Effect of Grape Seed Extract on Physicochemical Properties of Ground, Salted, Chicken Thigh Meat during Refrigerated Storage at Different Relative Humidity Levels

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ABSTRACT: The effect of grape seed extract (GSE, 0.1%) ± NaCl (1%) in ground chicken thigh meat during refrigerated storage at 59%, 76%, 88%, and 99% relative humidity (RH) was examined. Compared to the untreated control, GSE (0.1%) delayed the reduction of water activity (a_w) that occurred during refrigerated storage at different relative humidity levels but had no effect on moisture content or pH compared to the untreated control. GSE inhibited the formation of a secondary marker of lipid oxidation (TBARS) compared to the untreated control and altered the effect of NaCl on TBARS formation. The formation of TBARS was affected by RH level across all treatment groups in the order of 99% > 88% > 76% > 59%. Further analysis revealed that this effect likely is due to the presence of NaCl, which suggests that RH storage does not affect the formation of TBARS except in salted patties, the effect of which is mitigated by the addition of GSE. NaCl, but not GSE, increased both sarcoplasmic and myofibrillar protein solubility after 12 d of refrigerated storage, suggesting increased protein denaturation. This study shows that GSE is an effective antioxidant in ground chicken thigh meat that does not affect moisture content or pH during storage, inhibits TBARS formation, helps to mitigate the prooxidative effects of NaCl, and may alter the effect of NaCl on protein solubility in salted chicken patties. Future work is needed to determine how the physicochemical interactions of GSE affect important cooked meat quality attributes.

Keywords: antioxidant, grape seed extract, protein solubility, relative humidity, sodium chloride, TBARS, water activity

Introduction

Grape seed extract (GSE) is rich in polyphenolic compounds (Weber and others 2007) and has received a great deal of research attention in recent years, initially due to the discovery of its health promoting effects and later in the context of its antioxidative potential as a food ingredient. GSE polyphenolics are primarily condensed tannins, a.k.a. proanthocyanidins, usually oligomers and polymers of polyhydroxy flavan-3-ols such as (+)-catechin and (-)-epicatechin, many in the form of gallate esters or glycosides (Weber and others 2007). A characteristic of tannins long known to food science is their interaction with proteins. Tannins form both soluble and insoluble complexes with proteins and were generally regarded as factors that decreased the nutritional quality of food proteins (Bravo 1998). However, recent evidence strongly points to the health-promoting effect of polyphenolics in general and those found in GSE in particular. Several comprehensive reviews of the effects of GSE on health promotion exist (Cos and others 2004; Kar and others 2006). As a food ingredient, the efficacy of GSE or other grape extracts has been tested in a variety of systems, including systems rather than raw systems, although their experimental data and other research findings contradict this speculation. They report that nearly 6-fold higher TBARS values were observed for an untreated control compared to GSE-containing raw beef stored for 9 d at refrigeration. Brannan and Mah (2007) recently demonstrated that 0.1% GSE is an effective antioxidant in raw chicken breast and thigh during both frozen and refrigerated storage, which agrees with previous research in raw turkey (Lau and King 2003) and raw frozen fish (Pazos and others 2004).

In cooked meat systems, Ahn and others (2002, 2007) revealed that a commercial GSE (ActiVin®) was as effective as BHT/BHA and a commercial rosemary extract (Herbalox®) at reducing TBARS formation and warmed over flavor in cooked beef after 9 d of refrigerated storage. Brannan and Mah (2007) report that 0.1% GSE completely inhibited the formation of lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) in cooked beef, pork, chicken breast, and thigh after 7 d of refrigerated storage. Their research also revealed that GSE is an effective antioxidant in cooked chicken breast and thigh during frozen storage. At lower levels, GSE at 0.02% improved the sensory and chemical shelf life of refrigerated cooked beef and pork patties but did not affect the color (Rojas and Brever 2007). Research on the antioxidant effectiveness of GSE in food systems has largely focused on the effects of temperature and storage,
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while other details about the effect of GSE in conditions commonly encountered in food processing, especially meat processing, have been slow to emerge. The studies presented above clearly indicate that GSE is heat stable and will withstand meat processing temperatures such that its antioxidant efficacy in meat is observed during both refrigerated and frozen storage. The importance of pH on GSE effectiveness was revealed by Hu and others (2004), who showed that (+)-catechin monomers were prooxidative at pH 3 and not antioxidative at pH 7 in algae oil in water emulsions. No studies in the literature were found that tested the effects of muscle pH on the effectiveness of GSE. Two studies that used very high levels of GSE not likely to be commercially viable nonetheless found that infusion of chicken meat with high levels of GSE has been shown to reduce lipid oxidation caused by irradiation (Rababah and others 2006), and the addition of GSE (1%) to cooked and raw turkey containing a 50/50 mix of NaCl/KCl caused a reduction in TBARS and altered the expressible moisture (Lau and King 2003). No studies in the literature were found that describe the effects of relative humidity storage on the efficacy of GSE in meat. The objective of the present study was to determine the effect of GSE (0.1%) in salted and unsalted patties during refrigerated storage at different relative humidities on physicochemical factors that may affect meat quality, including water activity, moisture content, pH, lipid oxidation, and protein solubility.

Materials and Methods

Raw materials, sample preparation, and storage conditions

Commercially available GSE (Gravinol-S) was obtained from Kikkoman Intl. (San Francisco, Calif., U.S.A.). Bovine serum albumin was obtained from Sigma Aldrich (St. Louis, Mo., U.S.A.). All other chemicals and solvents were obtained from Fisher Scientific (Waltham, Mass., U.S.A.). Bone-in chicken thighs were obtained from a local retailer on the morning that each replication of the study was performed.

Chicken thigh meat was removed from the bone and cut by hand into small pieces (approximately 5-cm cubes), taking care to remove all visible fat, and then minced for 1 min on the highest speed of an Osterizer 12-speed blender with a dual blade food processor attachment (model 5900, Sunbeam Products, Boca Raton, Fla., U.S.A.). An aqueous stock solution of GSE, dry NaCl, and/or water was incorporated into the minced thigh meat at an appropriate volume/weight to standardize the systems to final reaction concentrations of 0.1% GSE and 1.0% NaCl by mixing by hand for 1 min. Four treatment groups were created, GSE (0.1%) only, NaCl (1.0%) only, GSE (0.1%) and NaCl (1.0%), and an untreated control. Minced meat (20 g) was formed into disc-shaped patties (5.1-cm dia × 1.3-cm height) using a mold.

Patties from each of the 4 treatment groups were assigned to each of the 4 pre-equilibrated RH chambers and stored refrigerated (4 °C) for as long as 12 d. RH chambers were created using saturated salt solutions to achieve 59%, 76%, 88%, and 99% RH at 4 °C using Mg(NO₃)₂, NaCl, KCl, and K₂SO₄, respectively (Pollio and others 1987). To accommodate the number of patties needed for the subsequent analysis, unwrapped patties were placed in small trays and stacked above the saturated salt solutions on open platforms within the RH chambers. Thus, only the top surface and the sides of the patties were exposed to the ambient RH and the drip was caught by the tray. The RH chambers were opened and closed once on each sampling day when the patties required for analysis were removed. This process took less than 1 min.

Measurement of water activity, moisture content, pH

Crude water activity (a_w) of the chicken thigh meat (3 g) was measured using a PawKit water activity meter (Decagon Devices Inc., Pullman, Wash., U.S.A.) with an accuracy of ±0.02 a_w units. Moisture content of chicken thigh meat (1 g) was measured as the difference in weight before and after drying in an oven (Fisher Isotemp Model 255D, Fisher Scientific) at 70 °C. The samples were dried until constant weight was achieved. A pH meter (Accumet AB15 Plus, Fisher Scientific) calibrated daily to pHs 4 and 7 was used to monitor the pH of a 5 g sample of chicken thigh meat mixed with 25 mL of water.

Measurement of TBARS, soluble protein

Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS) as described previously (Brannan and Decker 2001). Briefly, TBARS were extracted in 7.5% TCA/0.1% propyl gallate/100 M DTPA and reacted with 0.02 M TBA. After 15 min at boiling, absorbance was measured at 532 nm and TBARS quantified on the basis of a standard curve prepared from 1,1,3,3-tetramethoxypropane.

Soluble sarcoplasmic protein was extracted in 25 mM phosphate buffer (pH 7.2) while total soluble protein was extracted in 1.1 M KI in 0.1 M phosphate buffer (pH 7.2) according to an established procedure (Ryu and others 2005). Ground chicken thigh meat (0.25 g) was mixed with the appropriate buffer and vigorously mixed for 1 min. The mixture was stored for 24 h at 4 °C, then centrifuged (IEC HN-SII Table Top Centrifuge, Intl. Equipment Co., Needham Heights, Mass., U.S.A.) for 20 min at 1500 × g. Protein content of the supernatant was determined by the Lowry method and quantified based on a standard curve prepared from bovine serum albumin. Soluble myofibrillar protein is expressed as the difference between total soluble protein and soluble sarcoplasmic protein.

Statistical analysis

Two replications of the study were performed and measurements of a_w, pH and moisture content were made in duplicate while measurements for TBARS and protein solubility were made for each replicate in triplicate. SPSS (Chicago, Ill., U.S.A.) was used to analyze data using the general linear model procedure. The model, a 4 × 4 × 4 full factorial design, included the main effects of treatment (Control, GSE, NaCl, GSE + NaCl), RH (59%, 76%, 88%, 99%), storage time (0, 4, 8, 12 d), and replication (1, 2). Two-way, three-way, and four-way interactions were included in the analysis. For some analysis, slopes were first obtained using the multiple regression analysis on Microsoft® Office Excel 2003 and then the means of the slopes for each treatment were compared using the general linear model procedure of SPSS. The level of significance for all tests was set at 0.05. Means separations were achieved according to Duncan’s multiple range test.

Results and Discussion

Effect of relative humidity storage on water activity, pH, and moisture content of ground chicken thigh meat

Analysis of the main effects of storage time, RH, and treatment group revealed significant differences for a_w for each effect. A significant effect was observed for storage time (P < 0.001), with a_w at 12 d (0.960 a_w) significantly lower than 0, 4, and 8 d (0.986 a_w, 0.986 a_w, 0.999 a_w, respectively) and RH level (P < 0.001) in the order of 99% (0.993 a_w) > 88% (0.983 a_w) > 76% (0.981 a_w) > 56% (0.972 a_w). Treatment group also significantly affected a_w level (P < 0.001) as both NaCl-containing samples (1.0% NaCl +
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0.1% GSE—0.990 \( a_w \) 1.0% NaCl—0.985 \( a_w \) and exhibited higher \( a_w \) than the GSE sample (0.977 \( a_w \), which exhibited a higher \( a_w \) than the untreated control (0.967 \( a_w \). It appears that only the patties stored at 99% RH, which had an initial \( a_w \) near that level, achieved equilibrium. The 3-way interactions for \( a_w \), plotted as \( a_w \) against time for each treatment group within a RH level, are shown in Figure 1. Terminal water activity after 12-d storage ranged from 0.92 to 0.96 \( a_w \) at 59% RH, 0.93 to 0.96 \( a_w \) at 76% RH, 0.94 to 0.98 \( a_w \) at 88% RH, and 0.98 to 0.99 \( a_w \) at 99% RH. Research has shown that 54 d were necessary for pork meat to achieve equilibrium over saturated salt solutions from 11% to 98% RH (Comaposada and others 2000). In terms of percentage of equilibrium achieved, measured as the proportion of the difference between the initial and terminal \( a_w \) to the difference between the initial and target \( a_w \), patties stored at 59%, 76%, and 88% RH achieved 7.5% to 15%, 13% to 24%, and 9% to 42% of equilibrium, respectively.

The moisture content in ground chicken thigh meat treated with GSE and/or NaCl during refrigerated storage at 4 different RH levels was not significantly affected by day of storage, treatment group, or RH level, in spite of the fact that a drop in \( a_w \) was observed for some of the samples. However, these results do agree with previous research in which chicken breast with a moisture content between 70% and 80% stored at 4 \( ^\circ \)C was shown to equilibrate to a water activity between 0.926 and 0.932 (Delgado and Sun 2002).

Analysis of the main effects of storage time, RH, and treatment group revealed significant differences for pH for storage time \((P < 0.001)\) and treatment group \((P < 0.001)\), but not for RH level \((P = 0.791)\). Chicken thigh pH significantly increased over 0, 4, 8, and 12 d of refrigerated storage \((p = 6.38, pH 6.46, pH 6.60,\) and \(pH 7.01,\) respectively). Treatments with 1.0% NaCl alone \((pH 6.52)\) or with 0.1% GSE \((pH 6.58)\) exhibited lower pH values than 0.1% GSE \((pH 6.66)\) and the untreated control \((pH 6.70)\). The 2-way interactions for pH, plotted as pH against time for each treatment group, are shown in Figure 2. The pH increases observed in this study agree with previous research in which chicken thigh patties treated with 0.1% GSE with or without NaCl (0.54 and 0.72 \( \mu \)mol/kg, respectively) exhibited significantly lower TBARS values than the control \((1.35 \mu mol/kg)\) while patties treated with NaCl alone \((1.90 \mu mol/kg)\) exhibited significantly increased TBARS values compared to the untreated control. An effect of RH storage level as a main effect was observed, with significant TBARS formation occurring in the order of 99% \((1.45 \mu mol/kg) > 88% (1.28 \mu mol/kg) > 76% (0.97 \mu mol/kg) > 59% (0.78 \mu mol/kg)\). A significant increase in TBARS was observed at 8 d of storage, perhaps due to the fact that TBARS are a secondary product of lipid oxidation formed from the decomposition of oxidized lipid molecules, that is, lipid hydroperoxides. Previous research has shown that significant formation of TBARS in chicken thigh meat treated with GSE (0.1%) did not occur until after 7 d of refrigerated storage and corresponded with nearly total decomposition of lipid hydroperoxides (Brannan and Mah 2007).

The 3-way interactions of storage time, RH, and treatment group on the formation of TBARS in ground chicken meat stored at 4 \( ^\circ \) C under different RH values are presented in Table 1. GSE-containing patties inhibited TBARS formation after 12 d of storage by 62% to 72% compared to the untreated control, while NaCl-treated patties increased TBARS formation by 23%, 32%, and 52% at RH levels of 76%, 88%, and 99%, respectively. The addition of NaCl to patties held at 59% RH resulted in a decrease in TBARS formation of 26% compared to the untreated control. This suggests that GSE was antioxidative across all RH levels while NaCl was antioxidative at 59% RH and prooxidative at the higher RH levels. This result corresponds with the break point of the moisture sorption isotherm.

**Figure 1** — The 3-way interactions for water activity of raw chicken thigh patties during 4 \( ^\circ \)C storage at (1) 59%, (2) 76%, (3) 88%, and (4) 99% relative humidity and 4 \( ^\circ \)C.
observed for salted pork meat between 70% and 75% moisture, below which NaCl is likely to be crystallized and have only a slight effect and above which moisture content increases with increasing NaCl content (Comaposada and others 2000). At 59% RH, the surface of the patty will be drier and concentrate the NaCl. This may explain why NaCl alone was antioxidative at 59% RH, as previous research has shown that NaCl content at 2% and 3% was antioxidative in salted pork loin (Sarraqa and others 2002). GSE added to patties that contained NaCl enhanced the antioxidative effect of NaCl at 59% RH from 26% to 82% and completely reversed the prooxidant effect at 76%, 88%, and 99% RH, producing TBARS inhibition compared to the untreated control of 58%, 55%, and 66%, respectively.

To better understand the relationship between the patty treatments and RH storage, the rate of formation of TBARS values for chicken thigh meat ± GSE and/or NaCl held at different water activities during refrigerated storage was calculated; that is, the slopes from linear regression analysis were compared. As shown in Table 2, the rate of formation of TBARS for GSE-containing patties was not significantly affected by any of the RH levels. Untreated control patties exhibited a significantly higher rate of TBARS formation, roughly 4- to 6-fold higher than GSE-containing patties; however, the rate of formation was not significantly different across RH levels. Compared to the untreated control, the patties containing NaCl (1%) alone had a significantly higher rate of TBARS formation, and exhibited a significantly higher rate of TBARS formation with increasing RH level. These results suggest that differences observed between the main effects of RH with respect to TBARS formation were primarily due to either the prooxidant effect of NaCl or the interaction between RH and NaCl, rather than RH as an intrinsic factor.

**Effect of treatment on protein solubility in ground chicken thigh meat stored at different relative humidity values**

The effects of treatment group on mean sarcoplasmic and myofibrillar protein solubility in ground chicken thigh meat stored refrigerated for 0 and 12 d are presented in Table 3. Since the extraction of soluble proteins can be affected by a variety of factors, including mixing parameters, centrifugation speeds, and temperature (Regenstein and Stamm 1979), these factors were standardized in this study. No differences were observed among treatment groups for sarcoplasmic or myofibrillar protein solubility at 0 d. However, 1% NaCl caused an increase in the solubility of sarcoplasmic and myofibrillar proteins during storage as exhibited by the increase in both sarcoplasmic and myofibrillar protein solubility at 12 d of refrigerated storage compared to the other 3 treatment groups. Studies have established that the addition of salts can increase the solubility of muscle myofibrillar proteins (Regenstein and Stamm 1979, Agena and others 1999). These data also suggest that the addition of GSE altered the ability of NaCl to solubilize meat proteins since the treatment group containing both 0.1% GSE and 1.0% NaCl exhibited a significant 19% and 30% reduction in soluble sarcoplasmic and myofibrillar proteins, respectively, compared to the treatment group that contained 1.0% NaCl alone. This could be due to the ability of GSE to alter the susceptibility of the muscle to protein denaturation by NaCl. Alternatively, GSE may have an impact on the microstructure of the myofibrillar lattice in such a way that it affects the solubility of these nondenatured proteins in the same way that the microstructure of ground poultry is affected by factors that cause changes in water holding capacity, gel strength, and ultimate texture (Barbut 1997). In any event, an important finding from this study is that both TBARS formation (Table 1) and protein solubility (Table 3 and 4) induced by NaCl was altered by the presence of GSE.

The level of relative humidity exhibited a significant effect on protein solubility after 12 d of refrigerated storage that was different for sarcoplasmic and myofibrillar proteins (Table 4). Soluble sarcoplasmic protein solubility significantly decreased with increasing relative humidity in the order of 59% > 76% > 88% = 99%, while soluble myofibrillar protein levels were significantly higher at 99% RH.
### Table 2—Slopes generated from linear regression of TBARS values for chicken thigh meat + GSE and/or NaCl held at different water activities during storage (4°C).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>59% RH</th>
<th>76% RH</th>
<th>88% RH</th>
<th>99% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>398 cd</td>
<td>320 de</td>
<td>358 cde</td>
<td>411 bc</td>
</tr>
<tr>
<td>1.0% NaCl</td>
<td>287 e</td>
<td>405 bc</td>
<td>481 b</td>
<td>648 a</td>
</tr>
<tr>
<td>0.1% GSE</td>
<td>99 g</td>
<td>98 fg</td>
<td>113 fg</td>
<td>106 fg</td>
</tr>
<tr>
<td>1.0% GSE + 1.0% NaCl</td>
<td>60 g</td>
<td>118 fg</td>
<td>161 f</td>
<td>126 fg</td>
</tr>
</tbody>
</table>

Different lowercase letters represent significant differences (P < 0.05).

### Table 3—Effects of treatment on mean sarcoplasmic and myofibrillar protein solubility (mg/g tissue) in ground chicken thigh meat at 4°C for 0 and 12 d.

<table>
<thead>
<tr>
<th>Main effect</th>
<th>Soluble sarcoplasmic protein</th>
<th>Soluble myofibrillar protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 12</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>62 a</td>
<td>58 b</td>
</tr>
<tr>
<td>0.1% GSE</td>
<td>60 a</td>
<td>53 b</td>
</tr>
<tr>
<td>1% NaCl</td>
<td>53 a</td>
<td>69 a</td>
</tr>
<tr>
<td>0.1% GSE + 1% NaCl</td>
<td>63 a</td>
<td>56 b</td>
</tr>
</tbody>
</table>

Different lowercase letters within a day represent significant differences (P < 0.05).

### Table 4—Effects of relative humidity storage level on mean sarcoplasmic and myofibrillar protein solubility (mg/g tissue) in ground chicken thigh meat at 4°C for 0 and 12 d.

<table>
<thead>
<tr>
<th>Main effect</th>
<th>Soluble sarcoplasmic protein</th>
<th>Soluble myofibrillar protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 12</td>
</tr>
<tr>
<td>Relative humidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>59%</td>
<td>59^a</td>
<td>84 a</td>
</tr>
<tr>
<td>76%</td>
<td>78^a</td>
<td>73 b</td>
</tr>
<tr>
<td>88%</td>
<td>88^a</td>
<td>37 c</td>
</tr>
<tr>
<td>99%</td>
<td>99^a</td>
<td>40 c</td>
</tr>
</tbody>
</table>

^aSoluble protein levels for 0-d samples had not been subjected to differing RH levels.

Different lowercase letters within a day represent significant differences (P < 0.05).

than at the other 3 RH levels. Sun and others (2002) speculate that total protein solubility would increase at higher RH levels due to decreased protein denaturation, although these researchers show an increase in total protein solubility with increasing water activity (RH) in dried meat only at elevated storage temperatures (49°C). A confounding factor would be the interaction of sarcoplasmic and myofibrillar proteins with the polyphenolics found in GSE, since the phenolic compounds in GSE may lead to increased interactions with proteins.

### Conclusions

This study shows that GSE is an effective antioxidant in ground chicken thigh meat that does not affect moisture content or pH during storage, inhibits TBARS formation, helps to mitigate the prooxidative effects of NaCl, and may alter the effect of NaCl on protein solubility in salted chicken patties. As promising as these results are, additional research will be required to determine how the physicochemical interactions of GSE reported in this study and previous studies affect important cooked meat quality attributes (color, texture, flavor) and nutritional quality, especially in regard to the level, form, and health-promoting functionality of residual GSE in the meat after processing.

### References


Weber HA, Hodges AE, Cherif J. 1987. Prediction and measurement of the physicochemical interactions of GSE reported in this study and previous studies affect important cooked meat quality attributes (color, texture, flavor) and nutritional quality, especially in regard to the level, form, and health-promoting functionality of residual GSE in the meat after processing.