Food Research International xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace

S. Chamorro [a], A. Viveros [b], A. Rebolé [b], B.D. Rica [a], I. Arija [b], A. Brenes [a,*]

[a] Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), José Antonio Novais, 10, Ciudad Universitaria, 28040 Madrid, Spain
[b] Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form 11 November 2014
Accepted 27 November 2014
Available online xxxx

Keywords:
Polyphenols
Grape pomace
Enzymes
Polyunsaturated fatty acids
Antioxidant
Chickens

ABSTRACT

Grape pomace provides a rich source of polyphenols that have the capacity to act as powerful antioxidant. An experiment with ten dietary treatments (30 birds/treatment), was conducted to study the effect of inclusion of phenolic compounds from grape pomace (GP) added at different levels (0, 5, and 10%) and the addition (individually or combined) of hydrolyzing enzymes (carbohydrase enzyme complex and tannase at 500 ppm) in broiler chicks (1 to 21 days of age). A diet supplemented with 200 ppm of α-tocopheryl acetate (αT) was also used. Growth performance, ileal and fecal total polyphenol content, thigh meat lipid oxidation, and α-tocopherol and fatty acid content of thigh meat were determined. No differences were observed in body weight, feed consumption and feed efficiency among the different treatments. Birds fed GP diets showed a higher ileal and fecal polyphenol content. The inclusion of tannase in GP diets increased ileal polyphenol content and did not affect the fecal polyphenol content. Oxidative stability of thigh meat after 1 and 4 days of refrigerated storage increased with dietary addition of αT and GP, and was worsened when GP was supplemented with carbohydrases. Meat α-tocopherol content was increased by dietary addition of αT. Birds fed αT and GP diets showed higher meat polyunsaturated fatty acid content, while monounsaturated fatty acid was reduced. The addition of tannase to GP diets reversed the beneficial effect observed on fatty acid content obtained in GP diets. In conclusion, dietary GP reached the protective effect of α-tocopherol by reducing the susceptibility of meat to lipid oxidation and increasing the PUFA content. The inclusion of tannase in diets containing GP increased the amount of total polyphenol released in the intestine, but did not improve the stability to meat lipid oxidation.

1. Introduction

Supplementation of domestic animals with n-3 polyunsaturated fatty acids (n-3 PUFA) is becoming an accepted practice to improve the nutritional quality of lipids in animal products. However, this nutritional strategy also enhances the susceptibility to the lipoperoxidation of the meat. Poultry meat is susceptible to oxidative deterioration due to its high content of polyunsaturated fatty acids (Arshad et al., 2011; Huff-Lonergan & Lonergan, 2005; Tavárez et al., 2011). The oxidative stability of poultry meat may be improved by dietary addition of α-tocopherol, the active antioxidant form of Vitamin E (Avila-Ramos et al., 2012; Carreras et al., 2004). However, the bioefficiency of this vitamin is limited when PUFA intake is increased (Allard, Kurian, Aghdassi, Muggli, & Royall, 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, 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). Grape pomace (GP) is a wine by-product consisting of pressed seeds, skins, and stems, and is rich source in flavonoids including monomeric phenolic compounds, such as (+)-catechins, (−)-epicatechin, and (−)-epicatechin-3-gallate and dimeric and oligomeric proanthocyanidins. These compounds have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Yilmaz & Toledo, 2004). Previous reports (Brenes et al., 2008; Goñi et al., 2007) indicated that the intake of grape pomace increases the antioxidant capacity in breast and thigh meat of broiler of chickens in the same way as added vitamin E in experimental diets. The antioxidant activity of polyphenols in biological tissues is currently associated with their capacity to scavenge free radicals, chelate active redox metals and protection of the endogenous antioxidant system (Lourenco, Gago, Barbosa, Freitas, & Laranjinha, 2008; Pazos, Gallardo, Torres, & Medina, 2005).

The growing interest in the use of natural antioxidant grape-products in diets with high PUFA content may contribute to an improved oxidative stability of meat and meat products and provide greater potential for developing quality poultry foods for human consumption. However, the use of such natural antioxidants in animal nutrition could be limited

* Corresponding author. Tel.: +34 91 5434545.
E-mail address: abrenes@ictan.csic.es (A. Brenes).

http://dx.doi.org/10.1016/j.foodres.2014.11.054
0963-9969/

due to the low bioavailability of grape polyphenols, and might be improved by the use of exogenous enzymes. Enzymatic supplementation is a technique with increasing applicability for improving the nutritional characteristic of by-products and it is widely used in animal nutrition. Cell-wall degrading enzymes can improve the extraction of phenols. The GP cell wall is a complex network composed of 30% of neutral polysaccharides (cellulose, xyloglucan, arabinan, galactan, xylan and mannan), 20% of acidic pectin substances, 15% of insoluble proanthocyanidins, lignin and structural proteins and phenols, these two latter cross-linked to the lignin–carbohydrate framework (Pinelo, Arnous, & Meyer, 2006). A recent in vitro study (Chamorro, Viveros, Alvárez, Vega, & Brenes, 2012) reported that the addition of carbohydrases (pectinases and cellulases) and tannase released polyphenols and polysaccharides entrapped in grape pomace cell wall increasing its antioxidant activity. The hydrolysis of the complex polysaccharides and polyphenols into more digestible sugars and phenols might increase the amount of active substances that can be easily metabolized improving its nutritional value and render this by-product more suitable to be used as an animal ingredient. To our knowledge there is no information about the effect of the addition of enzymes to chicken diets containing grape pomace on the antioxidant status and lipid peroxidation of meat. The objective of this experiment was to study if dietary addition of enzymes could improve the polyphenol utilization, the antioxidant status and meat quality of chickens fed grape pomace.

2. Material and methods

2.1. Test product and enzymes

Red GP (*Vitis vinifera* var. Cencibel) was obtained from Grupo Matarromera (San Bernardo-Valbuena de Duero, Valladolid, Spain). Proximate composition of GP is shown in Table 1. GP was used as a source of dietary fiber and polyphenols in the chicken diets. The pomace, consisting of stems, skins and seeds from red grapes, was dried in a convection oven at 60 °C. Proximate composition of grape pomace was: protein 138.9 g/kg; fat 9.87 g/kg; fiber 343.0 g/kg and ash 24.1 g/kg. The α-tocopheryl acetate (αT) used in the diets was provided by DSM Nutritional Products Iberia S.A. (Alcalá de Henares, Madrid, Spain). Two different enzymatic preparations were selected on the basis of the structural composition of grape pomace. A feed enzyme complex (EC) Avyzyme®1505, donated by Danisco Animal Nutrition (Marlborough, UK), containing endo-1,4-beta-xylanase (1500 U/g, EC 3.2.1.8.), α-amylase (2000 U/g, EC 3.2.1.1) and subtilisin (20,000 U/g, EC 3.4.21.62) was added to diets containing GP. Another enzyme preparation with tannase activity (T) supplied by Kikkoman Foods Products Company (Edogawa Plant, Japan) containing tannin acylhydrolase (500 U/g, EC 3.1.20) was used.

Table 1
Ingredients and nutrient composition of experimental diets (% as fed).

	Control (C)	C + 5GP	C + 10GP
Maize	49.1	50.0	50.8
Soybean	19.0	16.9	14.4
Sunflower oil	9.70	9.64	10.5
Soybean concentrate	9.10	9.40	9.09
Straw	7.78	3.77	0
Grape pomace	0	5.0	10.0
Monocalcium phosphate	1.95	1.96	1.98
Calcium carbonate	1.42	1.37	1.31
Salt	0.30	0.30	0.30
Vitamin–mineral premix[a]	0.50	0.50	0.50
DL-Methionine	0.14	0.13	0.12
Enzymes[b]	–	−/+	−/+
Chemical composition			
Crude protein	21.1	21.1	21.1
Ether extract	12.0	12.0	12.0
Crude fiber	5.16	5.10	5.16
Ca	1.0	1.0	1.0
Available P	0.495	0.493	0.495
AME[c](Kcal/kg)	3000	3000	3000
SFA [d]	1.36	1.50	1.49
MUFA[d]	1.79	1.67	1.54
PUFA[d]	0.42	0.41	0.34

[a] Vitamin and mineral mix supplied the following per kilogram of diet: vitamin A, 8250 IU; cholecalciferol, 1000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B_{12}, 12.5 μg; riboflavin, 5.5 mg; Calcium pantothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; ethoxyquin, 125 mg; DL-methionine, 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, 2500 mg.
[b] Enzyme added to the diets were 500 mg/kg of enzyme complex (EC), 500 mg/kg of tannase (T) or the combination of both.
[c] AME: apparent metabolizable energy; calculated values (FEDNA Tables, 2003).
[d] Analyzed composition of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA).

2.2. Solvents and reagents

All solvents used for HPLC analysis were of liquid chromatography grade and the water was ultrapure. Standards for catechin (C), epicatechin (EC) and epicatechin O-gallate (ECG) procyanidin dimer B1 (PB1) and B2 (PB2) and cyanidin-3-O-glucoside were purchased from Extrasynthèse (Genay, France). Gallic acid (GA), Folin–Ciocalteu reagent, α-tocopherol, trolox, butylated hydroxytoluene, and 1,1,3,3-tetraethoxy propane were obtained from Sigma-Aldrich (St. Louis, MO). Acetone, butanol, isopropanol, hexane, acetonitrile and methanol were obtained from Panreac (Castellar del Vallés, Barcelona, Spain).

2.3. Birds and diets

A total of three 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 for 3 weeks. The chicks were allocated to 50 pens, each pen containing six chicks, to receive 10 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. Ingredients and nutrient composition of diets are shown in Table 1. All diets were formulated to meet or exceed the minimum (National Research Council, 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. Experimental diets were as follows: 1. Control corn soybean diet (C); 2. C + αT (200 mg/kg of α-tocopheryl acetate); 3. C + 5% of GP; 4. C + 5% GP + enzyme complex; 5. C + 5% GP + tannase; 6. C + 5% GP + enzyme complex + tannase; 7. C + 10% GP; 8. C + 10% GP + enzyme complex; 9. C + 10% GP + tannase, and 10. C + 10% GP + enzyme complex + tannase. At the end of the experimental period, birds were weighed and feed consumption was recorded for feed efficiency computation.

2.4. Collection of samples and measurements

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

At 21 days of age, fifteen birds per treatment were euthanized by carbon dioxide (100%), the ileum was quickly dissected out and the content expressed by gentle manipulation into a plastic container and stored at −20 °C. Digesta were pooled from three birds of each replicate within the same treatment. Ileal contents were freeze-dried and ground (1 mm screen) and used to determine the polyphenol content. Carcasses from ten birds per treatment were also immediately trimmed for thigh

Please cite this article as: Chamorro, S., et al., Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace, *Food Research International* (2014), http://dx.doi.org/10.1016/j.foodres.2014.11.054

meat, and tissues were individually sampled and used to determine lipid oxidation (5 birds/treatment) and fatty acid content (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 −20 °C until required. After thawed, the progress of lipid oxidation during storage in meat samples was determined after 1 and 4 days in a no illuminated refrigerated cabinet at 4 °C. Thigh meat collected for fatty acid determination was freeze-dried, methylated and analyzed by gas chromatograph.

2.5. Chemical analysis

2.5.1. Polyphenolic content

Total polyphenol content (TPC) and phenolic profile in GP, diet, ileal digesta and excreta were determined after the extraction with methanol/acetone/water following the procedure described by Chamorro et al. (2012) using an ultraviolet–visible spectrophotometer (Hitachi U-2000; Hitachi, Ltd., Tokyo, Japan). The results were expressed as grams of gallic acid equivalents (GAE) per 100 g of dry matter (DM) (Table 2).

The phenolic profile was determined as described by Chamorro et al. (2012) using an Agilent 1100 series LC (Agilent Technologies, Waldbronn, Germany), comprising quaternary pump with integrated degasser, autosampler, thermostated column compartment and diode array detector, coupled with an Agilent G1946D Quadrupole mass spectrometer (Agilent Technologies, Waldbroon, Germany). Extracted filtered samples were separated in Gemini C18 5 mm, 250 mm, and 34.6 mm i.d. column, (Phenomenex Inc., Torrance, CA, USA) and eluted with a mobile phase made of a mixture of deionized water and acetonitrile, both containing 0.1% formic acid, at a flow rate of 1 ml/min. Ionization was achieved by atmospheric pressure electrospray ionization source, operated in negative ion mode. Selected ion monitoring scan type was used for quantification. Data acquisition and analysis were carried out with an Agilent ChemStation Software. Phenolic yields were expressed as mg per 100 g of DM.

2.5.2. Meat α-tocopherol content

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 × 150 mm, 5 μm, Agilent Technologies) column

2.5.3. Meat lipid oxidation (TBARS)

The extent of lipid oxidation of meat was determined by measuring the thiobarbituric acid reacting substances (TBARS) after 1 and 4 days of storage using the procedure described by Botsoglou et al. (1994) using an ultraviolet–visible spectrophotometer Hitachi U-2000 (Hitachi, Ltd).

Table 2
Concentration of total extractable polyphenols (g Gallic Acid Equivalent/100 g DM), and phenolic profile (mg/100 g DM) of grape pomace (GP) and experimental diets containing GP at 5 and 10% (C + 5GP and C + 10GP, respectively).

	Control (C)	C + 5GP	C + 10GP	GP
Total extractable polyphenols	0.11 ± 0.007	0.14 ± 0.003	0.19 ± 0.007	2.34 ± 0.7
Phenolic profile				
Gallic acid	nd	0.86 ± 0.06	1.54 ± 0.15	19.3 ± 0.12
Catechin	nd	0.63 ± 0.07	1.15 ± 0.11	12.2 ± 0.18
Epicatechin	nd	0.71 ± 0.07	1.27 ± 0.14	12.0 ± 0.48
Procyanidin B1	nd	1.13 ± 0.07	1.88 ± 0.10	20.2 ± 2.1
Procyanidin B2	nd	1.79 ± 0.15	3.59 ± 0.28	36.9 ± 1.3
Epicatechin-*O*-gallate	nd	0.08 ± 0.01	0.14 ± 0.02	1.32 ± 0.03

Data are the mean of 4 determinations ± standard deviation.

Results were expressed as μg of malondialdehyde (MDA) per gram of muscle after the preparation of a standard curve of 1,1,3,3-tetraethoxy propane (TEP).

2.5.4. Fatty acid composition

Fatty acids were analyzed by duplicate in freeze-dried samples of thigh meat and diets after submitting the samples to a simplified direct bimethylation procedure (Lee, Tweed, Kim, & Scollan, 2012). Briefly, 0.2–0.3 g dry matter of the freeze-dried and ground samples was weighed in duplicate and added 4 ml 0.5 M sodium methoxide in anhydrous methanol and 1 ml heptane containing 1 mg/ml C13:0 (≥99.9%), as internal standard. The samples were then heated at 50 °C for 15 min before 4 ml 10% acetyl chloride in anhydrous methanol was added. Next, the samples were mixed thoroughly and heated for 1 h at 60 °C. Heptane and distilled water were added both at 2 ml before mixing and centrifuging for 5 min at 1500 ×*g*. The organic solvent top layer was pipetted into a second tube before further 2 ml of heptane was added to the original tube, mixed and centrifuged as previously. After pooling the organic layers in the second tube, anhydrous sodium sulfate (0.2 g) was added, mixed and then centrifuged. Finally, an aliquot was collected in an amber vial for subsequent gas chromatography analysis. Fatty acid methyl esters (FAME) were performed with a gas chromatograph (Agilent 7820A) equipped with a flame-ionization detector and an Agilent HP-88 column (60 m × 250 mm × 0.2 μm) with split injection (40:1) and Helium at a constant flow of 1.5 ml/min as the carrier gas. Detector temperature was set at 260 °C and injector oven temperature at 250 °C. The temperature profile of the oven was from 125 °C increasing by 8 °C/min to 145 °C followed by 2 °C/min to 220 °C. Identification was accomplished by comparing the retention times of peaks from samples with those of FAME standard mixtures. Quantification of FAME was based on the internal standard technique and on the conversion to relative peak areas to weight percentage, using the corrected response factor of each fatty acid (European Committee for Standardization, 1990). Fatty acids were expressed as percentage of the sum of identified fatty acids (% wt/wt).

2.6. Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) by using the general linear model procedure (Version 9.2, SAS Institute Inc., Cary, NC). When the effect was declared significant ($P < 0.05$), means were compared using a Tukey's Studentized range test. Nonorthogonal contrasts were used to test differences between the combined mean of several groups.

3. Results

3.1. Growth performance

The effect of feeding diets containing αT and GP supplemented with the enzyme complex and tannase on growth performance of chickens is shown in Table 3. No effect ($P > 0.05$) of dietary treatments were observed on body weight and feed consumption. Feed conversion was improved ($P < 0.05$) in birds fed α-T diet compared to those fed GP diets.

3.2. Ileal and excreta polyphenol content

The total ileal and excreta polyphenol content of birds fed diets containing αT, GP and enzymes is reported in Table 4. As expected, ileal and excreta content were not affected by dietary αT supplementation. Birds fed GP diets showed a higher ($P < 0.001$) ileal and excreta polyphenol content than those fed control diets, being higher in animals fed diets containing 10% GP. The inclusion of tannase in GP diets significantly increased ($P < 0.01$) ileal polyphenol content and did not affect the excreta polyphenol content. The addition of the enzyme complex (individually

Please cite this article as: Chamorro, S., et al., Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace, *Food Research International* (2014), http://dx.doi.org/10.1016/j.foodres.2014.11.054

Table 3
Effect of feeding diets containing α-tocopheryl acetate (αT), grape pomace (GP) and enzymes on growth performance of chicks at 21 days of age.

Dietary treatments[1]	Body weight	Feed consumption	Feed conversion
Control (C)	589	732	1.242
C + αT	588	745	1.268
C + 5GP	602	733	1.218
C + 5GP + EC	572	704	1.232
C + 5GP + T	597	724	1.215
C + 5GP + EC + T	566	681	1.204
C + 10GP	582	718	1.234
C + 10GP + EC	580	720	1.242
C + 10GP + T	563	697	1.238
C + 10GP + EC + T	593	734	1.239
SEM[2]	12.8	17.6	0.017
P-value of contrasts[3]:			
C vs. GP	ns	ns	ns
αT vs. GP	ns	ns	*
5% GP vs. 10% GP	ns	ns	ns
GP vs. GP + EC	ns	ns	ns
GP vs. GP + T	ns	ns	ns
GP vs. GP + EC + T	ns	ns	ns

[1] Diets supplemented (+) with: α-tocopheryl acetate (αT), 5 or 10% of grape pomace (5GP, 10GP); enzyme complex (EC), tannase (T), and enzyme complex + tannase (EC + T).
[2] SEM, standard error of means; number of replicates = 5 pens of 6 birds.
[3] ns: no significant effect (P > 0.05).
* P < 0.05.

or in combination with tannase) to GP diets did not affect the ileal and excreta polyphenol content.

3.3. Lipid oxidation of meat (TBARS)

The effect of dietary treatments on meat lipid oxidation is reported in Table 5. The extent of lipid oxidation measured as MDA (malondialdehyde) formation in thigh meat after 1 and 4 days of refrigerated storage was significantly lower (P < 0.05) in birds fed diets supplemented with αT (P < 0.01) or GP (P < 0.01) than in those fed the control diets. Thigh MDA concentration of birds fed GP diets was similar to those fed αT diets after 1 day of refrigerated storage, and higher after 4 days. No differences were observed in meat MDA values of birds fed with the different concentrations of GP, or with the enzyme supplementation to GP, except for the enzyme complex which increased the thigh MDA values.

Table 4
Effect of feeding diets containing α-tocopheryl acetate (αT), grape pomace (GP) and enzymes on ileal and excreta total polyphenol content (TP) expressed as g gallic acid equivalent (GAE) per 100 g of dry matter.

	TP (g GAE/100 g DM)	
Dietary treatments[1]	Ileal	Excreta
Control (C)	0.301^{d}	0.387^{cd}
C + αT	0.326^{cd}	0.377^{d}
C + 5GP	0.315^{d}	0.424^{bcd}
C + 5GP + EC	0.316^{d}	0.419^{bcd}
C + 5GP + T	0.320^{dc}	0.420^{bcd}
C + 5GP + EC + T	0.319^{dc}	0.443^{abc}
C + 10GP	0.357^{bc}	0.465^{ab}
C + 10GP + EC	0.376^{ab}	0.465^{ab}
C + 10GP + T	0.406^{a}	0.497^{a}
C + 10GP + EC + T	0.382^{ab}	0.472^{ab}
SEM[2]	0.081	0.128
P-value of contrasts[3]:		
C vs. GP	***	***
αT vs. GP	*	***
5% GP vs. 10% GP	***	***
GP vs. GP + EC	ns	ns
GP vs. GP + T	**	ns
GP vs. GP + EC + T	ns	ns

[a–d]Mean values within a column unlike superscript letter were significantly different.
[1] Diets supplemented (+) with: α-tocopheryl acetate (αT), 5 or 10% of grape pomace (5GP, 10GP); enzyme complex (EC), tannase (T), enzyme complex + tannase (EC + T).
[2] SEM, standard error of means; number of replicates = 5 pens. Each pen contained 3 (for ileal) and 6 (for fecal) birds.
[3] ns: no significant effect (P > 0.05).
* P < 0.05.
** P < 0.01.
*** P < 0.001.

Table 5
Effect of dietary addition of α-tocopheryl acetate (αT), grape pomace (GP) and enzymes, on lipid oxidation of thigh meat submitted to refrigerated storage for 1 and 4 days.

	MDA (μg/g meat)	
Dietary treatments [1]	1d	4d
Control (C)	0.082^{a}	0.217^{a}
C + αT	0.052^{b}	0.095^{b}
C + 5GP	0.056^{b}	0.122^{b}
C + 5GP + EC	0.081^{a}	0.195^{a}
C + 5GP + T	0.056^{b}	0.126^{b}
C + 5GP + EC + T	0.058^{b}	0.128^{b}
C + 10GP	0.055^{b}	0.114^{b}
C + 10GP + EC	0.080^{a}	0.206^{a}
C + 10GP + T	0.059^{b}	0.139^{b}
C + 10GP + EC + T	0.064^{ab}	0.141^{b}
SEM[2]	0.006	0.019
P-value of contrasts[3]:		
C vs. GP	*	**
αT vs. GP	ns	**
5% GP vs. 10% GP	ns	ns
GP vs. GP + EC	*	***
GP vs. GP + T	ns	ns
GP vs. GP + EC + T	ns	ns

[a,b]Mean values within a column unlike superscript letter were significantly different.
[1] Diets supplemented (+) with: α-tocopheryl acetate (αT), 5 or 10% of grape pomace (5GP, 10GP); enzyme complex (EC), tannase (T), enzyme complex + tannase (EC + T).
[2] SEM, standard error of means; number of replicates = 5 birds.
[3] ns: no significant effect (P > 0.05).
* P < 0.05.
** P < 0.01.
*** P < 0.001.

3.4. Alpha-tocopherol and fatty acid profile of thigh meat

The effect of dietary treatments on the composition of α-tocopherol and fatty acid of broiler thigh meat is presented in Table 6. A significant increase in thigh α-tocopherol concentration was observed in birds fed αT and GP supplemented diets compared with those fed the control diet. This effect was higher (P < 0.001) in birds receiving αT diets than in those fed GP diets. No effect on meat α-tocopherol was obtained when GP was supplemented with enzymes added individually (enzyme complex and tannase) or in combination.

Based on the results obtained from intramuscular fat, in comparison to the control diet, the dietary inclusion of αT, reduced (P < 0.001) the proportion of monounsaturated fatty acid (MUFA), increased (P < 0.001) that of polyunsaturated fatty acids (PUFA) and did not affect the saturated fatty acid (SFA) concentration of thigh meat. Birds fed GP diets reduced the proportion of total SFA, C14:0, C16:0, C17.0, C18:0 and C20:0 and increased that of C15:0 in comparison with the control group. A significant reduction was also observed in total MUFA based in the concentration of C14:1, C16:1, C18:1n9c, C181n11c and C20:1 in those birds fed GP diet. For total PUFA, when compared with the control group, the inclusion of GP increased the fatty acid concentration of C18:2n6c, C20:2, C18:3n3, C18:3n6, C20:3n6, and C20:4n6.

In comparison to the GP diet, the addition of αT increased the concentration of total SFA based on the fatty acid proportions of C17:0, C18:0 and C20:0. No significant differences in the total MUFA and PUFA content were obtained among birds fed GP and αT diets except for C:20:2 which was lower in those fed αT diets.

Please cite this article as: Chamorro, S., et al., Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace, *Food Research International* (2014), http://dx.doi.org/10.1016/j.foodres.2014.11.054

Table 6
Effect of dietary treatments on alpha-tocopherol content (μg/g) and fatty acid profile (%) of chicken thigh meat.

	Dietary treatments[1]								P-value of contrasts[3]	
	Control (C)	C + αT	C + 5GP	C + 10GP	C + 10GP + EC	C + 10GP + T	C + 10GP + EC + T	SEM[2]	C vs. GP	αT vs. GP
α-tocopherol	5.00^{c}	23.7^{a}	7.01^{c}	9.44^{cb}	15.1^{b}	10.7^{bc}	7.65^{c}	1.39	**	***
Total SFA[4]	21.5^{a}	20.4^{ab}	20.2^{ab}	18.2^{b}	17.7^{b}	18.8^{ab}	18.4^{b}	0.56	***	***
C12:0	0.029	0.024	0.027	0.025	0.027	0.030	0.028	0.003	ns	ns
C14:0	0.31^{a}	0.28^{ab}	0.28^{ab}	0.26^{ab}	0.24^{b}	0.28^{ab}	0.26^{ab}	0.013	**	ns
C15:0	0.019^{b}	0.026^{ab}	0.024^{ab}	0.027^{ab}	0.031^{a}	0.023^{ab}	0.028^{ab}	0.002	*	ns
C16:0	14.7^{a}	12.9^{ab}	13.1^{ab}	11.9^{b}	11.6^{b}	12.8^{ab}	12.2^{b}	0.449	***	ns
C17:0	0.12^{a}	0.13^{ab}	0.13^{ab}	0.11^{bc}	0.11^{bc}	0.10^{c}	0.10^{c}	0.004	**	***
C18:0	6.19^{abc}	6.92^{a}	6.49^{ab}	5.73^{bc}	5.63^{bc}	5.38^{c}	5.63^{bc}	0.168	*	***
C20:0	0.13^{ab}	0.15^{a}	0.14^{a}	0.11^{bc}	0.12^{bc}	0.11^{c}	0.11^{bc}	0.005	*	***
C24:0	0.036	0.036	0.038	0.028	0.026	0.033	0.025	0.003	ns	ns
Total MUFA[4]	41.5^{a}	30.1^{cd}	32.4^{c}	28.7^{d}	28.9^{d}	36.1^{b}	29.2^{cd}	0.610	***	ns
C14:1	0.062	0.042	0.046	0.042	0.039	0.050	0.043	0.005	**	ns
C16:1	1.92^{a}	1.20^{b}	1.35^{ab}	1.19^{b}	1.24^{b}	1.57^{ab}	1.34^{ab}	0.118	***	ns
C18:1n9c	38.0^{a}	27.7^{cd}	29.7^{c}	26.4^{d}	26.6^{d}	33.2^{b}	26.7^{d}	0.498	***	ns
C18:1n11c	1.23^{a}	0.95^{bc}	1.01^{bc}	0.89^{c}	0.89^{c}	1.05^{b}	0.93^{bc}	0.027	***	ns
C20:1	0.28^{a}	0.23^{b}	0.22^{bc}	0.20^{c}	0.20^{c}	0.23^{b}	0.20^{c}	0.005	***	ns
Total PUFA[4]	37.0^{c}	49.4^{ab}	47.4^{ab}	53.1^{a}	53.3^{a}	45.1^{b}	52.4^{a}	1.098	***	ns
C18:2n6c	34.81^{c}	46.7^{ab}	44.9^{b}	50.3^{a}	50.7^{a}	42.5^{b}	49.8^{a}	1.098	***	ns
C20:2	0.50^{a}	0.45^{b}	0.50^{a}	0.34^{ab}	0.32^{ab}	0.29^{b}	0.32^{ab}	0.033	**	*
C18:3n3	0.31^{b}	0.36^{ab}	0.34^{ab}	0.40^{a}	0.39^{a}	0.39^{a}	0.38^{ab}	0.015	***	ns
C18:3n6	0.22	0.31	0.30	0.32	0.31	0.32	0.30	0.025	***	ns
C20:3n6	0.22^{b}	0.31^{a}	0.28^{ab}	0.33^{a}	0.30^{ab}	0.31^{a}	0.31^{a}	0.016	***	ns
C20:4n6	0.79^{b}	1.23^{a}	1.01^{ab}	1.34^{a}	1.16^{ab}	1.11^{ab}	1.25^{a}	0.069	***	ns
C22:5n3	0.047	0.047	0.043	0.044	0.039	0.057	0.045	0.005	ns	ns
C22:6n3	0.062	0.065	0.062	0.057	0.050	0.054	0.045	0.008	ns	ns

[1] Diets supplemented (+) with: α-tocopheryl acetate (αT), 5 or 10% of grape pomace (5GP, 10GP); enzyme complex (EC), tannase (T), enzyme complex + tannase (EC + T).
[2] SEM, standard error of means; number of replicates = 5 birds.
[3] ns: no significant effect ($P > 0.05$).
[4] SFA, saturated fatty acid; MUFA monounsaturated fatty acid; PUFA, polyunsaturated fatty acids.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

4. Discussion

4.1. Growth performance and polyphenol utilization

Grape by-products have been proposed as potential functional ingredients in animal feed (Brenes et al., 2008; Viveros et al., 2011), but the polyphenol bioavailability of these by-products is rather low and could be enhanced by the addition of carbohydrases and tannase. Previous results published by our laboratory reported a positive antioxidant effect of GP in birds when added up to 6% (Brenes et al., 2008; Goñi et al., 2007) in chicken diets. Grape pomace contains high level of fiber and polymeric polyphenols as procyanidins with capacity to bind and precipitate both dietary and endogenous proteins, and therefore the incorporation of GP at high doses in chicken diets might impair nutrient digestion and growth. The utilization of enzymes with capacity to hydrolyse complex cell wall and polyphenols present in GP, might allow the use of higher doses of GP in chicken diets. Thus, in the present study, we used high doses of GP and, because all diets were formulated to contain the same fiber content, any difference should be attributed to the polyphenol content. Our results showed that dietary inclusion of GP up to 10% did not affect the chick growth performance. Consequently the use of the enzyme complex or tannase did not enhance the parameters studied.

Biological effect of polyphenols depends on their availability. Monomeric and some oligomeric polyphenols have been found to be absorbed at the small intestine while polymeric forms are poorly absorbed and must be hydrolyzed by the intestinal microbiota (Gonthier, Cheynier, et al., 2003). As expected, birds fed GP diets showed a higher intestinal (ileal and excreta) total polyphenol content than those fed control diets. The highest intestinal polyphenol concentrations were obtained in chicks fed diets containing 10% GP. Recent studies have estimated that the amount on non-absorbable polyphenols reaching the colon is very high and microbe-derived phenolic metabolites excreted in urine represent the largest proportion of polyphenol intake (Monagas et al., 2010). Bird lacks a specific urinary excretion system, and consequently the excreta of chicks are composed by both, urine and undigested compounds. Thus, the total polyphenols determined in excreta in this experiment were the addition of phenolic substances coming from both fractions, undigested and microbe-derived phenolic metabolites. This might explain why the amount of phenolic compounds in excreta was higher than those obtained at ileal content.

We hypothesized that the use of enzymes with carbohydrase and tannase activities might hydrolyze the complex carbohydrate and polyphenol structure of grape pomace into more simple nutrients and phenols enhancing the amount of bioactive available substances, and therefore, its antioxidant capacity. Lafay and Gil-Izquierdo (2008) reported that low molecular weight phenolic compounds can be much easier absorbed and may contribute more directly to the beneficial health effects. In our study, only the inclusion of tannase was able to release polyphenols of GP, and therefore, the addition of this enzyme increased the amount of total polyphenol in the intestine. Potential modifications on the polyphenol structures with tannase addition might explain the differences observed in total intestinal polyphenol content. In this context, we recently reported (Chamorro et al., 2012) modifications in the structure of polyphenols with the addition of tannase and pectinase. However, accordingly with this in vivo study, these modifications were only related with changes in total polyphenol content with the addition of tannase.

4.2. Meat lipid oxidation, α-tocopherol and fatty acid content

Lipid oxidation is one of the primary processes of quality deterioration in meat and meat products. Enhanced antioxidant capability in the muscle tends to improve meat quality and extend the shelf life (Tavárez

et al., 2011). In this study we investigated if a sustained consumption of GP supplemented or not with enzymes might reduce the lipid oxidation of the chicken meat, using a relatively rich diet in polyunsaturated fatty acid, in same way as supplemental dietary α-tocopheryl acetate. As expected, lipid oxidation (MDA concentration) increased with storage time, reflecting the reduction of meat capacity to resist against lipid oxidation during storage time and the tendency of PUFA oxidation. Dietary addition of αT (200 mg/kg) reduced the lipid oxidation of meat after 1 and 4 days of storage. The protection of dietary αT supplementation against lipid peroxidation of tissues has been extensively reported (De Winne & Dirinck, 1996; Giannenas, Florou-Paneri, Botsoglou, Christaki, & Spais, 2005).

Interestingly, in the present study dietary GP added up to 10% reached similar protective effect observed with the addition of αT, delaying the lipid oxidation of meat.

Protective effects of grape polyphenols were previously reported by Goñi et al. (2007), Brenes et al. (2008) and Sayago-Ayerdi, Brenes, and Goñi (2009) with dietary addition of up to 6% of GP. Different authors have successfully tested grape seed and grape pomace extracts as antioxidants in different lipid systems, and foodstuffs as vegetable and fish oil, beef, turkey, and chicken meat or fish muscle (Bañon, Diaz, Rodriguez, Garrido, & Price, 2007; Carpenter, O'Grady, O'Callaghan, O'Brien, & Kerry, 2007; Lau & King, 2003; Mielnick, Olsen, Vogt, Adeline, & Skrede, 2006; Pazos et al., 2005).

In the present experiment, meat α-tocopherol content was significantly increased (4 fold) in birds fed αT diets and was correlated with the reduction in MDA thigh content. Previous studies (Cortinas, Barroeta, Galobart, & Jensen, 2004; Voljc, Frankic, Levart, Nemec, & Salobir, 2011) also reported an increase in meat α-tocopherol contents (up to 4 fold) with dietary addition of 200 IU of vitamin E. Recently Iglesias, Pazos, Torres, and Medina (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. However, in the current study, meat α-tocopherol content was not increased in birds fed GP diets as was observed with αT diets.

Because grape products contain high amounts of polymeric polyphenols as procyanidins, we hypothesized that the use of an enzyme complex and tannase that hydrolyze the complex polyphenols into more simple phenols as gallic acid, might enhance the amount of bioactive available substances, and reduce the lipid oxidation. However, no additional protective effect was observed with the addition of these enzymes to GP diets on oxidative stability or in α-tocopherol content of meat. In fact, the addition of the carbohydrase complex to diets containing GP reversed the beneficial effect obtained with GP diets. We previously demonstrated in an in vitro study (Chamorro et al., 2012) that the addition of carbohydrases can exert important changes in the structure of GP, including the cell wall polysaccharide degradation, facilitating the release of several polyphenols entrapped in this matrix and increasing the antioxidant capacity. However, the present study shows that the positive effect of enzymes obtained under in vitro conditions cannot be extrapolated to in vivo studies.

These controversial results might also indicate a potential prooxidant effect of the polyphenols generated as a consequence of the modifications obtained in the structure and composition of polyphenols with the carbohydrases addition. It is well known that polyphenol compounds can display both antioxidant and prooxidant effects (Decker, 1997; Perron & Brumaghim, 2009) depending on several factors such as metal chelating potential, solubility characteristics, bioavailability and stability in tissues.

The fatty acid composition of meat is considered an important index for meat quality. In the current study the concentration of PUFA and the relation PUFA/MUFA in thigh meat were increased by dietary supplementation of αT when compared to the control diet. This effect was contributed mainly by a significant reduction in total MUFA and with a higher level of total n6 fatty acids. These results might indicate that dietary αT prevented a change in the PUFA content present in meat. In this sense, these results were correlated with a reduction in the susceptibility of the chicken meat to lipid peroxidation and an increase of meat α-tocopherol.

The influence of dietary α-tocopheryl acetate on fatty acid profile in chicken meat is controversial. Ajuyah, Ahn, Hardin, and Sim (1993), Zanini et al. (2004) and Rebolé et al. (2006) observed significant modifications, while other authors (Lin et al., 1994; O'Neill, Galvin, Morrissey, & Buckley, 1998) found no changes. Differences in the source and level of dietary fat and the inclusion of α-T might explain these differences. In our study, dietary addition of αT resulted in an increase of 36% in the meat content of total n6 mainly determined by an increase of C18:2n:6 and C20:4n6. Similar effects were also reported by Zanini et al. (2004) using diets containing sunflower oil and 400 mg/kg of vitamin E.

There is no available information about the supplemental effect of GP on chicken meat fatty acids profile. Results of this study confirm that dietary GP was also effective to modify thigh fatty acid composition as well as dietary α-tocopheryl acetate did. Dietary inclusion of GP reduced MUFA and increased PUFA meat content. Similar findings were reported in pigs fed fermented grape pomace (Yan & Kim, 2011) and in chickens fed a mixture of gallic and linoleic acids (Jung et al., 2010), essential oils (Hashemipour, Kermanshahi, Golian, & Veldkamp, 2013), flavonoids (Cao, Zhang, Yu, Zhao, & Wang, 2012; Kamboh & Zhu, 2013; Nkukwana et al., 2014) and herbs (Marcincakova et al., 2011). These results suggest that the intake of GP protect PUFA from lipid oxidation. This finding was also supported by a reduction in the MDA content, suggesting that GP could be applied in poultry industry as useful natural supplement to improve meat quality.

The addition of tannase to diets containing GP reversed the beneficial effect observed in GP diets. Birds fed with GP diet supplemented with this enzyme showed in the thigh a higher MUFA and a lower PUFA concentration in comparison to those birds fed the diet containing GP without enzyme. This could be justified by the modifications in the polyphenol structure observed in our previous study (Chamorro et al., 2012) with the addition of this enzyme. Tannase catalyzes the hydrolysis of the ester and depside bonds present in hydrolysable tannins and gallic esters, changing the galloylated form of catechin and releasing gallic acid (Lekha & Lonsane, 1997). The lower PUFA content observed in the meat of birds fed GP diets containing tannase compared to those fed GP diets might indicate that simple phenols generated by the action of tannase are less active than those more complex. Procyanidins may not require their intestinal absorption to be bioactive. Gut bacteria which are able to metabolize polymeric polyphenols may produce new phenolic compounds in situ, which could have better bioavailability and higher biological activity than their parent compounds (Requena et al., 2010). Dietary intake of polyphenol-rich grape products increased the biodiversity degree of intestinal bacteria in chicks (Viveros et al., 2011). Previous studies (Gonthier, Cheynier, et al., 2003; Gonthier, Verny, Besson, Rémésy, & Scalbert, 2003) also indicated that microbial metabolites resulting from the poor absorption of wine procyanidins strongly contribute to the biological effects of these polyphenols.

5. Conclusions

The present work shows that grape pomace might be included in chicken diets containing high PUFA to delay the meat lipid oxidation, reaching a similar protective effect observed with dietary α-tocopheryl acetate. This antioxidant effect was linked to an increase in the meat PUFA concentration. The inclusion of tannase in diets containing GP increased the amount of total polyphenol released in the intestine, but did not improve the stability of meat to lipid oxidation. These findings indicated that the polyphenol modifications generated by the action of enzymes presented a lower in vivo antioxidant capacity than natural compounds present in GP.

Please cite this article as: Chamorro, S., et al., Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace, *Food Research International* (2014), http://dx.doi.org/10.1016/j.foodres.2014.11.054

Acknowledgments

The authors wish to thank the Spanish Ministry of Science and Innovation (MICINN) and Comunidad Autónoma de Madrid (CAM) for financial support of this investigation, Projects AGL2012-31355 and S2013/ABI-2913 (MEDGAN), respectively, and to Dr. Inmaculada Alvarez for their excellent technical assistance.

References

Ajuyah, A.O., Ahn, D.U., Hardin, R.T., & Sim, J.S. (1993). Dietary antioxidants and storage affect chemical characteristics of ω-3 fatty acid enriched broiler chicken meat. *Journal of Food Science*, *58*, 43–61.

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.

Arshad, M.A., Anju, F.M., Khan, M.I., Yasin, M., Shahid, M., & El-Ghorab, M. (2011). Lipid stability and antioxidant profile of microsomal fraction of broiler meat enriched with α-lipoic acid and α-tocopherol. *Journal of Agricultural and Food Chemistry*, *59*, 7346–7352.

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., et al. (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.

Bañon, S., Diaz, P., Rodriguez, 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.

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.

Brenes, A., Viveros, A., Goñi, I., Centeno, C., Sáyago-Ayerdi, S., Arija, I., et al. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science*, *87*, 307–316.

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

Cao, F.L., Zhang, X.H., Yu, W.W., Zhao, L.G., & Wang, T. (2012). Effect of feeding fermented *Ginkgo biloba* leaves on growth performance, meat quality, and lipid metabolism in broilers. *Poultry Science*, *91*, 1210–1221.

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. *Meat Science*, *76*, 604–610.

Carreras, I., Guerrero, L., Guardia, M.D., Esteve-García, E., Garcia, J.A., Regueiro, J.A., et al. (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.

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

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

De Winne, A., & Dirinck, P. (1996). Studies on vitamin E and meat quality. 2. Effect of feeding high vitamin E levels on chicken meat quality. *Journal of Agricultural and Food Chemistry*, *44*, 1691–1696.

Decker, E.A. (1997). Phenolics: Prooxidants or antioxidants? *Nutrition Reviews*, *55*, 396–407.

European Committee for Standardization (1990). ES ISO 5508: Animal and vegetable fats and oils — Analysis by gas chromatography of methyl esters of fatty acids. Brussels, Belgium: European Standard ISO 5508. Eur. Comm. for Standardization.

Frank, J., Budek, A., Lundh, T., Parker, R.S., Swanson, J.E., & Lourenco (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.

Giannenas, I.A., Florou-Paneri, F., Botsoglou, N.A., Christaki, E., & Spais, A.B. (2005). Effect of supplementing feed with oregano and/or tocopheryl acetate on growth of broiler chickens and oxidative stability of meat. *Journal of Animal and Feed Sciences*, *14*, 521–535.

Gobert, M., Martin, B., Ferlay, A., Chilliard, Y., Graulet, B., Pradel, et al. (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.

Goñi, I., Brenes, A., Centeno, C., Viveros, A., Saura-Calixto, F., Rebolé, A., et al. (2007). Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility and susceptibility to meat lipid oxidation in chickens. *Poultry Science*, *86*, 508–516.

Gonthier, M.P., Cheynier, V., Donovan, J.L., Manach, C., Morand, C., & Mila, I. (2003). Microbial aromatic acid metabolites formed in the gut account for a major fraction of the polyphenols excreted in urine of rats fed red wine polyphenols. *Journal of Nutrition*, *133*, 461–467.

Gonthier, M.P., Verny, M.A., Besson, C., Rémésy, C., & Scalbert, A. (2003). Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *Journal of Nutrition*, *133*, 1853–1859.

Hashemipour, H., Kermanshahi, H., Golian, A., & Veldkamp, T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzymes activities, and immune response in broiler chickens. *Poultry Science*, *92*, 2059–2069.

Huff-Lonergan, E., & Lonergan, S.M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural change. *Meat Science*, *71*, 194–204.

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.

Jung, S., Choe, J.H., Kim, B., Yun, H., Kruk, Z.A., & Jo, B. (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.

Kamboh, A.A., & Zhu, W.Y. (2013). Effect of increasing levels of flavonoids in broiler feed on plasma oxidative potential, lipid metabolites, and fatty acid composition of meat. *Poultry Science*, *92*, 454–461.

Lafay, S., & Gil-Izquierdo, A. (2008). Bioavailability of phenolic acids. *Phytochemistry Reviews*, *7*, 301–311.

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.

Lee, M.R., Tweed, J.K., Kim, E.J., & Scollan, N.D. (2012). Beef, chicken and lamb fatty acid analysis — A simplified direct bimethylation procedure using freeze-dried material. *Meat Science*, *92*, 863–866.

Lekha, P., & Lonsane, K. (1997). Production and application of tannin acyl hydrolase: State of the art. *Advances in Applied Microbiology*, *44*, 215–260.

Lin, C.F., Gray, J.I., Asghar, A., Buckley, D.J., Booren, A.M., & Flegal, C.J. (1994). Effects of dietary oils and α-tocopherol supplementation on lipid composition and stability of broiler meat. *Journal of Food Science*, *54*, 1457–1460.

Lourenco, C.F., Gago, B., Barbosa, R.M., Freitas, V., & Laranjinha, J. (2008). LDL isolated from plasma-loaded red wine procyanidins resist lipid oxidation and tocopherol depletion. *Journal of Agricultural and Food Chemistry*, *56*, 3798–3804.

Marcincakova, D., Certik, M., Marcincak, S., Popelka, P., Simkova, J., Klempova, T., et al. (2011). Effect of dietary supplementation of *Melisa officinalis* and combination of *Achillea millefolium* and *Crataegus oxyacantha* on broiler growth performance, fatty acid composition and lipid oxidation of chicken meat. *Italian Journal of Animal Science*, *10*, 165–170.

Mielnick, 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.

Monagas, M., Urpi-Sarda, M., Sánchez-Patán, F., Llorach, R., Garrido, I., Gómez-Cordovés, C., et al. (2010). Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food & Function*, *1*, 233–253.

National Research Council (1994). *Nutrient requirements poultry* (9th rev. ed.). Washington, DC: Natl. Acad. Press.

Nkukwana, T.T., Muchenje, V., Masika, P.J., Hoffman, L.C., Dzama, K., & Descalzo, A.M. (2014). Fatty acids composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration. *Food Chemistry*, *142*, 255–261.

O'Neill, L.M., Galvin, K., Morrissey, P.A., & Buckley, D.J. (1998). Comparison of effects of dietary oil, tallow and vitamin E on the quality of broiler meat and meat products. *British Poultry Science*, *39*, 365–371.

Pazos, M., Gallardo, J.M., Torres, J.P., & Medina, I. (2005). Activity of grape polyphenols as inhibitors of fish lipids and frozen fish muscle. *Food Chemistry*, *92*, 547–557.

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.

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 & Technology*, *17*, 579–590.

Rebolé, A., Rodriguez, M.L., Ortiz, L.T., Alzueta, C., Centeno, C., Viveros, A., et al. (2006). Effect of dietary high-oleic acid sunflower seed, palm oil and vitamin E supplementation on broiler performance, fatty acid composition and oxidation susceptibility of meat. *British Poultry Science*, *47*, 581–591.

Requena, T., Monagas, M., Pozo-Bayón, M.A., Martín-Alvárez, P.J., Bartolomé, B., del Campo, R., et al. (2010). Perspectives of the potential implications of wine polyphenols on human oral and gut microbiota. *Trends in Food Science and Technology*, *21*, 332–344.

Sayago-Ayerdi, S.G., Brenes, A., & Goñi, I. (2009). Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburguers. *LWT Food Science and Technology*, *42*, 971–976.

Tavárez, M.A., Boler, D.D., Bess, K.N., Zhao, J., Yan, F., Dilger, A.C., et al. (2011). Effect of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. *Poultry Science*, *90*, 922–930.

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 recommendations 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.

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.

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.

Zanini, S.F., Torres, C.A.A., Bragagnolo, N., Turatti, J.M., Silva, M.G., & Zanini, M.S. (2004). Effect of oil sources and vitamin E levels in the diet on the composition of fatty acids in rooster thigh and chest meat. *Journal of the Science of Food and Agriculture*, *84*, 672–682.

Please cite this article as: Chamorro, S., et al., Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace, *Food Research International* (2014), http://dx.doi.org/10.1016/j.foodres.2014.11.054