorbic acid supplementation. *Meat Science*, 57, 197–208.

Gray, J. I., Monahan F.J. (1992). Measurement of lipid oxidation in meat and meat products. *Trends in Food Science and Technology*, 3, 315-319.

Green J. (1972). Vitamin E and the biological antioxidant theory. *Annals of the New York Academy of Sciences*, 203, 29–44

Guan Y.Q., Chu Q.C., Fu L., Ye J.N. (2005). Determination of antioxidants in cosmetics by micellar electrokinetic capillary chromatography with electrochemical detection. *Journal of Chromatography*, 1074(2):201– 204

Guendez R., Kallithraka S., Makris D. P., Kefalas P. (2005). Determination of low molecular weight polyphenolic constituents in grape (Vitis vinifer a sp.) seed extracts: Correlation with antiradical activity. *Food Chemistry*, 89 (1), 1-9.

Guo L., Xie M.Y., Yan A.P., Wan Y.Q., Wu Y.M. (2006). Simultaneous determination of five synthetic antioxidants in edible vegetable oil by GC–MS. *Analytical and Bioanalytical Chemistry*, 386(6), 1881–1887.

Gustafson R.H., Bowen R.E. (1997). Antiobiotic use in animal and agriculture. *Journal of Applied Microbiology*, 83, 531–541.

Halliwell B., Rafter J., Jenner A. (2005). Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *American Journal of Clinical Nutrition*, 81, 268-276.

Halliwell B.,. Gutteridge J.M.C. (2007). Free radicals in biology and medicine. Oxford University Press, Oxford, England

Hallman P.C.H., Katan M.B. (1997). Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed Pharmother*., 51, 305-316.

Hara H., Orita N., Hatano S., Ichikawa H., Hara Y., Matsumoto N., Kimura Y., Terada A., Mitsuoka T. (1995). Effect of tea polyphenols on fecal flora and fecal metabolic products of pigs. *The Journal of Veterinary Medical Science*, 57, 45–49.

Harborne J.B. (1989). Methods in plant biochemistry. In: Dey PM, Harborne JB. (ed.) Plant phenolics. *London: Academic Press*, 1.

Harborne J.B., Baxter H. (1999). The dandbook of natural flavonoids. Vol 2. US Department of Agriculture. USDA Database for Flavonoid Content of Selected Foods-2003. Wiley, West Sussex, UK.

Hargis P.S., Van Elswyk M.E. (1993). Manipulating the fatty acid com- position of poultry meat and eggs for the health conscious consumer. *World's Poultry Science,* J 49, 251-264.

Hatcher H.C., Singh R.N., Torti F.M., Torti S.V. (2009). Synthetic and natural iron chelators: therapeutic potential and clinical use. *Future Medicinal Chemistry*, 1, 1643-1670.

Heim K.E., Tagliaferro A.R., Bobilya D.J. (20029. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. *Journal of Nutritional Biochemistry*, 13, 572–84.

Heinz G., Hautzinger P. (2007). Meat processing technology for small- to medium-scale producers, processed products made of chicken meat, pp. 187.

Henchion M., McCarthy M., Resconi V.C., Troy D. (2014). Meat consumption: Trends and quality matters. *Meat Science*,98, 561-568.

Hernandez-Hernandez E., Ponce-Alquicira E., Jaramillo-Flores M.E., Guerrero-Legarreta I. (2009). Antioxidant effect rosemary (Rosmarinus officinalis L.) and

oregano (Origanum vulgare L.) extracts on TBARS and colour of model raw pork batters. *Meat Science*, 81, 2, 410-417.

Herrero A., de la Hoz L., Ordóñez J., Herranz B., Romero de Ávila M., Cambero M. (2008). Tensile properties of cooked meat sausages and their correlation with texture profile analysis (TPA) parameters and physico-chemical characteristics. *Meat Science*, 80, 690-698.

Hirano R., Sasamoto W., Matsumoto A., Itakura H., Igarashi O., Kondo K. (2001). Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *Journal of Nutritional Science and Vitaminology (Tokyo)*, 47:357–62.

Hu M., Mc Clements D.J., Decker E.A. (2004). Antioxidant activity of proanthocyanidin-rich extract from grape seed in whey protein isolate stabilized algae oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 52, 5272–5276.

Huda N., Shen Y.H., Huey Y.L. (2009). Proximate Composition, Colour, Texture Profile of Malaysian Chicken Balls. *Pakistan Journal of Nutrition*, 8, 1555-1558.

Hughes R.J., Brooker J.D., Smyl C. (2005) Growth rate of broiler chickens given condensed tannins extracted from grape seed. *Australian Poultry Sciences Symposium*, 17, 65–68.

ICMSF, International Commission for the Microbiological Specifications for Foods (2005). Microbial ecology of food commodities. Microorganisms in foods, pp. 1-101 and 174-249. Blackie Academic and Professional, London.

Igene J.O., King J.A., Pearson A.M., Gray J.I. (1979). Influence of heme pigments, nitrtite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. *Journal of Agricultural and Food Chemistry*, 27, 838-842.

Iglesias J., Pazos M., Torres J.L., Medina I. (2012). Antioxidant mechanism of grape procyanidins in muscle tissue: Redox interactions with endogenous ascorbic acid and α-tocopherol. *Food Chemistry*, 134, 1767–1774.

Ignat I., Volf I., Popa V.I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, 126(4), 1821–1835.

Ingr I. (1989). Meat quality: Defining the term by modern standards. *Fleisch* 69, 1268p.

Iqbal Z., Ali R., Sultan J.I., Ali A., Kamran Z., Khan S.A., Ahsan U. (2014). Impact of replacing grape polyphenol with vitamin E on growth performance, relative organs weight, and antioxidant status of broilers. *Journal of Animal and Plant Science*, 24, 1579-1583.

Irkin R., Esmer O. K. (2010). Control of Listeria monocytogenes in ground chicken breast meat under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of bay essential oil at 4°C. *Food Science and Technology Research*, 16, 285–290.

Ishihara N., Chu D.C., Akachi S., Juneja L.R. (2001). Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. *Livestock Production Science*, 68, 217–229.

ISO (1998) standard 15214. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of mesophilic lactic acid bacteria -- Colony-count technique at 30 degrees C.

ISO (2001) standard 16649-2. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli -- Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

Jackson R.S. (2008). Wine Science (Third Edition) Principles and Applications. Food Science and Technology.

Jakobek L., Sěruga M., Sěruga B., Novak I., Medvidović-Kosanovic M. (2009). Phenolic compound composition and antioxidant activity of fruits of Rubus and Prunus species from Croatia. *International Journal of Food Science and Technology*, 44, 860–868.

Jakobsen, K., Engberg R.M., Andersen J.O., Jensen S.K., Lauridsen C., Sorensen P., Henckel P., Bertelsen G., Skibsted L. H., Jensen, C. (1995). Supplementation of broiler diets with all-rac-alpha- or a mixture of natural source RRR-alpha-,gamma-,delta-tocopheryl acetate 1. Effect on vitamin E status of broilers in vivo and at slaughter. *Poultry Science*, 74, 1984–1994.

Jałosńska M., Wilczak J. (2009). Influence of plant extracts on the microbiological shelf life of meat products. *Polish Journal Of Food And Nutrition Sciences*, 59, 303–308.

Jamilah M.B., Abbas K.A., Rahman R.A. (2008). A review on some organic acids additives as shelf life extenders of fresh beef cuts. *American Journal of Agricultural and Biological Sciences*, 3, 566–574.

Jankun J., Selman S.H., Swiercz R. (1997). Why drinking green tea could prevent cancer. *Nature*, 387, 561.

Jassim J.M., Riyad K.M., Majid H.A., Yanzhang G. (2011). Evaluation of physical and chemical characteristics of male and female ducks carcasses at different ages. *Pakistan Journal of Nutrition*, 10 (2), 182-189.

Jeandet P., Bessis R., Gautheron B. (1991). The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. *American Journal of Enology and Viticulture*, *42*, 41–46.

Jensen S.K., Jensen C., Jakobsen K., Engberg R.M., Andersen J.O., Lauridsen C., Sorensen P., Skibsted L.H., Bertelsen G. (1998). Supplementation of broiler diets with retinol acetate, beta-carotene or canthaxanthin: Effect on vitamin status and oxidative status of broilers in vivo and on meat stability. *Acta Agriculturae Scandinavica, Section A*, 48, 28–37.

Jensen S.K., Lauridsen C. (2007). α-Tocopherol Stereoisomers. *Vitamins & Hormones*, 76, 281–308.

Jo C., Jang A., Jung S., Choe J.H., Kim B., Lee K.H. (2009). Effect of dietary herb extract mix on antioxidative activity of chicken thigh meat. *Journal of the Korean Society of Food Science and Nutrition*, 38, 302–308.

Jordão A. M., Ricardo da Silva J. M., Laureano O. (2001a). Evolution of proanthocyanidins in bunch stems during berry development (*Vitis* V*inifera* L.). *Vitis*, *40*, 17-22.

Jordão A.M., Ricardo da Silva J. M., Laureano O. (2001b). Evolution of catechins and oligomeric procyanidins during grape maturation of Castelão Frances and Touriga Francesa. *American Journal of Enology and Viticulture*, *52*, 230-234.

Jukna Č., Jukna V., Pečiulaitienė N., Kerzienė S. (2010). Influence of English Large White breed on fattening and meat characteristics and meat quality of Lithuanian White pigs. *Veterinary medicine and zootechnics*, 51(73), 17-22.

Jukna V., Jukna Č., Pečiulaitienė N. (2007). Genetic factors influence on pork quality. *Veterinary medicine and zootechnics*, 40(62), 35−38.

Jung S., Choe J.H., Kim B., Yun H., Kruk Z.A., Jo C. (2010). Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. *Meat Science*, 86, 520–526.

Just J., Daeschel M.A. (2003). Antimicrobial effects of wine on Escherichia coli O157:H7 and Salmonella typhimurium in a model stomach system. *Journal of Food Science*, 68, 285–290.

Kabir S.M.L. (2009). The role of probiotics in the poultry industry. International *Journal of Molecular Sciences*, 10, 3531–3546.

Kallithraka S., Salachaa M.I., Tzouroua I. (2009). Changes in phenolic composition and antioxidant activity of white wine during bottle storage: Accelerated browning test *versus* bottle storage. *Food Chemistry*, *113*, 500–505.

Kamal-Eldin, A., J. Frank, A. Razdan, S. Tengblad, S. Basu, and B. Vessby. (2000). Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. *Lipids*, 35, 427–435.

Kamboh A.A., Zhu W.Y. (2013). Effect of increasing levels of bioflavonoids in broiler feed on plasma anti-oxidative potential, lipid metabolites, and fatty acid composition of meat. *Poultry Science*, 92, 454-461.

Kammerer D., Claus A., Carle R. and Schieber A. (2004). Polyphenol Screening of Pomace from Red and White Grape Varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry*, 52, 4360.

Karabagias I., Badeka A., Kontominas M.G. (2011). Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Science*, 88, 109-116.

Karre L., Lopez K., Getty K. J. (2013). Natural antioxidants in meat and poultry products. *Meat Science*, 94(2), 220–227.

Kataliníc V., Možina S.S., Skroza D., Generalić I., Abramovič H., Miloš M., Ljubenkov I., Piskernik S., Terpinc, P., Boban M. (2010). Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). *Food Chemistry*, 119, 715–723.

Kaviarasan K., Kalaiarasi P., Pugalendi V. (2008). Antioxidant efficacy of flavonoid-rich fraction from Spermacoce hispida in hyperlipidemic rats. *Journal of Applied Biomedicine*, 6, 165–76.

Kemperman R.A., Gross G., Mondot S., Possemiers S., Marzorati M., Van de Wiele T., Dore J., Vaughan E.E. (2014). Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome. *Food Research International*, 53, 659–669.

Kennedy D.G., Rice D.A., Bruce E.A., Goodall E.A., McIlroy S.G. (1992). Economic effects of increased vitamin E supplementation of broiler diets on commercial broiler production. *British poultry science*, 33, 1015-1023.

Kennedy J.A., Jones G.P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry*, *49*, 1740-1746.

Kennedy J.A., Matthews M.A., Waterhouse A.L. (2000). Changes in grape seed polyphenols during ripening. *Phytochemistry*, 55, 77-85.

Kerem Z., Chertrit D., Shoseyov O., Regev-Shoshani G. (2006). Protection of lipids from oxidation by epicatechin, trans-resveratrol, and gallic and caffeic acids in intestinal model systems. *Journal of Agricultural and Food Chemistry*, 54, 10288-10293.

Khalil, A. (2002). Molecular mechanisms of the protective effect of vitamin E against atherosclerosis. Canadian Journal of Physiology and Pharmacology, 80, 662–669.

Khanbabaee K., Van Ree T. (2001). Tannins: classification and definition. Nat. Prod. Rep. 18, 641-649.

Khanbabaee K., van Ree T. (2001). Tannins: Classification and Definition. *Natural Product Reports*, 18, 641–649.

Kim S., Cho A.R., Ha, J. (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*, 29, 112–120.

Kolakowska A. (2002). Lipid oxidation in food systems, in chemical and functional properties of food lipids, edited by Sikorski ZE, and Kolakowska A. Chapter 8. CRC Press. ISBN: 978-1-58716-105-6.

Kolakowska A., Bratosz, G. (2010). Antioxidants. In: Chemical, biological and functional aspects of food lipids. Eds. ZE. Sikorski, A. Kolakowska. CRC Press, Boca Raton 163–184.

Korkina LG, Afanas'ev IB. (1997). Antioxidant and chelating properties of flavonoids. *Advances in Pharmacology*, 38, 151–163.

Kossah R., Zhang H., Chen W. (2011). Antimicrobial and antioxidant activities of Chinese sumac (Rhus typhina L.) fruit extract. *Food Control*, 22, 128-132.

Kresty L.A., Howell A.B., Maureen B. (2011). Cranberry proanthocyanidins mediate growth arrest of lung cancer cells through modulation of gene expression and rapid induction of apoptosis. *Molecules*, 16, 2375-2390.

Krishnan K.R., Babuskina S., AzhaguSaravanaBabu P., Sasikala M., Sabina K., Archana G., Sivarajan M., Sukumar M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology*, 171, 32–40.

Kumar V., Chatli M.K., Wagh R.V., Mehta N., Kumar P. (2015). Effect of the combination of natural antioxidants and packaging methods on quality of pork patties during storage. *Journal of Food Science and Technology*, 52, 10, 6230–6241.

Kurcubic V.S., Maskovic P.Z., Vujic M., Vranic D.V., Veskovic-Moracanin S.M., Okanovic D.G., Lilić SV. (2014). Antioxidant and antimicrobial activity of Kitaibelia vitifolia extract as alternative to the added nitrite in fermented dry sausage. *Meat Science*, 97, 459–467.

Ky I., Lorrain B., Kolbas N., Crozier A., Teissedre P.L. (2014). Wine by-products: Phenolic characterization and antioxidant activity evaluation of grapes and grape pomaces from six different french grape varieties. *Molecules*, *19*, 482–506.

Labadie J. (1999). Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science*, 52(3), 299-305.

Labarbe B., Cheynier V., Brossaud F. Souquet J.M., Moutounet M. (1999). Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *Journal of Agricultural and Food Chemistry*, *47*, 2719-2723.

Lafka T.I., Sinanoglou V., Lazos E.S. (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chemistry*, 104, 1206–1214.

Laguerre M., Lecomte J., Villeneuve P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Progress in Lipid Research,* 46, 244–282.

Landes M., Persaud S., Dyck J. (2004). "India's Poultry Sector: Development and Prospects". ERS, USDA, Agricultural and Trade Report WRS-04-03.

Lands W.E.M. (2003). Primary prevention in cardiovascular disease: moving out of the shadows of the truth about death. *Nutrition, Metabolism and Cardiovascular Diseases*,13, 154-164.

Laranjinha J. (2001). Redox cycles of caffeic acid with alphatocopherol and ascorbate. *Methods in Enzymology*, 335, 282–295.

Larrosa M., García-Conesa M.T., Espín J.C., Tomás-Barberán F.A. (2010). Review: ellagitannins, ellagic acid and vascular health. *Molecular Aspects of Medicin*e, 31, 513–539.

Larrosa M., Yañéz-Gascón M.J., Selma M.V., González-Sarrías A., Toti S., Cerón J.J., Tomás-Barberán F., Dolara P., Espín J.C. (2009). Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS874 induced colitis rat model. *Journal of Agricultural and Food Chemistry*, 57, 2211-2220.

Lau D.W., King A.J. (2003). Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *Journal of Agricultural and Food Chemistry,* 51, 1602-1607.

Laurent C., Besancon P., Caporiccio B. (2007). Flavonoids from a grape seed extract interact with digestive secretions and intestinal cells as assessed in an in vitro digestion/Caco-2 cell culture model. *Food Chemistry*, 100, 1704–1712.

Lauridsen C., Buckley D.J., Morrissey P.A. (1997). Influence of dietary fat and vitamin E supplementation on alpha-tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. *Meat Science*, 46, 9–22.

Lebert I., Begot C., Lebert A. (1998). Growth of Pseudomonas fluorescens and Pseudomonas fragi in a meat medium as affected by pH (5.8–7.0), water activity (0.97–1.00) and temperature (7–25°C). *International Journal of Food Microbiology,* 39 (1-2), 53-60.

Lecas, M., & Brillouet, J. M. (1994). Cell wall composition of grape berry skins. *Phytochemistry*, *35*(5), 1241-1243.

Lee S.H., Shindle P.L., Choi J.Y., Kwon I.K., Lee J.K., Pak S.I., Cho W.T, Chae B.J. (2010). Effects of tannic acid supplementation on growth performance, blood haematology, iron status and faecal microflora in weanling pigs. *Livestock Science*, 131, 281-286.

Leeson S. (2007). Vitamin requirements: Is there a basis for re-evaluating dietary specifications? *World's Poultry Science Journal*, 63, 255–266.

Leheska J.M., Boyce J., Brooks J.C., Hoover L.C., Thompson L.D., Miller M.F. (2006). Sensory attributes and phenolic content of precooked pork breakfast sausage with fruit purees. *Journal of Food Science*, 71, 249-252.

Lesiów T. , Oziemblowski M., Szkudlarek S. (2007). Incidence of PSE and DFD in chicken broiler breast muscles 24 h p.m. CD of Proc. XVII European Symposium on the Quality of Poultry Meat and XII European Symposium on the Quality of Eggs and Egg Products, Prague, Czech Republic, pp. 265–266.

Li W. J., Zhao G. P., Chen J. L., Zheng M. Q., Wen J. (2009). Influence of dietary vitamin E supplementation on meat quality traits and gene expression related to lipid metabolism in the Beijing-you chicken. *British poultry science*, 50, 188-198.

Lima M.T.R., Waffo-Téguo P., Teissedre P.L., Pujolas A., Vercauteren J., Cabanis J.C., Mérillon J.M. (1999). Determination of stilbenes (*trans*-astringin, *cis*- and *trans*-piceid, and *cis*- and *trans*-resveratrol) in Portuguese wines. *Journal of Agricultural and Food Chemistry*, *47*, 2666–2670.

Liu C., Wang L., Wang J., Wu B., Liu W.M., Fan P., Liang Z., Li S. (2013). Resveratrols in Vitis berry skins and leaves: Their extraction and analysis by HPLC. *Food Chemistry*, *136*, 643–649..

Liu L.L., He J.H. Xie H.B., Yang Y.S., Li J.C., Zou Y. (2014). Resveratrol induces antioxidant and heat shock protein mRNA expression in response to heat stress in black-boned chickens. *Poultry Science*, 93, 54-62.

Llobera A., Cañellas J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (Vitis vinifera): pomace and stem. *Food Chemistry*, 101, 659-666.

López-Bote C.J., Daza A., Soares M., Berges E. (2001). Dose response effect of dietary vitamin E concentration on meat quality characteristics in light-weight lambs. *Animal Science*, 73, 451−457.

López-López I., Cofrades S., Yakan A., Solas M.T., Jiménez-Colmenero F. (2010). Frozen storage characteristics of low-salt and low-fat beef patties as affected by Wakame addition and replacing pork backfat with olive oil-in-water emulsion in cooked ground beef patties. *Food Research International*, 43, 1244–1254.

Lorenzo J.M., Sineiro J., Amado I.R., Franco D. (2014). Influence of natural extracts on the shelf life of modified atmosphere-packaged pork patties. *Meat Science*, 96, 526-534.

Lotito S.B., Frei B. (2004). Relevance of apple polyphenols as antioxidants in human plasma: contrasting in vitro and in vivo effects. *Free Radical Biology and Medicine*, 36 (2), 201-11.

Lotito S.B., Frei B. (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radical Biology and Medicine*, 41, 1727–46.

Louli V., Ragoussis N., Magoulas K. (2004). Recovery of phenolic antioxidants from wine industry by-products. *Bioresource Technology*, 92, 201-208.

Lu J., Idris U., Harmon B., Hofacre C., Maurer J.J., Lee M.D. (2003). Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology*, 69, 6816–6824.

Lu Y., Yeap Foo L. (1998). The polyphenol constituents of grape pomace. Food Chemistry. *Food Research International*, 65(1) 1-8.

Luciano G., Vasta V., Monahan F.J., López-Andrés P., Biondi L., Lanza M., Priolo A. (2011). Antioxidant status, colour stability and myoglobin resistance to oxidation of longissimus dorsi muscle from lambs fed a tannin-containing diet. *Food Chemistry*, 124, 1036–1042.

Luciano, G., Monahan, F.J., Vasta, V., Biondi, L., Lanza, M., Priolo, A (2009). Dietary tannins improve lamb meat colour stability. *Meat Science*, 81, 120–125

Lusk J.L., Norwood F.B., Pruitt J.R. (2006). Consumer demand for a ban on antibiotic drug use in pork production. *American Journal of Agricultural Economics*, 88(4), 1015-1033.

Lynch M.P., Faustman C., Silbart L.K., Rood D., Furr H.C. (2001). Detection of lipidderived aldehydes and aldehyde: Protein adducts in vitro and in beef. *Journal of Food Science*, 66, 1093–1099.

Lyon B.G., Lyon C.E. (1996). Texture evaluations of cooked, diced broiler breast samples by sensory and mechanical methods. *Poultry Science*, 75, 812–819.

Lyon BG, Lyon CE. (2001). Meat quality: sensory and instrumental evaluations. In: Sams AR, editor. Poultry Meat Processing. CRC Press; New York, USA: 2001. pp. 97–120.

Madhavi D.L., Deshpande S.S., Salunkhe/Dekker D.K. (1996). Butylated hydroxyanisole (BHA; tert-butyl-4-hydroxyanisole) and butylated hydroxytoluene (BHT; 2,6-di-tert-butyl-pcresol). *Food Antioxidants: Technological, Toxicological, and Health Perspectives*.

Madhavi D.L., Salunkhe D.K. (1995). Toxicological aspects of food antioxidants. *In D. L. Madavi, S. S. Deshpande, & D. K. Salunkhe (Eds.),* Food antioxidants (pp. 267). New York: Marcel Dekker.

Maestre R., Micol V., Funes L., Medina I. (2010). Incorporation and interaction of grape seed extract in membrane and relation with efficacy in muscle food. *Journal of Agricultural and Food Chemistry*, 58, 8365–8374.

Magdelaine P., Spiess M.P., Valceschini E. (2008). Poultry meat consumption trends in Europe. *World's Poultry Science Journal*, 64, 53-64.

Maier T., Schieber A., Kammerer D.R., Carle R. (2009) Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants. *Food Chemistry*, 112, 3, 551-559.

Maiorano G., Sobolewska A., Cianciullo D., Walasik K., Elminowska-Wenda G., Sławińska A., Tavaniello S., Żylińska J., Bardowski J., Bednarczyk M. (2012). Influence of *in ovo* prebiotic and synbiotics administration on meat quality of broiler chickens. *Poultry Science,* 91, 2963-2969.

Makris D.P., Boskou G., Andrikopoulos N.K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal Of Food Composition And Analysi*, 20, 125-132.

Manach C., Scalbert A., Morand C., Remesy C., Jimenez L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79, 727–747.

Mancini R.A., Hunt M.C. (2005). Current research in meat color. *Meat Science*, 71, 100–121.

Mani-López E., Garcia H. S., López-Malo A. (2012). Organic acids as antimicrobials to control Salmonella in meat and poultry products. *Food Research International*, 45(2), 713–721.

Marouani N., Chahed A., Hedhili A., Hamdaoui M.H. (2007). Both aluminium and polyphenols in green tea decoction (Camelia sinensis) affect iron status and haematological parameters in rats. *European Journal of Nutrition*, 46, 453-459.

MartinCarron N., GarciaAlonso A., Goni I., SauraCalixto F. (1997). Nutritional and physiological properties of grape pomace as a potential food ingredient. *American Journal of Enology and Viticulture*, 48, 328-332.

Mattila P., Kumpulainen J. (2001). Coenzymes Q9 and Q10: Contents in Foods and Dietary Intake. *Journal of Food Compostion and Analysis*, 14, 409-417.

Maxwell S., Thorpe G. (2000). Impact of red wine on antioxidant status in vivo. *European Heart Journal*, 21, 1482–5.

Mayer R., Stecher G., Wuerzner R., Silva R.C., Sultana T. (2008). Proanthocyanidins: target compounds as antibacterial agents *Journal of Agricultural and Food Chemistry*, 56, 6959–6966.

Mead G.C. (1989). Microbes of the avian cecum: types present and substrates utilized. *The Journal of experimental zoology. Supplement*, 3, 48–54.

Mead G.C. (2004). Microbiological quality of poultry meat: a review. *Revista Brasileira de Ciência Avícola*, 6 (3) 135-142

Mendes J.A.S., Prozil S.O., Evtuguin D.V., Lopes L.P.C. (2013). Towards comprehensive utilization of winemaking residues: Characterization of grape skins from red grape pomaces of variety Touriga Nacional. *Industrial Crops and Products*, 43, 25–32.

Mielche M.M., Bertelsen G. (1994). Approaches to prevention of warmedover flavour. *Trends in Food Science & Technology*, 5, 322–327.

Mielnik M.B., Olsen .E, Vogt G., Adeline D., Skrede G. (2006). Grape seed extract as antioxidant in cooked, cold stored turkey meat. *LWT–Food Science and Technology*, 39, 191–198.

Mikova K. (2001). The regulation of antioxidants in food. In J. Pokorny, N. Yanisshlieva, & M. Gordon (Eds.), Antioxidants in foods. Cambridge, UK: Woodhead Publishing Ltd. (pp. 266–283).

Miller J.K., Brzexinska-Slebodzinska E., Madsen F.C. (1993). Oxidative stress, antioxidant, and animal function. *Journal of Dairy Science*, 76 (9), 2812–2823.

Min B., Ahn D.U. (2005). Mechanism of lipid peroxidation in meat and meat products-A review. *Food Science and Biotechnology*,14(1), 152-163.

Min B., Nam K.C., Cordray J., Ahn D.U. (2008). Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. *Journal of Food Science*, 73(6), C439-46.

Mitsumoto M. (2000). Dietary delivery versus exogenous addition of antioxidants. In: Antioxidants in muscle foods. Ed. E. Decker, C. Faustman, & C. J. Lopez-Bote. New York. Pages 315–328.

Mohamed H.M.H., Mansour H.A. (2012). Incorporating essential oils of marjoram and rosemary in the formulation of beef patties manufactured with mechanically deboned poultry meat to improve the lipid stability and sensory attributes. *Food Science and Technology*, 45, 79–87.

Monagas M., Bartolomé B., Gómez-Cordovés C. (2005). Updated knowledge about the presence of phenolic compounds in wine. *Critical Reviews in Food Science and Nutrition*, 45, 85 – 118.

Monagas M., Hernandez-Ledesma B., Garrido P., Martin-alvarez P.J., Gomez-Cordoves C., Bartolome B. (2005). Quality assessment of commercial dietary antioxidant products from Vitis vinifera L. grape seeds. *Nutrition and Cancer*, 53, 244–254.

Monagas M., Urpi-Sarda M, Sánchez-Patán F., Llorach R., Garrido I., Gómez-Cordovés C. (2010). Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food Function*, 1, 233-253.

Monaghan P., Metcalfe N.B., Torres R. (2009). Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters*, 12:75–92.

Monino I., Martínez C., Sotomayor J.A., Lafuente A., Jordán M.J. (2008). Polyphenolic transmission to segureno lamb meat from ewes' diet supplemented with the distillate from rosemary (Rosmarinus officinalis) leaves. *Journal of Agricultural and Food Chemistry*, 56, 3363–3367.

Montealegre P.R., Peces R.R., Vozmediano J.L.C., Gascueña J.M., Romero G. (2006). Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *Journal of Food Composition and Analysis*, *19*, 687–693.

Montreau F.R. (1972). Sur le dosage des compose´ s phe´ noliques totaux dans les vins par la methode Folin-Ciocalteau. *Connaissance Vigne Vin*, 24, 397–404.

Moreno-Labanda J.F., Mallavia R., Pérez-Fons L., Lizama V., Saura D., Micol V. (2004). Determination of piceid and resveratrol in Spanish wines deriving from Monastrell (*Vitis vinifera* L.) grape variety. *Journal of Agricultural and Food Chemistry*, *52*, 5396–5403.

Morrissey P.A., Buckley D.J., Gavin K. (2000). Vitamin E and the oxidation stability of pork and poultry. in Antioxidants in muscle foods. Ed. E. Decker, C. Faustman, & C. J. Lopez-Bote. New York. Pages 263–287.

Morrissey P.A., Sheehy P.J.A., Galvin K., Kerry J.P., Buckley D.J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49, S73–S86.

Mothershaw A.S., Gaffer T., Kadim I., Guizani N., Al-Amri I., Mahgoub O., Al-Bahry S. (2009). Quality characteristics of broiler chicken meat on salt at different temperatures. *International Journal of Food Properties*, 12, 681-690.

Muchenje V., Dzama K., Chimonyo M., Raats J.G., Strydom P.E. (2008). Meat quality of Nguni, Bonsmara and Angus steers raised on natural pasture in the Eastern Cape, South Africa. *Meat Science*, 79, 20-28.

Muchenje V., Dzama K., Chimonyo M., Strydom P.E., Hugo A., Raats J.G. (2009a). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chemistry*, 112, 279-289.

Muchenje V., Dzama K., Chimonyo M., Strydom P.E., Raats J.G. (2009b). Relationship between stress responsiveness and meat quality in three cattle breeds. *Meat Science*, 81, 653-657.

Mukai K., Mitani S., Ohara K., Nagaoka S. (2005). Structure-activity relationship of the tocopherol-regeneration reaction by catechins. *Free Radical Biology and Medicine*, 38, 1243–1256.

Mukai K., Sawada K., Kohno Y., Terao J. (1993). Kinetic study of the prooxidant effect of tocopherol. Hydrogen abstraction from lipid hydroperoxides by tocopheroxyls in solution. *Lipids*, 28, 747–752.

Murphy A., Kerry J.P., Buckley J., Gray R. (1998). The antioxidation properties of rosemary oleoresin and inhibition of off-flavors in precooked roast beef slices. *Journal of the Science of Food and Agriculture*, 77, 235-243.

Murray J.M., Delahunty C.M., Baxter I.A. (2001). Descriptive sensory analysis: past, present and future. *Food Research International*, 34, 461–471.

Murthy K.N.C. (2001). Evaluation of antioxidant activity of pomegranate (Punicagranatum) and grapes (Vitis vinifera), a thesis, Rajeev Gandhi Univ. of Health Science, (Banglore, India, 2001).

Na H.K., Surh Y.J. (2008). Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. *Food and Chemical Toxicology*, 46, 1271–1278.

Naczk M., Shahidi F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054, 95–111.

Nagata H., Takekoshi S., Takagi T., Honma T., Watanabe K. (1999). Antioxidative action of flavonoids, quercetin and catechin, mediated by the activation of glutathione peroxidise. *The Tokai journal of experimental and clinical medicine*, 24, 1–11.

Nagy S. (1980) Vitamin C Contents of Citrus Fruit and their Products: A Review. *Journal of Agricultural and Food Chemistry*, 28, 8-18.

Naidoo V., Mogaw L.J., Bisschop S.P.R., Duncan N., Eloff J.N. (2008). The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. *Veterinary Parasitology*, 153, 214–219.

Nair P.P. (1972). Vitamin E and metabolic regulation. *Annals of the New York Academy of Sciences*, 203, 53–61.

Narciso-Gaytán C., Shin D., Sams A.R., Bailey C.A., Miller R.K., Smith S.B., Leyva-Ovalle O.R., Sánchez-Plata M.X. (2010). Dietary lipid source and vitamin E effect on lipid oxidation stability of refrigerated fresh and cooked chicken meat. *Poultry Science*, 89, 726-2734.

Nassiri-Asl M., Hosseinzadeh H. (2009). Review of the pharmacological effects of *Vitis vinifera* (grape) and its bioactive compounds. *Phytotherapy Research*, *23*, 1197–1204.

Natella F., Nardini M., Giannetti I., Dattilo C., Scaccini C. (2002). Coffee drinking influences plasma antioxidant capacity in humans. *Journal of Agricultural and Food Chemistry*, 50, 6211–6.

Naveena B.M., Sen A.R., Vaithiyanathan S., Babji Y., Kondaiah N. (2008) Comparative efficacy of pomegranate juice, pomegranate rind powder and BHT in cooked chicken patties. *Meat Science*, 80, 1304–1308.

Naveena B.M., Sen A.R., Vaithiyanathan S., Babji Y., Kondaiah N. (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder and BHT in cooked chicken patties. *Meat Science*, 80, 1304–1308.

Nazni.P, Dharmaligam.R. (2013). Isolation And Separation Of Phenolic Compound From Coriander Flowers. *International Journal of Agricultural and Food Science*, 4(1), 13-21.

Negro C., Tommasi L., Miceli A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology*, *87*, 41–44.

Nerantzis E.T., Tartaridis P. (2006). Integrated enology-utilization of winery wastes for the production of high added value products. *e-Journal of Science and Technology*, 1, 79–89.

Newell-McGloughlin M., Burke J. (2014) .Regulatory challenges to commercializing the products of Ag Biotech. Encyclopedia of Agriculture and Food Systems, Second Edition, Neal K. Van Alfen, University of California, Davis, CA, USA, pp. 29.

Nijveldt R.J., van Nood .E, van Hoorn D.E.C., Boelens P.G., van Norren K., van Leeuwen P.A.M. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*, 74, 418–25.

Niki E. (2014) Antioxidants: basic principles, emerging concepts, and problems. *Biomedical Journal*, 37, 106-111.

Nissen L.R., Mansson L., Bertelsen G., Huynh-Ba T. And Skibsted L.H. (2000) Protection of dehydrated chicken meat by natural antioxidants as evaluated by electron spin resonance spectrometry. *Journal of Agricultural and Food Chemistry*, 48, 5548-5556.

NRC, National Research Council (1994) Nutrient Requirements of Poultry. 9th revised ed. The National Academies Press; Washington, DC, USA: pp. 1–157.

O'Grady M.N., Carpenter R., Lynch P.B., O'Brien N.M., Kerry J.P. (2008). Addition of grape seed extract and bearberry to porcine diets: Influence on quality attributes of raw and cooked pork. *Meat Science*, 78, 438-446.

Obst B.S., Diamond J.M. (1989). Interspecific variation in sugar and amino acid transport by the avian cecum. *Journal of experimental zoology. Supplement*, 3, 117–126.

OIV (2012). Organisation Internationale de la Vigne et du Vin. Retrieved September, from http://www.oiv.int/oiv/info/es

OIV (2014). Organisation Internationale de la Vigne et du Vin. Retrieved December,f rom http://www.oiv.int/oiv/info/es_OIV_Press_Conference_23_October_2014

Oliveira D.A., Salvador A.A.A.S., Smânia E.F.A., Maraschin M., Ferreira S.R.S. (2013). Antimicrobial activity and composition profile of grape (*Vitis vinifera*) pomace extracts obtained by supercritical fluids. *Journal of Biotechnology*, *164*, 423–432.

Olsen P., Meyer O., Bille N., Wurtzen G. (1986). Carcinogenicity study on butylated hydroxytoluene (BHT) in Wistar rats exposed in utero. *Food and Chemical Toxicology*, 24, 1-12.

Özkan G., Sagdic O., Baydar N.G., Kurumahmutoglu Z. (2004). Antibacterial activities and total phenolic contents of grape pomace extracts. Journal of the *Science of Food and Agriculture*, 84, 1807–1811.

Packer L., Weber S.U., Rimbach G. (2001). Molecular aspects of a-tocotrienol antioxidant action and cell signalling. *Journal of Nutrition*, 131 (Suppl.), 369–373.

Padda M.S., Picha D.H. (2008) Effect of low temperature storage on phenolics composition and antioxidant activity of sweet potatoes. *Postharvest Postharvest Biology and Technology*, 47, 176–180.

Palmieri B., Sblendorio V. (2007). Oxidative stress tests: Overview on reliability and use Part II. *European Review for Medical and Pharmacological Sciences*, 11, 383–399.

Papadopoulou C., Soulti K., Roussis I.G. (2005). Potential antimicrobial activity of red and white wine phenolic extracts against strains of Staphylococcus aureus, Escherichia coli and Candida albicans. *Food Technology and Biotechnology*, 43, 41–46.

Park S.J., Yoo S.O. (1999). Effects of supplementation of Chinese medicine refuse on performance and physiology in broiler chicks. Korea. *Jornal of Poultry Science*, 26, 195-201.

Passamonti S., Terdoslavich M., Franca R., Vanzo A., Tramer F., Braidot E., Petrussa E., Vianello A. (2009). Bioavailability of falvonoids. A review of their membrane tran sport ans the function of bilitranslocase in animal and plant organisms. *Current Drug Metabolism*, 10, 396-394.

Pastrana-Bonilla E., Akoh C.C., Sellappan S., Krewer G. (2003). Phenolic content and antioxidant capacity of muscadine grapes. *Journal of Agricultural and Food Chemistry*, 51, 5497-5503

Pazos M., Gallardo J.M., Torres J.L., Medina I. (2005). Activity of grape polyphenol as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chemistry*, 92, 547–557.

Pearson A.M., Love J.D., Shorland F. B. (1977). 'Warmed-over' flavour in meat. *Advances in Food Research*, 23, 1-74.

Pellerin P., Cabanis J.C. (2003). Los Glúcidos. In: C. Flanzy, Enología fundamentos científicos y tecnológicos Madrid, Spain: Mundi-Prensa., pp. 76-93.

Pennacchia C., Ercolini D., Villani F. (2011). Spoilage-related microbiota associated with chilled beef stored in air or vakuum pack. *Food Microbiology*, 28, 84-93.

Perez-Alvarez J.A., Sendra-Nadal E., Sanchez-Zapata E.J., Viuda-Martos M. (2010). Poultry flavour: General aspects and applications, in: Guerrero-Legarreta

I., Hui Y.H. (Eds) Handbook of Poultry Science and Technology: Secondary Processing, 2, 339-357 (New Jersey, John Wiley & Sons).

Perlo F., Bonato P., Fabre R., Teira G., Tisocco O. (2010). Meat quality evaluation of broiler breast fillets affected by aging time and marination. *International Journal of Poultry Science*, 9, 1063-1068.

Perron N.R., Brumaghim J.L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochemistry and Biophysics*, 53, 75–100.

Perumalla A.V.S., Hettiarachchy N.S. (2011). Green tea and grape seed extracts—Potential applicationsin food safety and quality. *Food Research International*, 44, 827–839.

Petracci M, Mudalal S., Soglia F., Cavani C. (2015). Meat quality in fast-growing broiler chickens. *World's Poultry Science Journal*, 71, 02, 363-374.

Petracci M., Betti M., Bianchi M., Cavani C. (2004). Color variation and characterization of broilers breast meat during processing in Italy. *Poultry Science*, 83, 2086-2092.

PFA (2008). Prevention of Food Adulteration. Pfa seventh amendment rules. The Universal law publishing co. pvt. Ltd. pp 102-103.

Pietrasik Z., Janz .JA.M. (2009). Influence of freezing and thawing on the hydration characteristics, quality, and consumer acceptance of whole muscle beef injected with solutions of salt and phosphate. *Meat Science*, 81 (3), 523−532.

Pietta PG. (2000). Flavonoids as antioxidants. *Journal of Natural Products*, 63, 1035–42.

Pikul J., Leszczynski D.E., Kummerow F.A. (1984). Relative role of phospholipids, triacylglycerols, and cholesterol esters on malonaldehyde formation in fat extracted from chicken meat. *Journal of Food Science*, 49, 704-708.

Pinelo M., Arnous A., Meyer A.S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science and Technology*, *17*(11), 579-590.

Ping L., Brosse N., Chrusciel L., Navarrete P., Pizzi A. (2011). Extraction of condensed tannins from grape pomace for use as wood adhesives. *Industrial Crops and Products*, 33 (1), 253-257.

Pokorny J. (2007). Are natural antioxidants better – and safer –than synthetic antioxidants? *European Journal of Lipid Science and Technology*, 109, 629–642.

Poudel, P.R.; Tamura, H.; Kataoka, I.; Mochioka, R. (2008). Phenolic compounds and antioxidant activities of skins and seeds of five wild grapes and two hybrids native to Japan. *Journal of Food Composition and Analysis*, 21, 622–625.

Pozuelo M.J., Agis-Torres A., Hervert-Hernández D., López-Oliva M.E., Múnoz-Martínez E., Rotger R., Goñi I. (2012). Grape antioxidant dietary fiber stimulates Lactobacillus growth in rat cecum. *Journal of Food Science*, 77, H59-H52.

Pridham J.B. (1960). Phenolics in Plants in Health and Disease; Pergamon Press: New York, NY, USA.

Prieur C., Rigaud J, Cheynier V., Moutounet M. (1994). Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry*, *36*, 781-784.

Probst Y. (2009). Nutrient composition of chicken meat. *RIRDC* Publication 8/210.

Procházková D., Boušová I., Wilhelmová N. (2011). Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 82, 513–523.

Püssa T., Floren J., Kuldkepp P., Raal A. (2006). Survey of grapevine *Vitis vinifera* stem polyphenols by liquid chromatography-diode array detection-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, *54*, 7488–7494.

Quideau S., Deffieux D., Douat-Casassus C., Pouysegu L. (2011). Plant polyphenols: chemical properties, biological activities, and synthesis. *Angewandte Chemie International Edition*, 50, 586-621.

Rababah T.M., Ereifej K.I., Al-Mahasneh M.A., Al-Rababah M.A. (2006). Effect of plant extract on physicochemical properties of chicken breast meat cooked using conventional electric oven or microwave. *Poultry Science*, 85, 148-154.

Rababah T.M., Navam S., Horax R. (2004). Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. Journal of Agricultural and *Food Chemistry*, 52, 5183.

Regenhard P., Nakov D., Sauerwein H. (2014). Applicability of a spectrophotometric method for assessment of oxidative stress in poultry. *Macedonian Veterinary Review*, 37 (1), 43-47.

Rekitt, M., Sauerwein, H., Andresen, U. (2002). Orientierende Untersuchungen zum oxidativen Stress bei Reitpferden. *Tierarztliche umschau*, 57,471- 48.

Requena T., Monagas M., Pozo-Bayón M.A., Martín-Álvarez P.J., Bartolomé B., Campo R., Ávila M., Martínez-Cuesta M.C., Peláez C., Moreno-Arribas M.V. (2010). Perspectives of the potential implications of wine polyphenols on human oral and gut microbiota. *Trends in Food Science and Technology*, 21, 332-344.

Ribéreau-Gayon P., Dubourdieu D., Donèche B., Lonvaud A. (2004). Traité d'Oenologie. Paris: Dunod.

Ricardo da Silva J.M., Rigaud, J., Cheynier V., Cheminat A., Moutounet M. (1991). Procyanidin dimers and trimers from grape seeds. *Phytochemistry* , *30*, 1259-1264.

Ricardo da Silva J.M., Rosec J. Ph., Bourzeix M., Mourgues J., Moutounet M. (1992). Dimer and trimer procyanidins in Carignan and Mourve`dre grapes and red wines. *Vitis*, *31*, 55-63.

Rice-Evans C.A., Miller N.J. (1996). Antioxidant activities of flavonoids as bioactive components of food. *Biochemical Society Transactions*, 24, 790–795.

Rijswijk W.V., Frewer L.J. (2008). Consumer perceptions of food quality and safety and their relation to traceability. *British Food Journal*, 110, 1034-1046.

Rinttila T., Apajalahti J. (2013). Intestinal microbiota and metabolites implications for broiler chicken health and performance. *Journal of Applied Poultry Research*, 22, 647–658.

Ripoll G., González-Calvo L., Molino F., Calvo J.H., Joy M. (2013). Effects of finishing period length with vitamin E supplementation and alfalfa grazing on carcass color and the evaluation of meat color and the lipid oxidation of light lambs. *Meat Science*, 93, 906–913.

Rockenbach I.I., Gonzaga L.V., Rizelio V.M., Gonçalves A.E.S.S., Genovese M.I., Fett R. (2011a). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. *Food Research International*, *44*, 897–901

Rockenbach I.I., Rodrigues E., Gonzaga L.V., Caliari V., Genovese M.I., Gonçalves A.E.S.S., Fett R.(2011b). Phenolic compounds content and antioxidant

activity in pomace from selected red grapes (*Vitis vinifera* L. and *Vitis labrusca* L.) widely produced in Brazil. *Food Chem*istry, *127*, 174–179.

Rodríguez-Carpena J.G., Morcuende D., Estévez M. (2011). Avocado by-products as inhibitors of colour deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage. *Meat Science*, 89, 166–173.

Rodríguez-Vaquero M.J., Alberto M.R., Manca de Nadra M.C. (2007). Antibacterial effect of phenolic compounds from different wines. *Food Control*, 18, 93–101.

Rojas M.C, Brewer M.S. (2008) Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. *Journal of Food Quality*, 31, 173-188.

Rojas M.C., Brewer M.S. (2007). Effect of natural antioxidants on oxidative stability of cooked, refrigerated beef and pork. *Journal of Food Science*, 72, S282-S288.

Rondeau P., Gambier F., Jolibert F., Brosse N. (2013). Compositions and chemical variability of grape pomaces from French vineyard. *Industrial Crops and Products*, 43, 251–254.

Rotava R., Zanella I., Gonçalves Campos E., Pain A., Palermo Manfron M., Hartz Alves S. (2009) Subproductos da uva comopromotores decrescimento emdietas de frangos de corte de 1 a 21 dias de edad. *Acta Scientiarum. Animal Sciences. Maringá*, 31, 145–150.

Ruberto G., Renda A., Daquino C., Amico V., Spatafora C., Tringali C., Tommasi N. (2007). Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars. *Food Chem*istry, *100*, 203–210.

Ruiz-Capillas C., Jimenez Colmenero F. (2008). Application of flow injection analysis for determining sulphites in food and beverages: A review. *Food Chemistry*, 112, 487–493.

Ruiz-Capillas C., Nollet L. (2016). Book: Flow Injection. Analysis of Food Additives. Edit by Ruiz-Capillas C. and Nollet L. CRCpress Taylor and Francis group.

Rule D.C., Broughton K.S., Shellito S.M., Maiorano G. (2002). Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk and chicken. *Journal of Animal Science*, 80, 1202-1211.

Ruxton C.H.S., Reed S.C., Simpson M.J.A., Millington K.J. (2007). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of Human Nutrition and Dietetics*, 20, 275-285.

Sahin K., Akdemir F., Orhan C. (2010). Effect of dietary resveratrol supplementation on egg production and antioxidante status. *Poultry Science*, 89, 1190-1198.

Saláková A., Straková E., Válková V., Buchtová H., Steinhauserová I. (2009). Quality indicators of chicken broiler raw and cooked meat depending on their sex. *Acta Veterinaria Brno*, 78, 497-504.

Salanitro J.P., Fairchilds I.G., Zgornicki Y.D. (1974). Isolation, culture characteristics, and identification of anaerobic bacteria from the chicken cecum. *Journal of Applied Microbiology*, 27, 678–687.

Salobir J., Rezar V., Pajk T., Levart A. (2005). Effect of nucleotide supplementation on lymphocyte DNA damage induced by dietary oxidative stress in pigs. *Animal Science*, 81,135–140.

Sample S. (2013). Oxidation and antioxidants in fish and meat from farm to fork. In I. Muzzalupo (Ed.), Food industry (114–144). Croatia: InTech Publishing.

Sánchez-Alonso I., Borderías A.J. (2008). Technological effect of red grape antioxidant dietary fibre added to minced fish muscle. *International Journal of Food Science and Technology*, 43, 1009–1018.

Sánchez-Alonso I., Jiménez-Escrig A., Saura-Calixto F., Borderías A.J. (2008). Antioxidant protection of white grape pomace on restructured fish products during frozen storage. *LWT-Food Science and Technology*, 41, 42–50.

Sánchez-Moreno C. (2002). Compuestos polifenólicos: estructura y classificación: presencia en alimentos y consumo: biodisponibilidad y metabolismo. *Alimentaria*, 329, 19-28.

Sanchez-Muniz F.J., Olivero-David R., Triki M., Salcedo L., Gonzalez-Munoz M.J., Cofrades S., Ruiz-Capillas C., Jiménez-Colmenero F., Benedi J. (2012). Antioxidant activity of Hypericum perforatum L. extract in enriched n-3 PUFA pork meat systems during chilled storage. *Food Research International*, 48(2), 909–915.

Sánchez-Patán F., Cueva ., Monagas M., Walton G.E., Gibson G.R., Martín-Álvarez P.J., Moreno-Arribas M.V., Bartolomé B. (2012). Gut microbial

catabolism of grape seed flavan-3-ols by human faecal microbiota. Targeted analysis of precursor compounds, intermediate metabolites and end products. *Food Chemistry*, 131 (1), 337–347.

Sant'Ana A.S., Franco B.D.G.M., Schaffner D.W. (2014). Risk of infection with Salmonella and Listeriamonocytogenes due to consumption of ready-to-eat leafy vegetables in Brazil. *Food Control*, 42, 1–8.

Santé V., Fernandez X., Monin G., Renou J.P. (2001). Nouvelles méthodes de mesure de la qualité de la viande de volaille. *INRA Productions animals*, 14 (4), 247-254.

Santiago H.L., Denbow D.M., Emmerson D.A., Denbow C., Graham P., Hohenboken W. (2005). Effects of strain, plane of nutrition, and age at slaughter on performance and meat quality traits of broilers. *Poultry Science*, 84, 1, 128.

Santos V.A.C., Silva A.O., Cardoso J.V.F., Silvestre A.J.D., Silva S.R., Martins C., Azevedo J.M.T. (2007). Genotype and sex effects on carcass and meat quality of suckling kids protected by the PGI ‘‘Cabrito de Barroso’’. *Meat Science*, 75, 725-736.

Sarantópoulos C., Soler R. (1991). Embalagens com atmosfera modificada/controlada. *Revista nacional da carne*, 209, 32-42.

Sarantópoulos C.I.G.L., De Oliveira L.M., Canavesi E. (2001). Carnes, aves, pescados e derivados. Requisitos de conservação de alimentos em embalagens flexíveis. *Campinas*, SP: CETEA, 151-174-

Sasse A., Colindres P., Brewer M.S. (2009) Effect of natural and synthetic antioxidants on the oxidative stability of cooked, frozen pork patties. Journal of *Food Science*, 74, S30-S35.

Sauerwein H., Schmitz S., Hiss S. (2005). The acute phase protein haptoglobin and its relation to oxidative status in piglets undergoing weaning induced stress. *Redox Report*, 10 (6), 295-302.

Saunders M. S., Takeda F., Bates R.P., Regulski F. (1982). Composition and Utilization of Florida Grape Pomace. *Florida State Horticultural Society*, 95, 107-109.

Saura-Calixto F. (2011) Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *Journal of Agricultural and Food Chemistry*, 59, 43-9.

Sayago-Ayerdi S.G., Brenes A., Goñi I. (2009a). Antioxidative effect of dietary grape pomace concentrate on lipid oxidation of chilled and long-term frozen stored chicken patties. *Meat Science*, 83, 528–533.

Sayago-Ayerdi S.G., Brenes A., Goñi I. (2009b) Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. *Food Science and Technology*, 42, 971–976.

Scalbert A. (1991). Antimicrobial properties of tannins. Phytochemistry, 30, 3875–3883.

Scalbert A., Williamson G. (2000). Dietary intake and bioavailability of polyphenols. **Journal of Nutrition**, 130, 2073–2085.

Schieber A., Stintzing F.C., Carle R. (2001). By-products of plant food processing as a source of functional compounds-recent developments. *Trends in Food Science & Technology*, 12, 401–413.

Schoefer L., Mohan R., Braune A., Birringer M., Blaut M. (2002). Anaerobic C-ring cleavage of genistein and daidzein by Eubacterium ramulus. *FEMS Microbiology Letters*, 208, 197–202.

Schroepfer G.J. (2002). Oxysterols: Modulators of cholesterol metabolism and other processes. *Physiological Reviews*, 80, 361–554.

Seabra R.M., Andrade P.B., Valentao P., Fernandes E., Carvalho F., Bastos M.L. (2006) Biomaterials from aquatic and terrestrial organisms. Eds.: Science Publishers; Enfield, NH, USA.

Seal B.S., Lillehoj H.S., Donovan D.M., Gay C.G. (2013). Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. *Animal Health Research Reviews*, 14, 78–87.

Selani M.M., Contreras-Castillo C.J., Shirahigue L.D., Gallo C.R., Plata-Oviedo M., Montes-Villanueva N.D. (2011). Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. *Meat Science*, 88, 397-403.

Selma M. V., Espín J. C., Tomás-Barberán F. A. (2009). Interaction between phenolics and gut microbiota: role in human health. *Journal of Agricultural and Food Chemistry*, 57, 15, 6485–6501.

Sgorlon S., Stradaioli G., Zanin D., Stefanon B. (2006). Biochemical and molecular responses to antioxidant supplementation in sheep. *Small Ruminant Research*, 74, 17-33.

Shahidi F. (1989). Flavor of cooked meats, in: Teranishi R., Buttery R.G., Shahidi, F. (Eds) Flavor Chemistry: Trends and Developments, pp. 188-201 (Washington, American Chemical Society).

Shahidi F., Janitha P.K, Wanasundara P.D. (1992) Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, 32, 67-103.

Shahidi F., Miraliakbari H. (2005). Omega-3 fatty acids in health and disease: Part 2 - Health effects of omega-3 fatty acids in autoimmune diseases, mental health, and gene expression. *Journal of Medicinal Food*, 8, 133-148.

Shahidi F., Zhong,Y. (2005). Antioxidants: Regulatory status. In F. Shahidi (Ed.), Bailey's industrial oil and fat products. New Jersey: John Wiley & Sons, Inc. (pp. 491–511).

Shaker E.S. (2006). Antioxidative effect of extracts from red grape seed and peel on lipid oxidation in oils of sunflower. *Journal of Food Science and Technology*, 39, 883–892.

Shalini S., Dorstyn L., Wilson C., Puccini J., Ho L. Kumar S. (2012). Impaired antioxidant defence and accumulation of oxidative stress in caspase-2-deficient mice. Cell Death and Differentiation, 19, 1370–1380.

Sharma A.K., Gangwar M., Tilak R., Nath G., Sinha A.S.K., Tripathi Y.B., Kumar D. (2012). Comparative in vitro antimicrobial and phytochemical evaluation of methanolic extract of root, stem and leaf of Jatropha curcas Linn. *Pharmacognosy Journal*, 4(30), 34–40.

Sharoni Y., Danilenko M., Levy J. (2000) Molecular Mechanisms for the Anticancer Activity of the Carotenoid Lycopene. *Drug Development Research*, 50, 448-456.

Shi J., Yu J., Pohorly J.E., Kakuda Y. (2003). Polyphenolics in grape seeds-Biochemistry and Functionality. *Journal of Medicinal Food*, 6(4), 291-299

Shirahigue L.D., Plata-Oviedo M., Alencar S.M., Regitano-D'Arce M.A.B., Vieira T.M.F.S., Oldoni T.L.C., Contreras-Castillo C.J. (2010) Wine industry residue as antioxidant in cooked chicken meat. *International Journal of Food Science and Technology*, 45, 863-870.

Shirzadegan K., Falahpour P. (2014). The physicochemical properties and anti-oxidative potential of raw thigh meat from broilers fed a dietary medicinal herb extract mixture. *Open Veterinary Journal*, 4, 2, 69-77.

Sijben J.W., Sharama J.W., Nieuwland M.G., Hovenier R., Beynen A.C., Verstegen M.W., Parmentier H.K. (2002). Interactions of dietary polyunsaturated fatty acid and vitamin E with regard to vitamin status, fat composition and antibody responsiveness in layer hens. *British Poultry Science*, 43, 297-305.

Silván J.M., Mingo E., Hidalgo M., Pascual-Teresa S., Carrascosa A.V., Martinez-Rodriguez A.J. (2013). Antibacterial activity of a grape seed extract and its fractions against Campylobacter spp. *Food Control*, 29, 25–31.

Simeonovová J. (1999). Technology of Poultry, Eggs and other Minor Animal Products. MZLU, Brno. 247.

Simitzis P.E., Deligeorgis S.G., Bizelis J.A., Dardamani A., Theodosiou I., Fegeros K. (2008). Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Science*, 79, 217–223.

Sims M.D., Dawson K.A., Newman K.E., Spring P., Hooge D.M. (2004). Effects of dietary mannan oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. *Poultry Science*, 83, 1148–1154.

Singleton V.L., Orthofer R., Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.

Siriwan P., Bryden W.L., Mollah Y., Annison E.F. (1993). Measurements of endogenous amino acid losses in poultry. *British Poultry Science*, 34, 939–949.

Sirk T.W., Brown E.F., Fiedman M., Sum A.K. (2009). Molecular binding of catechins to biomembranes: relationship to biological activity. *Journal of Agricultural and Food Chemistry*, 57, 6720–6728.

Sitz B.M., Calkins C.R., Feuz D.M., Umberger W.J., Eskridge K.M. (2005). Consumer sensory acceptance and value of domestic, Canadian, and Australian grass-fed beef steaks. *Journal of Animal Science,* 83, 2863-2868

Sklan D., Shachaf B., Baron J., Hurwitz S. (1978). Retrograde movement of digesta in the duodenum of the chick: extent, frequency, and nutritional implications. *Journal of Nutrition*, 108, 1485–1490.

Smet K., Raes K., Huyghebaert G., Haak L., Arnotus S., De Smet S. (2008). Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. *Poultry Science*, 87, 1682-1688.

Soobrattee M.A., Neergheena V.S., Luximon-Rammaa A., Aruomab O.I., Bahoruna T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 579, 200–213.

Souquet J.M., Cheynier V., Brossaud F., Moutounet M. (1996). Polymeric proanthocyanidins from grape skins. *Phytochemistry*, 43, 509-512.

Souquet J.M., Labarbe B., Le Guerneve C., Cheynier V., Moutounet M. (2000). Phenolic composition of grape stems. *Journal of Agricultural and Food Chemistry*, 48, 1076–1080.

Spranger I., Sun B.S., Mateus A.M., de Freitas V., Ricardo-da-Silva A.M. (2008). Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. *Food Chemistry*, 108, 519–532.

Steinhauser L., Benes J., Ingr I. (2000). Produkce masa. Last, Tišnov, 464 s.

Stone H., Bleibaum R. N., Thomas H. A. (2012). Sensory Evaluation Practices, Waltham, MA : Academic Press.

Stoupi S., Williamson G., Drynan J.W., Barron D., Clifford M.N. (2010). Procyanidin B2 catabolism by human fecal microflora: partial characterization of dimeric intermediates. *Archives of Biochemistry and Biophysics*, 501, 73-78.

Su M., Silva J. (2006). Antioxidant activity, anthocyanins, and phenolics of rabbit eye blueberry (Vaccinium ashei) by-products as affected by fermentation. Food Chemistry. *Food Research International*, 97 (3), 447-451.

Suchý P., Jelínek P., Straková E., Hucl J. (2002). Chemical composition of muscles of hybrid broiler chickens during prolonged feeding. *Czech Journal of Animal Science*, 47(12), 511–518.

Sun B.S., Pinto T., Leandro M.C., Ricardo da Silva J. M., Spranger M.I. (1999). Transfer of catechins and proanthocyanidins from solid parts of the grape cluster into wine**.** *American Journal of Enology and Viticulture*, 50, 179-184.

Surai P.F. (2002) Natural antioxidants in Avian nutrition and reproduction. Notthingam University Press, Notthingam UK.

Surai P.F. (2006). Selenium in Nutrition and Health. Notthingam University Press, Notthingam UK.

Surai P.F. (2014). Polyphenol compounds in the chicken/animal diet: from the past to the future. *Journal of Animal Physiology and Animal Nutrition*, 98, 19–31.

Surai P.F. (2016). Antioxidant Systems in Poultry Biology: Superoxide Dismutase. *Animal Nutrition*, 1, 8, 1-17.

Surai P.F., Fisinin V.I. (2015). Antioxidant-Prooxidant Balance in the Intestine: Applications in Chick Placement and Pig Weaning. *Journal of Veterinary Science & Medicine*, 3 (1) 1-16.

Surai P.F., Sparks N.H.C. (2000). Tissue specific fatty acid and α-tocopherol profile in male chickens depending on dietary tuna oil and vitamin E provision. *Poultry Science*, 79, 1132-1142.

Taha F.A. (2003). "The Poultry Sector in Middle-income Countries and its Feed Requirements: The Case of Egypt". Outlook Report No. WRS03-02. Economic Research Service, USDA. Http://www.ers.usda.gov/ publications/WRS03/dec03/ wrs0302.

Teixeira A., Baenas N., Dominguez-Perles R., Barros A., Rosa E., Moreno D.A., Garcia-Viguera C. (2014). Natural bioactive compounds from winery by-products as health promoters: a review. *International Journal of Molecular Sciences*, 15, 15638-15678

Thorngate J.H., Singleton V. L. (1994). Localization of procyanidins in grape seeds. *American Journal of Enology and Viticulture*, 45 (2): 259-262

Torok V.A., Ophel-Keller K., Loo M., Hughes R.J. (2008). Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. *Applied and Environmental Microbiology*, 74, 783–791.

Torres J., Bobet B. (2001). New flavanol derivatives from grape (Vitis vinifera) byproducts antioxidant aminoethylthio-flavan-3-ol conjugates from a polymeric

waste fraction used as a source of flavanols. *Journal of Agricultural and Food Chemistry*, 49, 4627-34.

Toscano G., Riva G., Duca D., Pedretti E.F., Corinaldesi F., Rossini G. (2013). Analysis of the characteristics of the residues of the wine production chain finalized to their industrial and energy recovery. *Biomass and Bioenergy*, 55, 260-267.

Touraille C. (1994). Influence of muscle characteristics on sensory properties of meat. *Rencontres autour des Recherches sur les Ruminants*, 1, 169 – 176.

Travaglia F., Bordiga M., Locatelli M., Coïson Arlorio M. (2011). Polymeric proanthiocyanidins in skins and sedes of 37 Vitis vinífera L. cultivars: A methodological comparative study. *Journal of Food Science*, 76, C742-C749.

Triki M., Herrero A.M., Colmenero F.J., RuizCapillas C. (2013). Effect of preformed konjac gels, with and without olive oil, on the technological attributes and storage stability of merguez sausage. *Meat Science*, 93, 3, 351-360.

Trotti R., Carratelli M., Barbieri M., Micieli G., Bosone D., Rondanelli M., Bo P. (2001). Oxidative stress and a thrombophilic condition in alcoholics without severe liver disease. *Haematologica*, 86, 85-91.

Troy D.J., Kerry J.P. (2010). Consumer perception and the role of science in the meat industry. *Meat Science*, 86, 214-226.

Tsang C., Auger C., Mullen W., Bornet A., Rouanet J.M., Crozier A., Teissedre P.L. (2005). The absorption, metabolism and excretion of flavan-3-ols and procyanidins following the ingestion of a grape seed extract by rats. *British Journal of Nutrition*, 94, 170-181.

Tsao R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients*, *2*, 1231–1246.

Tzounis X., Vulevic J., Kuhnic G.G.C., George T., Leonczak J., Gibson C.R., Kwik-Uribe C., Spencer,J.P.E. (2008). Flavanol monomer-induced changes to the human faecal microflora. *British Journal of Nutrition*, 99, 782–792.

USDA (2015). United States Department of Agriculture. GAIN Report: EU-28 Poultry and Products Annual, EU-28 Broiler Production and Exports to Grow Again in 2016

Valceschini E. (2006). Poultry Meat Trends and Consumer Attitudes; Proceedings of the XII European Poultry Conference [CD-ROMs]; Verona, Italy. September 2006; Verona, Italy: Verona Fiere.

Valiente C., Arrigoni C., Esteban R.M., Amado R. (1995). Grape pomace as a potential food fiber. J*ournal of Food Science, 60*, 818–820.

Valsta L.M., Tapanainen H., Mannisto S. (2005). Meat fats in nutrition. A rewiew. *Meat Science,* 70, 525-530.

Van de Crommenacker J., Horrocks N.P.C., Versteegh M.A., Komdeur J., Tieleman B.I., Matson K.D. (2010). Effects of immune supplementation and immune challenge on oxidative status and physiology in a model bird: implications for ecologists. *Journal of Experimental Biology*, 213, 3527–3535.

van der Wielen P.W., Keuzenkamp D.A., Lipman L.J., van Knapen F., Biesterveld S. (2002). Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. *Microbial Ecology,* 44, 286–293.

Vergara-Salinas J.R., Bulnes P., Zuñiga M.C., Perez-Jimenez J., Torres J.L., Mateos-Martin M.L., Agosin E., Perez-Correa J.R. (2013). Effect of pressurized hot water extraction on antioxidants from grape pomace before and after enological fermentation. *Journal of Agricultural and Food Chemistry*, 61, 6929-6936.

Verhelst R., Schroyen M., Buys N., Niewold T. (2014). Dietary polyphenols reduce diarrhea in enterotoxigenic Escherichia coli (ETEC) infected post-weaning piglets. *Livestock Scien*ce, 160, 138–140.

Vermerris W., Nicholson R. (2006). Phenolic Compound Biochemistry. West Lafayette IN: Springer; pp: 1-34

Vidal S., Hayasaka Y., Meudec E., Cheynier V., Skouroumounis G. (2004). Fractionation of grape anthocyanin classes using multilayer coil countercurrent chromatography with step gradient elution. *Journal of Agricultural and Food Chemistry*, 52, 713–719.

Viveros A., Chamorro S., Pizarro M., Arija I., Centeno C., Brenes A. (2011). Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science*, 90, 566-578.

Voljc M., Frankic T., Levart A., Nemec M., Salobir J. (2011). Evaluation of different vitamin E recommendation and bioactivity of α-tocopherol isomers in broiler nutrition by measuring oxidative stress in vivo and the oxidative stability of meat. *Poultry Science*, 90, 78-88.

Vossen E., Ntawubizi M., Raes K., Smet K., Huyghebaert G., Arnouts S., De Smet S. (2011). Effect of dietary antioxidant supplementation on the oxidative status of plasma in broilers. *Journal of Animal Physiology and Animal Nutrition*, 95, 198–205.

Wallace R.J., Oleszek W., Franz C., Hahn I., Baser K.H.C., Mathe A., Teichmann K. (2010). Dietary plant bioactives for poultry health and productivity. *British Poultry Science*, 51, 461-487.

Wang M.L., Suo X., Gu J.H., Zhang W.W., Fang Q., Wang X. (2008). Influence of grape seed proanthocyanidin extract in broiler chickens: Effect on chicken coccidiosis and antioxidant status. *Poultry Science*, 87, 2273-2280.

Ward N.C., Croft K.D., Puddey I.B., Hodgson J. M. (2004). Supplementation with grape seed polyphenols results in increased urinary excretion of 3-hydroxyphenylpropionic acid, an important metabolite of proanthocyanidins in humans. *Journal of Agricultural and Food Chemistry*, 52, 5545–5549.

Waterman P.G., Mole S. (1994). Analysis of phenolic plant metabolites. In Methods in ecology (ed. J H Lawton and GE Likens), pp. 83–85. Blackwell, Oxford.

Wei S., Morrison M., Yu Z. (2013). Bacterial census of poultry intestinal microbiome. *Poultry Science*, 92, 671–683.

Wilfred V., Nicholson R. (2006). Phenolic compound biochemistry. Springer. Dordrecht, The Netherlands.

Wilkins L.J., Brown S.N., Phillips A.J., Warriss P.D. (2000). Variation in the colour of broilers breast fillets. *British Poultry Science*, 41, 308–312.

Williams R.J., Spencer J.P.E, Rice-Evans C. (2004). Flavonoids: Antioxidants or signaling molecules? *Free Radical Biology and Medicine*, 36, 838-849.

Williamson G., Manach C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *The American Journal of Clinical Nutrition*, 81, 243S–255S.

Windisch W., Schedle K., Plitzner C., Kroismayr A. (2008). Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science*, 86 (E. Suppl.), E140–E148.

Woelfel R.L., Owens C.M., Hirschler E.M., Martinez-Dawson R., Sams A.R. (2002). The characterization and incidence of pale, soft, and exudative broilers meat in a commercial processing plant. *Poultry Science*, 81, 579–584.

Wollgast J., Anklam E. (2000). Review on polyphenols in Theobroma cacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International*, 33, 423-447.

Wood J.D., Enser M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, 78, S49– S60.

Xia E.Q., Deng G.F., Ya-Jun Guo Y.J., Hua-Bin Li H.B. (2010). Biological activities of polyphenols from grapes. *International Journal of Molecular Sciences* ,11, 622-646.

Xiao R., Power R.F., Mallonee D., Crowdus C., Brennan K. M., Ao T., Pierce J. L., Dawson K. A. (2011). A comparative transcriptomic study of vitamin E and an algae-based antioxidant as antioxidative agents: Investigation of replacing vitamin E with the algae-based antioxidant in broiler diets. *Poultry Science*, 90, 136-146.

Yan L., Kim I.H. (2011). Effect of dietary grape pomace fermented by Saccharomyces boulardii on the growth performance, nutrient digestibility and meat quality in finishing pigs. *Asian-Australasian Journal of Animal Sciences*, 24, 1763-1770.

Yegani M., Korver D.R. (2008). Factors affecting intestinal health in poultry. *Poultry Science*, 87, 2052-2063.

Yilmaz E.E., Ozvural E.B., Vural H. (2011). Extraction and identification of proanthocyanidins from grape seed (Vitis vinifera) using supercritical carbon dioxide. *Journal of Supercritical Fluids*, 55, 924–928.

Yilmaz Y., Toledo R.T. (2004). Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin and gallic acid. *Journal of Agricultural and Food Chemistry*, 52, 255–260.

Yong H.I., Kim H.J., Jung S., Jayasena D.D., Bae Y.S., Lee S.K., Jo C. (2013). Effect of Dietary Supplementation of Wild Grape on the Antioxidative Potential of

the Breast and Leg Meat of Broilers. *Korean Journal of Food Science*, 33, 1, 83-88.

Young I., Woodside J. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology*, 54, 176–186.

Yuksel Z., Avci E., Erdem Y.K. (2010). Characterization of binding interactions between green tea flavonoids and milk proteins. *Food Chemistry*, 121, 450–456.

Zakeri A., Kashefi P. (2011). The comparative effects of five growth promoters on broiler chickens humoral immunity and performance. *Journal of Animal and Veterinary Advances*, 10, 1097–1101.

Zhang H.J., Jiang X.R., Mantovani G., Valdez A.E., Comi M., Alborali G., Savoini G., Dell'Orto V., Bontempo V. (2014). Modulation of plasma antioxidant activity in weaned pigs by plant polyphenols. *Italian Journal of Animal Science*, 13, 424-430.

Zhang W., Xiao S., Lee E.J., Ahn D.U. (2011). Consumption of oxidized oil increases oxidative stress in broilers and affects the quality of breast meat. *Journal of Agricultural and Food Chemistry*, 59(3), 969-674.

Zhang W., Xiao S., Samaraweera H., Lee E.J., Ahn, D.U. (2010). Improving functional value of meat products. *Meat Science*, 86, 15–31.

Zhao J., Wang J., Chen Y., Agarwal R. (1999). Anti-tumor-promoting activity of a polyphenolic fraction isolated from Grape Seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3′-gallate as the most effective antioxidant constituent. *Carcinogenesis*, 20, 1737– 1745.

Zhou B., Wu L.M., Yang L., Liu Z.L. (2005). Evidence for alphatocopherol regeneration reaction of green tea polyphenols in SDS micelles. *Free Radical Biology and Medicine*, 38, 78–84.

Zhu L., Zhang Y., Lu J. (2012). Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. *International Journal of Molecular Sciences*, *13*, 3492–3510.

Zhu Q.Y., Huang Y., Chen Z.Y. (2000). Interaction between flavonoids and α-tocopherol in human low density lipoprotein. *Journal of Nutritional Biochemistry*, 11, 14–21.

Zhu Q.Y., Huang Y., Tsang D., Chen Z.Y. (1999). Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechins. *Journal of Agricultural and Food Chemistry*, 47, 2020–5.

Zinoviadou K.G., Koutsoumanis K.P., Biliaderis C.G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science*, 82, 338–345.

LIST OF ABBREVIATIONS

4HR	4-hexylresorcinol
ABTS	2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
AFNOR	association francaise de normalisation
AIA	acid insoluble ash
AID	apparent ileal digestibility
AME	apparent metabolisable energy
ANOVA	one-way analysis of variance
BHA	butylated hydroxyl anisole
BHT	butylated hydroxytoluene
BSE	bovine spongiform encephalopathyù
CAT	catalase
CFU	colony-forming unit
CM	chroma meter
CP	crude protein
CSIC	consejo superior de investigaciones científicas
DFD	dark firm dry
DM	dry matter
DNA	deoxyribonucleic acid
DNPH	2,4 dinitrophenylhy-drazine
DP	polymerization degree
EC	european communities
EDTA	ethylenediaminetetraacetic acid
EU	european union
FA	fatty acid
FAO	food and agriculture organization
FC	folin–ciocalteu
FCR	feed conversion ratio
FDA	food and drug administration
FEDNA	fundación española para el desarrollo de la nutrición animal
FS	fermented grape skin

GA	gallic acid
GAE	gallic acid equivalent
GC	gas chromatograph
GI	gastro intestinal
GIT	gastro-intestinal tract
GP	grape pomace
GPx	glutathione peroxidase
GS	grape seed
GSE	grape seed extract
HPLC	high-performance (high-pressure) liquid chromatography
HPLC-MS	high performance liquid chromatography-mass spectrometry
HSD	honest significant difference
ICTAN	institute of food science, technology and nutrition
ISO	international organization for standardization
IU	international unit
KSF	kramer shear force
LAB	lactic acid bacteria
LC–MS	liquid chromatographic mass spectrometry
LC–MS/MS	liquid chromatography–tandem mass spectrophotometry
LDL	low density lipoprotein
MAP	modified atmosphere packaging
MDA	malondialdehyde
mDP	average polymerization degree
MRS	de man, rogosa, sharp agar
MS	mass spectrometry
n3 PUFA	omega-3 polyunsaturated fatty acids
NAD(P)H	nicotinamide adenine dinucleotide phosphate
NDGA	nordihydroguaiaretic acid
NRC	national research cuncil
OG	octyl gallate
PCA	plate count agar
PG	propyl gallate

PGE	polyphenolic grape extracts
PSE	pale soft exudative
PUFA	polyunsaturated fatty acids
ROMs	reactive oxygen metabolites
ROS	reactive oxygen species
rRNA	ribosomal ribonucleic acid
SAS	statistical analysis system
SD	standard deviation
SEM	standard error of mean
SFA	saturated fatty acids
SOD	superoxide dismutase
SPSS	statistical package for social science
SS	grape skin
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reacting substances
TBHQ	tertiary butyl hydroquinone
TC	tannins content
TCA	trichloroacetic acid
TEP	1,1,3,3-tetraethoxy propane
TEPC	total exctractable polyphenols content
THBP	2,4,5-trihydroxybutyrophenone
TPA	texture profile analysis
TRFLP	terminal restriction fragment length polymorphism
TVC	total viable count
UDP	uridine diphosphate
UFA	unsaturated fatty acids
UFS	unfermented grape skin
UK	united kingdom
USA	united states of America
USDA	united states department of agriculture
UV-VIS	ultraviolet–visible
VRBG	violet red bile glucose agar

WB	warner-Bratzler
WHO	world health organization
α-T	α-Tocopherol
γ-T	γ tocopherol

LIST OF PUBLICATIONS

Publications at Conference/Congress

F. Vizzarri, C. Corino, M. Palazzo, **M. Nardoia**, D. Casamassima. "Effect of dietary lippia citriodora extract on various metabolites and productive performance in lacaune suckling lambs." Conference Paper. XXI Congresso Nazionale S.I.P.A.O.C. Società Italiana di Patologia ed Allevamento degli Ovini e dei Caprini, Foggia, Italy; Sptember 2014

M. Nardoia, S. Chamorro, A. Viveros, I. Arija, D. Casamassima, A. Brenes "Effect of dietary fermented and unfermented grape skin and vitamin E on digestibility of polyphenols, antioxidant and antimicrobial activity in chickens" International PhD Workshop on "Welfare, biotechnology and quality of animal production" Campobasso, Italy , 2015, 26th, November.

F. Vizzarri, M. Palazzo, **M. Nardoia**, M. Cinone, D. Casamassima "Dietary effecr of *Lippia citriodora* extract on semen quality characteristics in male hares (Lepus europaeus Pallas, 1778)" The 3rd International Scientific Conference "Animal Biotechnology" Slovak J. Anim. Sci., 48, 2015 (4):181-194

Publications in International Journals with I.F.

D. Casamassima, M. Palazzo, **M. Nardoia,** V. Longo, L. Pozzo, F. Vizzarri "Dietary effect of fermented wheat powder (Lisosan G ®) on productive performance and meat quality in intensively-reared rabbit" Pakistan Journal of Zoology. (2016) (*I.F. 0.400*).

M. Palazzo, F. Vizzarri, **M. Nardoia**, S. Ratti, G. Pastorelli, D. Casamassima,. "Dietary Lippia citriodora extract in rabbit feeding: effects on quality of carcass and meat". (2015), Arch. Anim. Breed., 58, 355-364. (*I.F 0.503*)

L. Pozzo, F. Vizzarri, M. Ciardi, **M. Nardoia**, M.Palazzo, D. Casamassima, Dr. Vincenzo Longo. "The effects of fermented wheat powder (Lisosan G) on the blood lipids and oxidative status of healthy rabbits". Food and chemical toxicology, (2015); (84) 1-7. (*I.F. 2.895*)

D. Casamassima, **M. Nardoia**, M. Palazzo, F. Vizzarri, C. Corino. "Effect of dietary extruded linseed, verbascoside and vitamin E supplements on selected serum

biochemical parameters and plasma oxidative status in Lacaune ewes". Slovenian Veterinary Research, (2014); 51(2):89-100. (*I.F. 0.314*).

F. Vizzarri, **M. Nardoia**, M. Palazzo. "Effect of dietary Lippia citriodora extract on productive performance and meat quality parameters in hares (*Lepus europaeus Pall.*)". Archiv Tierzucht 57 (2014) 20, 1-7. (*I.F. 0.503*)

D. Casamassima, **M. Nardoia**, M. Palazzo, F. Vizzarri, A.G. D'Alessandro, C. Corino. "Effect of dietary extruded linseed, verbascoside and vitamin E supplements on yield and quality of milk in Lacaune ewes" Journal of Diary Research 81 (2014) 485-493. (*I.F. 1.598*).

F. Vizzarri, D. Casamassima, **M. Nardoia,** M. Palazzo "Water-restriction effect on various physiological parameters in Lacaune ewes intensively reared" *Submitted* Veterinarni Medicina (2016) (*I.F. 0.639*)

D. Casamassima, M. Palazzo, **M. Nardoia,** A.G. D'Alessandro, F. Vizzarri "Effect of water-restriction on milk yield and quality in Lacaune breed ewes" *Submitted* Tropical Animal Healt and Production (2016) (*I.F. 0.817*)

M. Nardoia, S. Chamorro, A. Viveros, I. Arija, C. Romero, D. Casamassima, A. Brenes "Effects of dietary fermented and unfermented grape skin and vitamin E on digestibility of polyphenol, antioxidant and antimicrobial activity in chickens" *Submitted* Animal (2016) (*I.F. 1.841*)