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# Pre- and Post-Mortem Use of Grape Seed Extract in Dark Poultry Meat To Inhibit Development of Thiobarbituric Acid Reactive Substances

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Diets containing grape seed extract (GSE)—control, GSE [low GSE, low GSE + methionine, high GSE, and high GSE + methionine], or α-tocopherol—were fed to broiler chicks to estimate the antioxidative activity of GSE in processed meat. GSE was detrimental to the growth of chicks, and methionine did not reverse the detrimental effect. GSE with 85.4 g of gallic acid equiv/100 g (GAE 85.4) was added to ground dark turkey meat to obtain treatments with no GSE, 1.0% GSE, and 2.0% GSE and then processed as unsalted or salted and unheated or heated. Processed treatments were analyzed for thiobarbituric acid reactive substances (TBARS) and percent expressible moisture (%EM). GSE at 1.0 and 2.0% decreased TBARS values nearly 10-fold as compared to the control. GSE (1.0%) had a %EM value significantly greater than that of the control. GAE 85.4 decreased TBARS values more than GAE 88.9.

**KEYWORDS: Grape seed extract (GSE); poultry; turkey; chicken; lipid oxidation; thiobarbituric acid reactive substances (TBARS); antioxidant**

## INTRODUCTION

In 1979, the hypothesis that high wine consumption reduced coronary heart disease emerged (*1*). This was followed in 1992 by the expansion of the French paradox hypothesis suggesting that the French population, despite their relatively high fat consumption, had low incidences of heart disease due to alcohol consumption from wine (*2*). Their risk of coronary heart disease was similar to that of people consuming Mediterranean diets, which are much lower in fat. In 1993, phenolic compounds in the nonalcoholic fraction of red wine were found to have potent antioxidant activity toward oxidation of human low-density lipoprotein (LDL) (*3*). This was considered to be important because oxidized LDL had been shown to lead to atherosclerosis (*4*).

Flavonoids are the most abundant group of phenolics (*5*) and are antioxidants (*6*−*8*). The multiple mechanisms of flavonoid and polyphenol antioxidant functions include radical scavenging, metal chelation, and synergism with other antioxidants (*9*, *10*). More than 5000 compounds can be divided into 13 classes (*9*). Short chains (four or fewer monomers) are termed oligomeric procyanidins, whereas longer chains (five or more monomers) are known as tannins or proanthocyanidins (*11*). Flavonoids are found in foods derived from plant sources such as vegetables (cabbage), fruits (apples and grapes), herbs, legumes, grains, and tea leaves (*12*−*21*).

Byproducts of wine/grape juice processing provide an abundant source of flavonoid compounds (*22*−*27*). After grapes are pressed and the juice is collected, the remaining material is known as pomace. This material contains grape seeds, skins, and/or stems (*22*, *23*). Grape seeds (rich in proanthocyanidins) from grape juice and wine processing can be separated, extracted, dried, and purified into grape seed extract (GSE), which contains phenolic compounds (*11*, *25*, *28*−*30*).

GSE is reputed to have antioxidant activity when fed to animals (*31*). Grape seed tannins or proanthocyanidins have been shown to have a hypocholesterolemic, antiatherosclerotic, and antioxidant effect in vivo when fed to rats receiving diets with cholesterol (*31*−*33*). Fasted rats were administered GSE via intragastric intubation, and plasma was collected and incubated with oxidants (*34*). Results suggest that GSE protects blood plasma from oxidative stresses (*34*). Also, addition of grape seed proanthocyanidins (GSPC) to a system containing polyunsaturated fatty acids and mice liver or brain microsomes inhibited oxidation by UV light peroxidation (*35*).

Many in vitro studies have been conducted to examine the antioxidative properties of GSE. Grape extracts inhibited conjugated diene and hexanal formation in lecithin liposomes (*36*). Total phenolic content was highly correlated with relative percent inhibition of conjugated diene and hexanal (*36*). Another study showed that fresh grape extracts inhibited human LDL oxidation in vitro (*37*).

GSE has been suggested to have potential inhibitory effects in inflammation-related diseases (*38*). Reportedly, it has anticancer properties in mouse epidermis (*39*, *40*), mouse liver cells (*41*), and human prostate cells (tested in vitro) (*42*). In an

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experiment in which rats were given dietary GSE, it was shown to have a preventative effect against colon cancer but not against liver or mammary gland cancers (*43*). When rats were given GSE orally (along with sugars, fat, fiber, ash, organic acids, and protein) dissolved in water, the oligomers were found to have antiulcer properties (*44*). Others have reported that GSE was cytotoxic to some cancer cells while enhancing the growth of others (*45*).

GSE has been evaluated for its antioxidative effect in food. Nissen et al. (*46*) tested the oxidative stability of four natural antioxidants in dehydrated chicken meat that was mechanically deboned. The polyphenol content (millimoles per gram) of the antioxidants in grape skin (1.60), coffee (1.23), rosemary (0.92), and green tea (0.57) was determined (*46*). Analyses performed to determine the extent of lipid oxidation include electron spin resonance, hexanal, thiobarbituric acid reactive substances (TBARS), sensory evaluation, and conjugated dienes. The investigators found that the order of efficiency in inhibiting lipid oxidation was rosemary ~ synthetic antioxidants > coffee ~ tea > grape skin > control. Although grape skin was least efficient in decreasing lipid oxidation, it was considerably more effective in retarding it than the control sample (no antioxidants).

The molar ratio of polyunsaturated fatty acids to tocopherol in dark poultry meat causes it to become more susceptible to lipid oxidation than light meat, especially during processing when meat is exposed to prooxidant conditions such as light, heat, and grinding (*47*). To retard lipid oxidation in stored poultry products made from dark meat, many antioxidants [α-tocopherol and rosemary (*48*−*52*) and green tea catechins and herbs (*53*−*55*)] have been added pre- and post-mortem.

Due to its reported antioxidative properties, it was hypothesized that GSE would inhibit lipid oxidation in dark poultry meat. For the pre-mortem addition, GSE with amino acid supplementation was fed to birds to estimate its activity as an antioxidant in harvested, further processed meat. For post-mortem addition, GSE was added, without or with 50% NaCl/50% KCl, and after processing and storage, its antioxidative capacity was estimated.

## MATERIALS AND METHODS

**Materials.** DL-Methionine (99% purity) and α-tocopherol acetate (500 IU/g) were obtained from Aventis (Antony, France) and Hoffmann-La Roche Inc. (Nutley, NJ), respectively. Turkey thigh meat was obtained from a local processor. Grape seed extract was obtained from Polyphenolics, Inc. (Burlingame, CA). The three lots of GSE used contained the following amounts of total phenolics expressed as gallic acid equivalents (GAE) by the Folin method (grams of GAE per 100 g): 85.4, 88.9, and 90.2 g of GAE/100 g. Future reference to lots with the above concentrations will be GAE 85.4, GAE 88.9, and GAE 90.2. GAE 85.4 and GAE 88.9 were used in preparing meat patties, whereas GAE 90.2 was used in the preliminary feeding trial. A 50/50 mixture of NaCl and KCl was obtained from Morton Salt (Chicago, IL). Butylated hydroxyanisole (BHA), 1,1,3,3-tetraethoxypropane (TEP), and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO). Perchloric acid and ethanol were purchased from Fisher Scientific (Pittsburgh, PA). Whatman (Clifton, NJ) filter papers 2V (150 mm), 3 (55 mm), and 50 (70 mm) were used. Tulle (netting) was purchased from a local fabric store.

GSE and 50% NaCl/50% KCl (a prooxidant) were always added to meat on a w/w basis, weight of substance by weight of meat. NaCl/KCl is subsequently referred to as salt.

**Preliminary Feeding Trial.** Ninety Cobb × Cobb male and female 4-day-old chicks were separated into 18 pens of 5 birds. Three replicates (15 birds total) received one of the following six diets after receiving the control diet for 1 week: control, low GSE (2.59% of diet), low GSE (2.59%) plus supplemental methionine (0.15%), high GSE (5.18%), high GSE (5.18%) plus supplemental methionine (0.15%), or α-tocopherol (0.01%). As noted above, GSE used in feed was GAE 90.2. Basal diets were prepared according to the Nutrient Requirements of Poultry (*56*). Feed and water were administered ad libitum. Conditions for light, heat, housing, and slaughtering were approved by the University of California, Davis, Animal Care and Use Committee (protocol 8655). Feed and bird weights were recorded on the seventh day of each week for 4 weeks.

**Preliminary Experiment: Preparation of Dark Poultry Meat Patties for GAE 85.4 Added Post-mortem at 0−0.1%.** Raw, skinless, boneless turkey thigh meat was stored on ice in plastic bags, packed in ice-containing coolers, and transported from a local processor to the laboratory within 4 h after slaughter. Meat was stored at −80 °C until further processed in the laboratory. Meat was allowed to thaw overnight, protected from light, at room temperature (~23 °C). Excess fat and membrane were removed from the meat (1 kg), and the meat was cut into 2.5 $cm^2$ cubes before being ground for 20 s in a food processor (Cuisinart, model DLC 7 Super Pro, Norwich, CT). All meat was mixed with a hand-held utensil for 5 min before the addition of GSE. After the addition of ultrapure water (2.0% v/w), meat was mixed for an additional 5 min.

Samples were prepared for a 2 × 2 × 6 factorial experiment with variables of unheated/heated; storage periods of 3 and 6 days at 7 °C with fluorescent light; and 0 (control), 0.005, 0.01, 0.03, 0.05, or 0.10% GSE. At least two patties (90 g each) were prepared for each time period within each treatment and stored for 3 days at 7 °C with light. Patties were heated (if necessary) for 12 min according to the procedure of King and Bosch (*57*) and stored for an additional 3 or 6 days before further analyses (see below) were performed. At least duplicate samples were analyzed; the experiment was not repeated.

**Preparation of Dark Poultry Meat Patties for GAE 85.4 Added Post-mortem at 0−2.0%.** The delivery and handling of meat were as noted above. The preparation was similar except that each treatment of 0, 1.0, and 2.0% GSE included a factor for unsalted heated, salted unheated, and salted heated. After grinding, salt was added and mixed for 5 min before the addition of GSE and ultrapure water.

The same storage periods and conditions noted above were followed. Two to three investigators analyzed samples in duplicate. The entire experiment was repeated. There were no fewer than eight observations for each salt/heat combination within each storage condition for each treatment.

**Preparation of Dark Poultry Meat Patties for GAE 88.9 Added Post-mortem at 0−2.0%.** GAE 88.9 contained 3.5% more total phenolics than GAE 85.4. Therefore, analyses were performed to determine whether the lots would differ in their ability to affect TBARS values. Meat source and meat handling were the same as noted above. Meat preparation was similar except that meat samples were prepared only as salted without heat. The same storage time and conditions were followed. The resulting experimental design was a 2 × 3 factorial with variables of storage periods of 3 and 6 days at 7 °C with fluorescent light and 0, 1.0, or 2.0% GSE. Triplicate samples (patties) were analyzed by two investigators. The entire experiment was repeated. There were no fewer than 12 observations for each storage condition within treatment.

**Thiobarbituric Acid Reactive Substances and Percent Expressible Moisture.** The TBARS method (*58*) is a colorimetric method that primarily measures malonaldehyde, which complexes with thiobarbituric acid. However, other substances (metals, decomposition and breakdown products of protein, and carbohydrates) may react with thiobarbituric acid, causing an overestimation of TBARS. Thus, this method was used to *approximate* lipid oxidation in variously processed meat. Ten milliliters of aqueous solutions containing meat with 1.0 or 2.0% GSE, perchloric acid, BHA, and thiobarbituric acid was analyzed.

The method of Earl et al. (*59*) was used to determine percent expressible moisture (%EM) for 0 and 1.0% GSE with salt/heat combinations of unsalted unheated, unsalted heated, salted unheated, and salted heated. Quadruplicate samples were analyzed by two investigators resulting in 16 observations for each salt/heat combination within treatments.

**Preliminary Sensory Evaluation.** An untrained sensory panel of three evaluators compared patties containing 1.0% GSE and the control.

**Table 1.** Average Final Weight and Weight Gain of 4-Week-Old Birds Fed GAE 90.2 for 3 Weeks[a]

| diet[b] | av final bird wt (g) | av wt gain (g)/bird[c] |
|---|---|---|
| control | 843 | 644 |
| low GSE (2.59%) | 452 | 245 |
| low GSE + methionine (0.15%)[d] | 541 | 330 |
| high GSE (5.18%) | 217 | 11 |
| high GSE + methionine (0.15%)[d] | 228 | 5 |
| α-tocopherol (0.01%) | 998 | 785 |

[a] $n = 15$. [b] All birds were fed the control diet during the first week. Grape seed extract (GSE) and methionine were added as a percent of the diet. [c] Cumulative data from weeks 2–4, when birds were fed experimental diets.

**Statistical Analysis.** Data for TBARS values and %EM for all repeated experiments were analyzed by methods of ANOVA and PROC GLM (SAS Institute, version 8.1, Cary, NC). Means were compared by Tukey's HSD Student *t* test to determine significance at $p < 0.05$.

## RESULTS AND DISCUSSION

**Feeding Experiment.** A preliminary study was conducted to test the efficacy of GSE, added as a feed ingredient, in the post-mortem dark meat of birds. Due to the severity of growth depression observed for the treatments containing GSE, the feeding study was not repeated. Dietary addition of an antioxidant, such as α-tocopherol, rather than post-mortem addition to meat, allows it to be positioned closely to unsaturated lipids in membranes to exert its greatest effect as an antioxidant (*60*−*62*). Although statistical analysis could not be performed on the single preliminary study, some observations from it, however, are relevant to the future use of GSE as a feed ingredient for poultry. High-GSE diets seemed to result in poor growth as compared to a control **(Table 1)**. α-Tocopherol diets had the highest numerical value for growth. Dietary methionine supplementation seemed to be ineffective in counteracting the tannin effect.

Previously, Elkin et al. (*63*) fed ducks and chicks diets with sorghum grain (contains tannins). These diets contained between 0.05 and 5.60% catechin equivalents (CE), a measure of the amount of tannins. Addition of supplemental methionine to high-CE diets eliminated the negative weight gain effect caused by tannin consumption. Observations from the preliminary study reported here are different from those of Elkin et al. (*63*) possibly because GSE used in the present study was in a purer form than that in grains. Contrary to results demonstrating alleviation of the tannin effect with supplemental methionine (*63*, *64*), another study indicated that tannins depressed growth in chicks despite methionine supplementation (*65*). Our observations seemed to be in agreement with the latter results (*65*).

**Thiobarbituric Acid Reactive Substances Values.** *Preliminary Study: GAE 85.4 Added Post-mortem at 0*−*0.1%*. As shown in **Table 2**, the overall results for 0.005−0.05% GSE were not clear. However, at 0.10% GSE, reduced TBARS values were observed as compared to the control and 0.005% GSE. Results from this preliminary study as well as that from another experiment (*66*) indicated that a quantity of GSE >0.1% would decrease TBARS values. Due to the inconsistency of TBARS values, larger quantities of GSE were used to assess its antioxidative capacity; 1.0 and 2.0% GSE were used in subsequent studies.

*GAE 85.4 Added Post-mortem at 0*−*2.0%*. Results showed significant differences among investigators performing the experiments. However, the trends for results from each analyst were always the same. For the main effect of treatment, addition of GSE at 1.0 and 2.0% decreased TBARS values nearly 10-fold as compared to the control (**Table 3**). In general, TBARS values at 1.0% GSE were numerically lower than values at 2.0% GSE. Results from **Table 3** along with that from the preliminary experiment for GAE 85.4 suggest that the optimum GSE level in poultry meat is probably between 0.1 and 1.0%. Other investigators have noted that in a bulk oil system, greater hydroperoxide inhibition corresponded to a lower concentration of α-tocopherol rather than a higher concentration (*67*). One possibility is that phenolic compounds, even in different systems, exhibit similar trends. More experiments need to be conducted to test this hypothesis. It is also likely that phenolic antioxidants, such as α-tocopherol and most likely GSE, can become prooxidants at high concentrations because of their propensity to act as chain carriers (*68*). In addition, high concentrations of antioxidants can reduce metals to a more catalytically active lower valence state, thus promoting oxidation (*68*).

*GAE 88.9 Added Post-mortem at 0*−*2.0%*. Results for TBARS values for 1.0 and 2.0% GSE were significantly ($p < 0.05$) lower than the control (**Table 4**). Also, 1.0% GSE produced values significantly ($p < 0.05$) lower than 2.0% GSE. This latter finding supports the observation noted above that GSE at higher levels becomes prooxidative.

*GAE 85.4 Compared to GAE 88.9 Added Post-mortem at 0*−*2.0%*. The TBARS values for the two lots were significantly ($p < 0.05$) different from each other (**Table 4**). Despite having 3.5% less total phenolics, GAE 85.4 decreased TBARS values more than GAE 88.9. One possible explanation is that the procyanidolic value (an indicator of oligomeric procyanidins,

**Table 2.** Preliminary Thiobarbituric Acid Reactive Substances (TBARS) Values for GAE 85.4 Added Post-mortem to Dark Poultry Meat[a]

| | TBARS values (mg of malonaldehyde/kg of meat) | | |
|---|---|---|---|
| treatment | unheated | heated | overall effect |
| control | $_{y}0.680 \pm 0.566^{b}$ | $_{y}0.952 \pm 0.233^{bc}$ | $0.831^{bc}$ |
| 0.005% GSE | $_{y}0.816 \pm 0.720^{b}$ | $_{y}1.089 \pm 0.214^{b}$ | $0.952^{b}$ |
| 0.01% GSE | $_{y}0.609 \pm 0.506^{b}$ | $_{z}0.976 \pm 0.777^{bc}$ | $0.792^{bc}$ |
| 0.03% GSE | $_{y}0.708 \pm 0.610^{b}$ | $_{z}0.405 \pm 0.448^{c}$ | $0.556^{cd}$ |
| 0.05% GSE | $_{y}0.517 \pm 0.479^{b}$ | $_{y}0.798 \pm 0.651^{bc}$ | $0.645^{bcd}$ |
| 0.10% GSE | $_{y}0.000 \pm 0.000^{c}$ | $_{z}0.567 \pm 0.228^{bc}$ | $0.354^{d}$ |

[a] $n = 6$ for unheated (0.005, 0.01, 0.03, and 0.05%) and heated (0.005, 0.01, and 0.03); $n = 5$ for heated (0, 0.5, and 0.10%); $n = 4$ for unheated (0%); and $n = 3$ for unheated (0.10%). GSE was added on a w/w basis, weight of substance by weight of meat. Means with different superscripts (b–d) in columns are significantly different at $p < 0.05$. Means with different subscripts (y–z) in rows are significantly different at $p < 0.05$.

**Table 3.** Overall Thiobarbituric Acid Reactive Substances (TBARS) Values for GAE 85.4 Added Post-mortem to Dark Poultry Meat[a]

| | TBARS values (mg of malonaldehyde/kg of meat) | | | |
|---|---|---|---|---|
| | unsalted | | salted | |
| treatment | unheated | heated | unheated | heated |
| control | *b* | $13.929 \pm 3.647^{c}$ | $9.886 \pm 1.686^{c}$ | $10.556 \pm 1.597^{c}$ |
| 1.0% GSE | *b* | $0.484 \pm 0.102^{d}$ | $1.096 \pm 0.335^{d}$ | $0.708 \pm 0.227^{d}$ |
| 2.0% GSE | *b* | $0.738 \pm 0.157^{d}$ | $1.071 \pm 0.397^{d}$ | $1.123 \pm 0.451^{e}$ |

[a] GSE and 50% NaCl/50% KCl were always added to meat on a w/w basis, weight of substance by weight of meat. 50% NaCl/50% KCl is referred to as salt. $n = 36$ for salted and heated control; $n = 32$ for salted and heated 1.0% GSE; $n = 28$ for salted and heated 2.0% GSE; $n = 24$ for unsalted and heated control and salted and unheated control and 1.0% GSE; and $n = 21$ for unsalted and heated 1.0% GSE and salted and unheated 2.0% GSE; $n = 18$ for unsalted and heated 2.0% GSE. Means with different superscripts (c–e) in columns are significantly different at $p < 0.05$. [b] Data not available.

**Table 4.** Comparison of Thiobarbituric Acid Reactive Substances (TBARS) Values for GAE 85.4 and GAE 88.9 Added Post-mortem to Dark Poultry Meat[a]

| | TBARS values (mg of malonaldehyde/kg of meat) | |
|---|---|---|
| treatment[b] | GAE 85.4 | GAE 88.9 |
| control | $_y9.886 \pm 1.686^c$ | $_z7.420 \pm 1.762^c$ |
| 1.0% GSE | $_y1.096 \pm 0.335^d$ | $_z1.610 \pm 0.314^e$ |
| 2.0% GSE | $_y1.071 \pm 0.397^d$ | $_z2.513 \pm 0.792^d$ |

[a] GSE and 50% NaCl/50% KCl were always added to meat on a w/w basis, weight of substance by weight of meat. 50% NaCl/50% KCl is referred to as salt. $n = 24$ for all values, combined over 3 and 6 days, except for GAE 85.4, 2.0%, where $n = 21$. Means with different superscripts (c–e) in columns are significantly different at $p < 0.05$. Means with different subscripts (y–z) in rows are significantly different at $p < 0.05$. [b] All treatments contained salt and were unheated.

**Table 5.** Percent Expressible Moisture (%EM) for GAE 85.4 Added Post-mortem to Dark Poultry Meat[a]

| | % EM | | | |
|---|---|---|---|---|
| | unsalted | | salted | |
| treatment | unheated | heated | unheated | heated |
| control | $35.145 \pm 3.633^b$ | $25.168 \pm 2.082^b$ | $20.747 \pm 2.917^b$ | $29.305 \pm 4.188^b$ |
| 1.0% GSE | $43.836 \pm 3.333^c$ | $29.084 \pm 2.253^c$ | $37.474 \pm 3.635^c$ | $34.078 \pm 2.056^c$ |

[a] GSE and 50% NaCl/50% KCl were always added to meat on a w/w basis, weight of substance by weight of meat. 50% NaCl/50% KCl is referred to as salt. $n = 16$. Means with different superscripts (b–c) in columns are significantly different at $p < 0.05$.

dry basis) for GAE 85.4 was 141.5, whereas that for GAE 88.9 was 107.7. Oligomers of procyanidins were found to be more effective antioxidants than monomers in a radical scavenging assay (*69*) and in in vivo experiments in which rats were fed cholesterol and GSE (*31*, *32*).

**Percent Expressible Moisture, GAE 85.4.** All control samples had significantly lower ($p < 0.05$) %EM than 1.0% GSE, indicating that the additive decreased the overall water-holding capacity of the meat (**Table 5**). Observations revealed that GSE is a dry powdery substance. Its addition to meat resulted in a crumbly patty, lacking cohesion. Thus, the consistency of GSE seems to be detrimental to the water-holding capacity of meat. Technological manipulation is needed to improve its commercial applicability to the meat industry.

**Other Observations.** GSE patties made with 0 and 1.0% GAE 85.4 were evaluated for taste. Observations by three untrained sensory evaluators noted an odor of wine, a masking of the mild chicken flavor, and a slightly bitter aftertaste in GSE patties. However, samples with GSE were not rated as objectionable compared to a control. In addition, GSE patties were darker in color as compared to the control.

## ABBREVIATIONS USED

LDL, low-density lipoprotein; GSE, grape seed extract; NaCl, sodium chloride; KCl, potassium chloride; GAE, gallic acid equivalents; BHA, butylated hydroxyanisole; TEP, 1,1,3,3-tetraethoxypropane; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; CE, catechin equivalents; %EM, percent expressible moisture.

## LITERATURE CITED

(1) St. Leger, A. S.; Cochrane, A. L.; Moore, F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet* **1979**, *1*, 1017−1020.

(2) Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

(3) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

(4) Witztum, J. L.; Steinberg, D. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends Cardiovasc. Med.* **2001**, *11*, 93−102.

(5) Thomson, R. H. Structure and reactivity of phenolic compounds. In *Biochemistry of Phenolic Compounds*; Harborne, J. B., Ed.; Academic Press: London, U.K., 1964.

(6) Richardson, G. A.; El-Rafey, M. S.; Long, M. L. Flavones and flavone derivatives as antioxidants. *J. Dairy Sci.* **1947**, *30*, 397−413.

(7) Mehta, A. C.; Seshadri, T. R. Flavonoids as antioxidants. *J. Sci. Ind. Res.* **1958**, *18B*, 24−28.

(8) Rice-Evans, C. A.; Miller, N. J. Antioxidant activities of flavonoids as bioactive components of food. *Biochem. Soc. Trans.* **1996**, *24*, 790−795.

(9) Bors, W.; Heller, W.; Michel, C.; Stettmaier, K. Flavonoids and polyphenols: chemistry and biology. In *Handbook of Antioxidants*; Cadenas, E., Packer, L., Eds.; Dekker: New York, 1996.

(10) Cadenas, E. Basic mechanisms of antioxidant activity. *BioFactors* **1997**, *6*, 391−397.

(11) Peng, Z.; Hayasaka, Y.; Iland, P. G.; Sefton, M.; Hoj, P.; Waters, E. J. Quantitative analysis of polymeric procyanidins (tannins) from grape (*Vitis vinifera*) seeds by reverse phase high-performance liquid chromatography. *J. Agric. Food Chem.* **2001**, *49*, 26−31.

(12) Shahidi, F.; Naczk, M. *Food Phenolics: Sources, Chemistry, Effects, Applications*; Technomic Publishing: Lancaster, PA, 1995.

(13) Pratt, D. E.; Watts, B. M. The antioxidant activity of vegetable extracts I. Flavone aglycones. *J. Food Sci.* **1964**, *29*, 27−33.

(14) Miyake, T.; Shibamoto, T. Antioxidative activities of natural compounds found in plants. *J. Agric. Food Chem.* **1997**, *45*, 1819−1822.

(15) MacNeil, J. H.; Dimick, P. S.; Mast, M. G. Use of chemical compounds and a rosemary spice extract in quality maintenance of deboned poultry meat. *J. Food Sci.* **1973**, *38*, 1080−1081.

(16) Pearson, D. A.; Frankel, E. N.; Aeschbach, R.; German, J. B. Inhibition of endothelial cell-mediated oxidation of low-density lipoprotein by rosemary and plant phenolics. *J. Agric. Food Chem.* **1997**, *45*, 578−582.

(17) Wang, S. Y.; Lin, H.-S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140−146.

(18) Chang, S.; Tan, C.; Frankel, E. N.; Barrett, D. M. Low-density lipoprotein antioxidant activity of phenolic compounds and polyphenol oxidase activity in selected clingstone peach cultivars. *J. Agric. Food Chem.* **2000**, *48*, 147−151.

(19) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.

(20) Lakenbrink, C.; Lapczynski, S.; Maiwald, B.; Engelhardt, U. H. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *J. Agric. Food Chem.* **2000**, *48*, 2848−2852.

(21) Toschi, T. G.; Bordoni, A.; Hrelia, S.; Bendini, A.; Lercker, G.; Biagi, P. L. The protective role of different green tea extracts after oxidative damage is related to their catechin composition. *J. Agric. Food Chem.* **2000**, *48*, 3973−3978.

(22) Waterhouse, A. L.; Walzem, R. L. Nutrition of grape phenolics. In *Flavonoids in Health and Disease*; Rice-Evans, C., Packer, L., Eds.; Dekker: New York, 1998; pp 359−385.

(23) El Boushy, A. R. Y.; van der Poel, A. F. B. Fruit, vegetable and brewers' waste. In *Poultry Feed from Waste: Processing and Use*; Chapman and Hall: London, U.K., 1994; pp 187−340.

(24) Lu, Y.; Yeap Foo, L. The polyphenol constituents of grape pomace. *Food Chem.* **1999**, *65*, 1−8.

(25) Ricardo da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidin dimers and trimers from grape seeds. *Phytochemistry* **1991**, *30*, 1259−1264.

(26) Yang, Y.; Chien, M.; Yang, Y.; Chien, M. J. Characterization of grape procyanidins using high-performance liquid chromatography/mass spectrometry and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 3990−3996.

(27) Souquet, J.-M.; Labarbe, B.; Le Guerneve, C.; Cheynier, V.; Moutounet, M. Phenolic composition of grape stems. *J. Agric. Food Chem.* **2000**, *48*, 1076−1080.

(28) Masquelier, J. Plant extract with a proanthocyanidins content as therapeutic agent having radical scavenger effect and use thereof. In *United States Patent and Trademark Office*; Societe Civile d'Investigations Pharmacologiques d'Aquitaine (Bordeaux, FR); Horphag Overseas: St. Peter Port, Guernsey, 1987.

(29) de Freitas, V. A. P.; Glories, Y.; Bourgeois, G.; Vitry, C. Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. *Phytochemistry* **1998**, *49*, 1435−1441.

(30) Waterhouse, A. L.; Ignelzi, S.; Shirley, J. R. A comparison of methods for quantifying oligomeric proanthocyanidins from grape seed extracts. *Am. J. Enol. Vitic.* **2000**, *51*, 383−389.

(31) Tebib, K.; Rouanet, J. M.; Besancon, P. Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol−vitamin E-deficient diet. *Food Chem.* **1997**, *59*, 135−141.

(32) Tebib, K.; Bitri, L.; Besancon, P.; Rouanet, J. M. Polymeric grape seed tannins prevent plasma cholesterol changes in high-cholesterol-fed rats. *Food Chem.* **1994**, *49*, 403−406.

(33) Yamakoshi, J.; Kataoka, S.; Koga, T.; Ariga, T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* **1999**, *142*, 139−149.

(34) Koga, T.; Moro, K.; Nakamori, K.; Yamakoshi, J.; Hosoyama, H.; Kataoka, S.; Ariga, T. Increase of antioxidative potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape seeds. *J. Agric. Food Chem.* **1999**, *47*, 1892−1897.

(35) Bouhamidi, R.; Prevost, V.; Nouvelot, A. High protection by grape seed proanthocyanidins (GSPC) of polyunsaturated fatty acids against UV-C induced peroxidation. *C. R. Acad. Sci. III* **1998**, *321*, 31−38.

(36) Yi, O.; Meyer, A. S.; Frankel, E. N.; Yi, O. S. Antioxidant activity of grape extracts in a lecithin liposome system. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1301−1307.

(37) Frankel, E. N.; Meyer, A. S. Antioxidants in grapes and grape juices and their potential health effects. *Pharm. Biol.* **1998**, *36*, 14−20.

(38) Sen, C. K.; Bagchi, D. Regulation of inducible adhesion molecule expression in human endothelial cells by grape seed proanthocyanidin extract. *Mol. Cell. Biochem.* **2001**, *216*, 1−7.

(39) Bomser, J. A.; Singletary, K. W.; Wallig, M. A.; Smith, M. A. Inhibition of TPA-induced tumor promotion in CD-1 mouse epidermis by a polyphenolic fraction from grape seeds. *Cancer Lett.* **1999**, *135*, 151−157.

(40) Zhao, J.; Wang, J.; Chen, Y.; Agarwal, R. 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* **1999**, *20*, 1737−1745.

(41) Ray, S. D.; Kumar, M. A.; Bagchi, D. A novel proanthocyanidin IH636 grape seed extract increases in vivo Bcl-XL expression and prevents acetaminophen-induced programmed and unprogrammed cell death in mouse liver. *Arch. Biochem. Biophys.* **1999**, *369*, 42−58.

(42) Agarwal, C.; Sharma, Y.; Agarwal, R. Anticarcinogenic effect of a polyphenolic fraction isolated from grape seeds in human prostate carcinoma DU145 cells: modulation of mitogenic signaling and cell-cycle regulators and induction of G1 arrest and apoptosis. *Mol. Carcinog.* **2000**, *28*, 129−138.

(43) Singletary, K. W.; Meline, B. Effect of grape seed proanthocyanidins on colon aberrant crypts and breast tumors in a rat dual-organ tumor model. *Nutr. Cancer* **2001**, *39*, 252−258.

(44) Saito, M.; Hosoyama, H.; Ariga, T.; Kataoka, S.; Yamaji, N. Antiulcer activity of grape seed extract and procyanidins. *J. Agric. Food Chem.* **1998**, *46*, 1460−1464.

(45) Ye, X.; Krohn, R. L.; Liu, W.; Joshi, S. S.; Kuszynski, C. A.; McGinn, T. R.; Bagchi, M.; Preuss, H. G.; Stohs, S. J.; Bagchi, D. The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells. *Mol. Cell. Biochem.* **1999**, *196*, 99−108.

(46) Nissen, L. R.; Mansson, L.; Bertelsen, G.; Tuong Huynh, B.; Skibsted, L. H. Protection of dehydrated chicken meat by natural antioxidants as evaluated by electron spin resonance spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 5548−5556.

(47) Botsoglou, N. A.; Florou-Paneri, P.; Christaki, E.; Fletouris, D. J.; Spais, A. B. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *Br. Poult. Sci.* **2002**, *43*, 223−230.

(48) King, A. J.; Uijttenboogaart, T. G.; deVries, A. W. α-Tocopherol, β-carotene and ascorbic acid as antioxidants in stored poultry muscle. *J. Food Sci.* **1995**, *60*, 1009−1012.

(49) Lopez Bote, C. J.; Gray, J. I.; Gomaa, E. A.; Flegal, C. J. Effect of dietary oat administration on lipid stability in broiler meat. *Br. Poult. Sci.* **1998**, *39*, 57−61.

(50) Barbut, S.; Josephson, D. B.; Maurer, A. J. Antioxidant properties of rosemary oleoresin in turkey sausage. *J. Food Sci.* **1985**, *50*, 1356−1359.

(51) Wong, J. W.; Hashimoto, K.; Shibamoto, T. Antioxidant activities of rosemary and sage extracts and vitamin E in a model meat system. *J. Agric. Food Chem.* **1995**, *43*, 2707−2712.

(52) Lopez Bote, C. J.; Gray, J. I.; Gomaa, E. A.; Flegal, C. J. Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. *Br. Poult. Sci.* **1998**, *39*, 235−240.

(53) Sano, M.; Sasaki, K.; Tomita, I.; Ikeya, M.; Torii, Y.; Koizumi, Y.; Kodomari, S. Effects of tea leaf (*Camellia sinensis* L.) feeding on the freshness of broiler chickens. *J. Food Hyg. Soc. Jpn.* **1996**, *37*, 38−42.

(54) Shahidi, F.; Alexander, D. M. Green tea catechins as inhibitors of oxidation of meat lipids. *J. Food Lipids* **1998**, *5*, 125−133.

(55) El-Alim, S. S. L. A.; Lugasi, A.; Hovari, J.; Dworschak, E. Culinary herbs inhibit lipid oxidation in raw and cooked minced meat patties during storage. *J. Sci. Food Agric.* **1999**, *79*, 277−285.

(56) *Nutrient Requirements of Poultry*, 9th revised ed.; National Research Council (U.S.) Subcommittee on Poultry Nutrition; National Academy Press: Washington, DC, 1994.

(57) King, A. J.; Bosch, N. Effect of NaCl and KCl on rancidity of dark turkey meat heated by microwave. *J. Food Sci.* **1990**, *55*, 1549−1551.

(58) Salih, A. M.; Smith, D. M.; Price, J. F.; Dawson, L. E. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poult. Sci.* **1987**, *66*, 1483−1488.

(59) Earl, L. A.; King, A. J.; Fitzpatrick, D. P.; Cooper, J. E. A modification of a method to determine expressible moisture in ground, dark poultry meat. *Poult. Sci.* **1996**, *75*, 1433−1436.

(60) Marusich, W. L.; De Ritter, E.; Ogrinz, E. F.; Keating, J.; Mitrovic, M.; Bunnell, R. H. Effect of supplemental vitamin E in control of rancidity in poultry meat. *Poult. Sci.* **1975**, *54*, 831−844.

(61) Higgins, F. M.; Kerry, J. P.; Buckley, D. J.; Morrissey, P. A. Dietary supplementation versus direct post mortem addition of α-tocopherol on lipid and colour stability in cooked turkey breast patties. *Food Res. Int.* **1998**, *31*, 205−209.
(62) Renerre, M.; Poncet, K.; Mercier, Y.; Gatellier, P.; Metro, B. Influence of dietary fat and vitamin E on antioxidant status of muscles of turkey. *J. Agric. Food Chem.* **1999**, *47*, 237−244.
(63) Elkin, R. G.; Rogler, J. C.; Sullivan, T. W. Comparative effects of dietary tannins in ducks, chicks, and rats. *Poult. Sci.* **1990**, *69*, 1685−1693.
(64) Fuller, H. L.; Chang, S. I.; Potter, D. K. Detoxication of dietary tannic acid by chicks. *J. Nutr.* **1967**, *91*, 477−481.
(65) Vohra, P.; Kratzer, F. H.; Joslyn, M. A. The growth depressing and toxic effects of tannins to chicks. *Poult. Sci.* **1966**, *45*, 135−142.
(66) Harel, S., Personal correspondence; ARO Volcani Center, Department of Food Science, Israel, 1998.
(67) Frankel, E. N.; Huang, S.-W.; Kanner, J.; German, J. B. Interfacial phenomena in the evaluation of antioxidants: Bulk oils vs emulsions. *J. Agric. Food Chem.* **1994**, *42*, 1054−1059.
(68) Frankel, E. N. *Lipid Oxidation*; Oily Press: Dundee, Scotland, 1998.
(69) Castillo, J.; Benavente-Garcia, O.; Lorente, J.; Alcaraz, M.; Redondo, A.; Ortuno, A.; Del Rio, J. A. Antioxidant activity and radioprotective effects against chromosomal damage induced in vivo by X-rays of flavan-3-ols (procyanidins) from grape seeds (*Vitis vinifera*): Comparative study versus other phenolic and organic compounds. *J. Agric. Food Chem.* **2000**, *48*, 1738−1745.

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