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# Evaluation of the antioxidant effects of different forms of *Schisandra chinesis* in emulsion-type sausages during chilled storage

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**The effects of different forms of *Schisandra chinesis* extract (20% *S. chinensis* juice, 20% *S. chinensis* ethanol extract, and 20% *S. chinensis* water extract) on physico-chemical characteristics and color in emulsion-type sausage were evaluated. Physico-chemical characteristics and color were determined during storage. Thiobarbituric acid reactive substance (TBARS) and residual nitrite (RN) values decreased significantly in treatments with 20% *S. chinensis* juice and 20% *S. chinensis* ethanol extract due to a lower pH. However, control treatments and treatment with 20% *S. chinensis* water extract did not have a significant effect on physico-chemical characteristics during storage. In addition, no remarkable differences were observed in total plate counts (TPCs, antimicrobial effect) or color in any treatment as the storage time increased. Our results suggest that 20% *S. chinensis* ethanol extract was the best antioxidant agent to reduce lipid oxidation and RN during storage.**

**Key words:** *Schisandra chinensis* juice, *Schisandra chinensis* ethanol extract, *Schisandra chinensis* boiling water extract, physico-chemical characteristics, color, emulsion-type sausage.

## INTRODUCTION

The appearance of meat, especially its color, is an indicator of freshness (Ismail et al., 2008). Therefore, discoloration and lipid oxidation in meat products are important parameters that determine quality and acceptability by consumers. Consumers may be concerned about the application of antioxidants that prevent color change and lipid oxidation in meat and meat products during storage. In recent years, natural antioxidants have been accepted as alternatives to widely used synthetic antioxidants. For example, in some regions, the use of nitrite, butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) have been restricted and the use of synthetic antioxidants has been reduced, primarily due to their toxic properties (Hettiararchchy et al., 1996; European

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**Abbreviations: TBARS**, Thiobarbituric acid reactive substance; **RN**, residual nitrite; **TPCs**, total plate counts.

Union, 2006). Thus, using natural agents as *Schisandra chinensis* (*S. chinensis*) has very good antioxidant potential, as they are considered natural.

*S. chinensis* is a perennial climbing plant that grows abundantly in the northern regions of China, Korea, Japan and Russia. The berries of *S. chinensis* are called 'wu wei zi' in China or 'omija' in Korea because it possesses all five basic flavors: sour, sweet, salty, sharp, and bitter (Panossian and Wikman, 2008; Hwang, 2012). *S. chinensis* is extensively used as a therapeutic drug, tea, wine and functional food (Hwang, 2012), as well as an alternative medicine to treat liver diseases (Sheng et al., 2011). Bioactive compounds from *S. chinensis* include lignans (types A-E), volatile oil (includes ingredients such as cadinene, ylangene and chamigrenol) and polysaccharides (Hwang, 2012; Yang et al., 2011). The lignans isolated from *S. chinensis* might be effective in the treatment or prevention of a variety of human diseases, including cardiac diseases and immune diseases (Hwang, 2012). *S. chinensis* polysaccharides have antioxidant activities that protect cells from the damaging effects of reactive species and pharmacological properties that prevent chronic diseases (Song et al., 2010). Furthermore, Sheng et al. (2011) reported that the use of *S. chinensis* as a traditional Chinese medicine (TCM) as anti-lipid peroxidative properties could prevent free radical induced problems. However, knowledge about *S. chinensis* on antimicrobial effect in meat industry has not been reported.

Although the effects of *S. chinensis* in animals have been studied, there are few studies on the use of *S. chinensis* extracts on emulsion-type sausages during chilled storage. The objective of the present work was to determine the effectiveness of different forms of *S. chinesis* as antioxidant agent in emulsion-type sausage during chilled storage.

## MATERIALS AND METHODS

### Preparation of *S. chinensis* extracts

The *S. chinensis* fruits used in this study were purchased from a local market (Munkyong, South Korea). To obtain *S. chinensis* juice, approximately 500 g of *S. chinensis* fruit was processed in a juicer (KJ-303, Kwang Jin Co., South Korea). To obtain *S. chinensis* ethanol extract and *S. chinensis* water extract, 500 g of *S. chinensis* fruit was suspended in 1,000 mL 70% ethanol and distilled water for 30 min at room temperature and extracted by steam distillation at 60°C, respectively.

### Sausage preparation

Sausages were made following standard methods used in emulsion-type sausages. Fresh boneless pork was obtained from a local meat market and excess fat and connective tissue were removed. Pork meat was ground using a 5-mm grinder plate. The ground pork was divided into four groups: control (no additives), T1 (20% *S. chinensis* juice), T2 (20% *S. chinensis* ethanol extract), and T3 (20% *S. chinensis* water extract). The ingredients used in the sausage formulations were: 55% ground pork meat, 15% fat, 5.3% cornstarch, 3% sausage seasoning (containing 0.4% nitrite), 1.5% salt, 0.2% polyphosphate, and 20% iced water. The control was the basal sausage containing 2000 mL ice water (20% ice water) as 20% for a 10 kg sausage. To make 20% *S. chinensis* juice, 20% *S. chinensis* ethanol extract, or 20% *S. chinensis* water extract, the extract was added to ice water, adjusted to 2000 ml and then mixed together. All other ingredients were mixed with ground pork using a cutting chopper. When the emulsion was processed, heat was generated. The generated heat was absorbed by the iced water. During the chopping process, the meat was cut into finer particle sizes which encourage protein extraction. Fat was added to solubilized meat proteins when emulsions were thoroughly formed. The batter was then mixed for 5 min in an emulsifier (Kenmix Electronic, model FP800, Kenwood Ltd., New Hampshire, UK). The emulsified meat batter was stuffed into polyvinylidene chloride casings (50 mm in diameter, Viskase Corporation, Chicago, IL) and cooked at 75°C for 70 min in a cooking chamber (NU-VUES-3, Food Service System, USA). All samples were cooled in ice water for 2 h and stored at 4°C. The samples were analyzed at 0, 10, 20, and 30 days of storage at 4°C. All experiments were performed in triplicate.

### pH measurement

pH was determined following the AOAC method (1990). To measure pH, 10 g of sausage was homogenized in a blender with 90 mL of distilled water. A digital pH meter (Model 520A, Orion, USA) was used to record pH.

### Thiobarbituric acid reactive substance (TBARS)

Lipid oxidation was determined by the TBARS assay following the method described Witte et al. (1970). A 20 g sample was homogenized with 50 mL of 20% trichloroacetic acid solution (in 2 M phosphate solution) in a blender and mixed with 50 mL of distilled water. After samples were filtered through No. 1 filter paper; 5 mL of TBA solution (0.005 M in water) was mixed with 5 mL of the filtered solution in a test tube. The test tubes were placed in darkness at room temperature. After 15 h, the absorbance of the supernatant was recorded at 532 nm using an ultra-violet/visible (UV/VIS) spectrophotometer (UV-24D, Shimadzu, Tokyo, Japan). TBARS values were expressed as mg malondialdehyde (MDA) per kg sausage.

### Residual nitrite (RN) content

The RN measurement was estimated using the colorimetric procedure described in AOAC (1990). A 5 g sausage sample was homogenized with 50 mL of distilled water for 2 min. The mixture was heated for 10 min at 40°C in a boiling water bath; 5 mL of saturated HgCl solution was added to the mixture and the resulting mixture was heated for 2 h at 80°C in a boiling water bath, and then cooled to room temperature. A 10-mL supernatant solution sample was mixed with 1 mL of sulfanilamide and kept at room temperature for 15 min. Absorbance was measured at 540 nm using a UV-VIS spectrophotometer (UV-24D). The RN content was expressed as mg per kg of sausage.

### Total plate counts (TPCs)

A 10 g sausage sample was homogenized with 90 mL sterile peptone water using a stomacher (Laboratory Equipment, London,

**Table 1.** Effect of different forms of *S. chinensis* on physico-chemical characteristics of emulsion- type sausages during storage at 4°C.

| Item | Treatments[1)] | Storage time (days) | | | |
|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 30 |
| pH | Control | 6.65±0.03$^{aA}$ | 6.46±0.03$^{bA}$ | 6.30±0.05$^{cAB}$ | 6.28±0.10$^{cA}$ |
| | T1 | 6.47±0.04$^{aB}$ | 6.34±0.04$^{bB}$ | 6.23±0.08$^{cB}$ | 6.16±0.04$^{cB}$ |
| | T2 | 6.41±0.04$^{aB}$ | 6.28±0.04$^{bB}$ | 6.21±0.06$^{bB}$ | 6.12±0.04$^{cB}$ |
| | T3 | 6.59±0.04$^{aA}$ | 6.46±0.04$^{bA}$ | 6.38±0.026$^{cA}$ | 6.40±0.04$^{bcA}$ |
| TBARS (mg MDA/kg) | Control | 0.29±0.02$^{dA}$ | 0.39±0.06$^{cA}$ | 0.43±0.02$^{bA}$ | 0.50±0.02$^{aA}$ |
| | T1 | 0.23±0.02$^{dB}$ | 0.29±0.03$^{cB}$ | 0.37±0.01$^{bB}$ | 0.42±0.02$^{aB}$ |
| | T2 | 0.23±0.02$^{dB}$ | 0.29±0.03$^{cB}$ | 0.35±0.01$^{bB}$ | 0.39±0.02$^{aC}$ |
| | T3 | 0.28±0.03$^{cA}$ | 0.38±0.02$^{bA}$ | 0.41±0.02$^{bA}$ | 0.48±0.01$^{aAB}$ |
| Residual nitrite (mg/kg) | Control | 7.36±0.11$^{aA}$ | 7.12±0.09$^{bA}$ | 5.76±0.16$^{cA}$ | 5.16±0.12$^{dA}$ |
| | T1 | 6.00±0.39$^{aB}$ | 5.61±0.19$^{bB}$ | 4.73±0.11$^{cB}$ | 4.18±0.07$^{dC}$ |
| | T2 | 5.76±0.13$^{aB}$ | 5.13±0.07$^{bC}$ | 4.11±0.10$^{cC}$ | 3.76±0.14$^{dD}$ |
| | T3 | 7.29±0.25$^{aA}$ | 7.03±0.14$^{bA}$ | 5.74±0.06$^{cA}$ | 4.81±0.08$^{dB}$ |
| TPCs (log CFU/g) | Control | 1.36±0.01$^{dA}$ | 3.59±0.29$^{cA}$ | 5.45±0.04$^{bA}$ | 7.02±0.04$^{aA}$ |
| | T1 | 1.37±0.01$^{dA}$ | 3.73±0.07$^{cA}$ | 5.39±0.07$^{bA}$ | 6.53±0.09$^{aB}$ |
| | T2 | 1.37±0.01$^{dA}$ | 3.65±0.10$^{cA}$ | 5.18±0.07$^{bA}$ | 6.47±0.06$^{aB}$ |
| | T3 | 1.36±0.02$^{dA}$ | 3.76±0.14$^{cA}$ | 5.41±0.05$^{bA}$ | 6.80±0.04$^{aA}$ |

$^{a-d}$Means within row with different superscripts are significantly different ($p$<0.05). $^{A-D}$Means within columns with different superscripts are significantly different ($p$<0.05). [1]Control: no *S. chinensis*, T1: 20% *S. chinensis* juice. T2: 20% *S. chinensis* extract, T3: 20% *S. chinensis* water extract.

UK). The samples were serially diluted ($10^{-1}$ to $10^{-8}$) and 100 µL of dilutions ($10^{-4}$ to $10^{-8}$) were spread-plated on plate count agar (Difco Laboratory, Detroit, MI). The agar plates were incubated at 35°C for 48 h. The colonies were counted and expressed as logarithmic colony forming units (CFU) per g sausage.

#### Color evaluation

Color was evaluated using a Minolta Chromameter (Minolta CR-300, Tokyo, Japan) that had been standardized with a white plate (lightness, L* = 96.16; redness, a* = 0.10; yellowness, b* = 1.90). Five random readings were taken for each sausage point.

#### Statistical analysis

All data were analyzed by analysis of variance (ANOVA) using the SAS General Linear Model (GLM) procedure (SAS, 2002). Duncan's multiple range test was used to determine significant differences among treatment means at the 5% level (Duncan, 1955).

## RESULTS AND DISCUSSION

### Physico-chemical characteristics of emulsion-type sausage

Table 1 shows the effects of different forms of *S. chinensis* on physico-chemical characteristics of emulsion-type sausage during chilled storage. Statistical differences (P < 0.05) were observed in pH, TBARS, RN, and TPCs between treatments and storage times. However, TPCs did not differ in any treatment at 0, 10 or 20 days of storage.

For pH, the lowest pH value was observed in T2 (20% *S. chinensis* ethanol extract), followed by T1 (20% *S. chinensis* juice). Treatments with 20% *S. chinensis* water extract (T3) and the control had similar pH values (6.65 through 6.28) during storage. These differences have been attributed to the spectrum of compounds extracted with the different materials used. Overall, pH values decreased as storage time increased in all treatments. Antioxidant effectiveness relies on pH (Xiong et al., 1993). Therefore, the low pH values may be due to the anti-oxidants present in *S. chinensis*. An increase in meat pH could be due to the accumulation of metabolites by bacteria and deamination of proteins (Jay, 1996). Ibrahim et al. (2011) reported that lamb patties containing ginseng extract as a source of antioxidants had the lowest pH values after nine days of storage.

TBARS values, which are indicators of the degree of lipid oxidation, increased significantly over time in all treatments. The order of effectiveness of different forms of *S. chinensis* as antioxidants in decreasing TBARS values is T2 >T1 > T3 = control. Thus, the 20% *S. chinensis* ethanol extract (T2) had the highest antioxidant properties. The antioxidant activity of *S. chinensis* has been attributed to a group of lignans or phenolic compounds

**Table 2.** Effect of different forms of *S. chinensis* on color of emulsion-type sausages during storage at 4°C.

| Item | Treatment[1] | Storage time (days) | | | |
|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 30 |
| L* (lightness) | Control | 67.75±0.54$^{aA}$ | 67.13±0.23$^{aA}$ | 65.57±0.31$^{bA}$ | 64.40±0.36$^{cA}$ |
| | T1 | 68.09±0.27$^{aA}$ | 65.63±0.31$^{bB}$ | 63.99±0.38$^{cB}$ | 63.84±0.18$^{cAB}$ |
| | T2 | 67.81±0.56$^{aA}$ | 65.21±0.09$^{bB}$ | 64.07±0.81$^{cB}$ | 63.61±0.37$^{cB}$ |
| | T3 | 67.93±0.11$^{aA}$ | 65.61±0.15$^{bB}$ | 65.70±0.28$^{bA}$ | 64.35±0.26$^{cA}$ |
| a* (redness) | Control | 8.13±0.08$^{cA}$ | 8.26±0.05$^{bAB}$ | 8.37±0.13$^{abB}$ | 8.55±0.14$^{aB}$ |
| | T1 | 8.12±0.05$^{dA}$ | 8.36±0.10$^{cA}$ | 8.61±0.10$^{bA}$ | 8.82±0.08$^{aA}$ |
| | T2 | 8.04±0.12$^{dA}$ | 8.38±0.06$^{cA}$ | 8.61±0.06$^{bA}$ | 8.79±0.12$^{aA}$ |
| | T3 | 8.09±0.06$^{cA}$ | 8.21±0.09$^{bcB}$ | 8.37±0.06$^{bB}$ | 8.55±0.13$^{aB}$ |
| b* (yellowness) | Control | 8.02±0.10$^{aA}$ | 7.73±0.21$^{abA}$ | 7.47±0.29$^{bA}$ | 7.56±0.09$^{bA}$ |
| | T1 | 8.05±0.04$^{aA}$ | 7.98±0.06$^{aA}$ | 7.45±0.21$^{bA}$ | 7.72±0.07$^{cA}$ |
| | T2 | 8.11±0.06$^{aA}$ | 7.91±0.15$^{abA}$ | 7.71±0.28$^{abA}$ | 7.76±0.16$^{bA}$ |
| | T3 | 8.05±0.04$^{aA}$ | 7.79±0.17$^{abA}$ | 7.53±0.41$^{abA}$ | 7.76±0.20$^{bA}$ |

$^{a-c}$Means within row with different superscripts are significantly different ($p$<0.05). $^{A-B}$Means within columns with different superscripts are significantly different ($p$<0.05). [1]Control: no *S. chinensis*, T1: 20% *S. chinensis* juice. T2: 20% *S. chinensis* extract, T3: 20% *S. chinensis* water extract.

(Song et al., 2010; Toda et al., 1988). According to Osada et al. (2000), the antioxidant action of *S. chinensis* powder (SCP) might inhibit cholesterol oxidation in meat and meat products during storage or processing because of their phenolic compounds. Our results agrees with the results of Jin and Park (2013), who reported that TBARS values decreased with increasing levels of *S. chinensis* powder in cooked pork sausages.

As the storage time increased, the RN content declined in all treatments. The RN content was affected (P < 0.05) by both storage days and treatments. The efficacy of the different forms of *S. chinensis* extracts in decreasing the RN content was greatest in T2 (20% *S. chinensis* ethanol extract), followed by T1, T3 and the control. Adding *S. chinensis* extract to emulsion-type sausages increased the antioxidant activity, which was similar to the results of Hah et al. (2006). The antioxidant effects of *S. chinensis* in meat products have been reported by several researchers (Kim et al., 2000; Kim et al., 2008). For example, the phenolic compounds in *S. chinensis* increase the nitrite scavenging activity. Furthermore, nitrites could react with certain amines in some foods to form carcinogenic nitrosamines, leading to various cancers (Van Maanen et al., 1998; Mirvish et al., 2000). RN reduction in emulsion type sausages may be related to a lower pH and the presence of *S. chinensis*, both of which are important factors controlling nitrite reactions.

In the current study, all treatments showed an increase in TPCs values over storage. Our observation is that after 30 days of storage, the TPCs in all treatments exceeded the maximum permissible levels (6 $\log_{10}$ cfu/g) recommended by ICMSF (1986) for human consumption. Overall, these results indicate that the use of *S. chinensis* extract in emulsion-type sausages did not bring antimicrobial activity.

## The color of emulsion-type sausages

The effects of different forms of *S. chinensis* on the color of emulsion-type sausages during storage are presented in Table 2. At 10, 20 and 30 days of storage, all *S. chinensis* treatments had some effects (P < 0.05) on L* and a* values in emulsion type sausages. However, no differences were observed for L* and a* values at 0 d or for b* values at 0 through 30 days. There were statistically differences (P < 0.05) in L*, a*, and b* values in all treatments as the number of storage days increased. In general, meat discoloration is closely related to myoglobin oxidation caused by lipid oxidation (Yin and Faustman, 1993). In the current study, using different forms of *S. chinensis* extracts did not improve the color of emulsion-type sausages during storage.

## Conclusions

During storage, the addition of 20% *S. chinensis* juice and 20% *S. chinensis* ethanol extracts as antioxidant agents decreased TBRAS (lipid oxidation) and residual nitrite (RN) in emulsion-type sausages, due to a decrease in pH. However, our findings do not confirm a benefit of different forms of *S. chinensis* extracts on total plate counts (TPCs) or color stability of emulsion-type sausages during storage. *S. chinensis* may be a promising source of antioxidant agents that extend the shelf life of emulsion-type sausages

and prevent lipid oxidation during storage.

## Conflict of Interests

The author(s) have not declared any conflict of interest.

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