



## Utilization of Dried Garlic Powder and $\alpha$ -Tocopherol to Improve the Shelf-life of Emulsion-type Sausage during Refrigerated Storage

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### Abstract

This study investigated the shelf life of emulsion-type sausages containing garlic powder and/or  $\alpha$ -tocopherol during storage at 4°C for 0, 10, 20, and 30 d. Six groups of emulsion-type sausages were included: control (no additives), GP1 (1% garlic powder), GP3 (3% garlic powder), AT100 (100 IU of  $\alpha$ -tocopherol/kg of sausage), AT200 (200 IU of  $\alpha$ -tocopherol/kg of sausage), and GP1+AT100 (1% garlic powder+100 IU of  $\alpha$ -tocopherol/kg of sausage). During storage, the pH, thiobarbituric acid reactive substances, and residual nitrite content were reduced by the addition of garlic powder and/or  $\alpha$ -tocopherol relative to the control ( $p < 0.05$ ). In addition, emulsion-type sausages supplemented with garlic powder and/or  $\alpha$ -tocopherol improved color stability ( $p < 0.05$ ). The results suggest that a higher amount of garlic powder and their different combinations could improve the shelf life of emulsion-type sausages and protect against lipid oxidation.

**Key words:** emulsion-type sausage, garlic powder,  $\alpha$ -tocopherol, TBARS, residual nitrite, meat color

### Introduction

Lipid oxidation and discoloration, which are the major causes of deterioration in meat quality during storage, are 2 of the greatest concerns in the meat industry (Fernández-López *et al.*, 2005; Kanner, 1994). Lipid oxidation and discoloration reduce both the nutritional quality and consumer acceptability (Brewer *et al.*, 2002; Buckley *et al.*, 1995; Fernandez-Lopez *et al.*, 2005; Ryu *et al.*, 2005, 2006). This has led to great interest in the application of additives or antioxidants that prevent the oxidative deterioration of meat and meat products. Accordingly, the application of natural products with antioxidant activities in meat and meat products may be necessary to extend their storage shelf life and prevent diseases (Yin and Cheng, 2003). Many studies have demonstrated that various antioxidants such as nitrite, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), garlic, and  $\alpha$ -tocopherol, are effective in food products (Fernández-López *et al.*, 2005; Kahl and Kappus, 1993; Martinez-Tome *et al.*, 2001; Sammet *et al.*, 2006). However, use of

the synthetic antioxidants nitrite, BHA, and BHT in the meat industry has begun to be restricted due to their toxic properties and potential health hazards (Kahl and Kappus, 1993; Luecke, 1999). Consequently, a number of consumers are concerned about the safety of synthetic food additives. Therefore, replacement of these synthetic antioxidants with natural antioxidative substances, such as garlic and  $\alpha$ -tocopherol, that can prolong the shelf life of both processed and unprocessed meat products is being considered.

Garlic is commonly used as a flavor enhancement in sausage and is also appreciated for its medicinal properties (Sallam *et al.*, 2004). It has long been known that garlic has beneficial effects in animals, including antioxidant, antiviral, and antifungal activities (Harris *et al.*, 2001; Jackson *et al.*, 2002). Previous research has suggested that these functions are mainly attributed to the bioactive components present in garlic, including sulfur-containing compounds, such as alliin, allicin, and diallyl sulfides (Amagase *et al.*, 2001).

$\alpha$ -Tocopherol is the most effective chain breaking lipid antioxidant present in cell membranes, and it protects cellular structures against damage from oxygen free radicals and the reactive products of lipid peroxidation (Sodhi *et al.*, 2008). In several studies, lipid stability was shown to be greater in meats and sausages when pork and lamb

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diets were supplemented with  $\alpha$ -tocopherol (Harms *et al.*, 2003; Lauzurica *et al.*, 2005). In addition, the use of antioxidant blends in animal diets has a potential economic advantage for the meat industry because a combination of antioxidants will require lower production costs than single pure compounds (Shahidi, 1996). Therefore, determining the antioxidant activity of mixtures of garlic and  $\alpha$ -tocopherol will provide fundamental information that may lead to new sausage formulations to gradually increase consumer acceptance.

Although many studies have focused on the supplementation of feed with garlic powder and  $\alpha$ -tocopherol to improve lipid and color stability in chicken, pork, and beef, scientific literature on the shelf life properties of emulsion-type sausage supplemented with garlic powder,  $\alpha$ -tocopherol, or both, is still scarce. Therefore, the aim of the present study was to evaluate the effect of garlic powder,  $\alpha$ -tocopherol, or both, on the pH, thiobarbituric acid reactive substances (TBARS), residual nitrite (RN), and color properties of emulsion-type sausage during storage.

## Materials and Methods

### Materials

About 20 kg of fresh garlic samples produced in May or June were purchased from a local market (Eui-Sung local National Agriculture Co-operation Federation, Korea). Fresh garlic was prepared following the procedure previously described by Kim *et al.* (2010). Briefly, fresh garlic was peeled, cut into slices, and subsequently thinly spread on a mat in direct sunlight at 30 to 35°C to make garlic powder. The drying process continued for 8-10 h to ensure the appropriate consistency of the garlic. After drying, the garlic was further dried in an oven at 50°C for 8 h, ground to a fine powder, and stored immediately at 4°C until use. The dried garlic powder used in this study contained 905 g dry matter/kg, 128 g crude protein/kg, 38 g crude fat/kg, and 57 g crude ash/kg.  $\alpha$ -Tocopherol (*all-rac*- $\alpha$ -tocopheryl acetate) was purchased from Sigma (USA).

### Sausage Making

To produce emulsion-type sausages, fresh boneless pork, purchased from a local market, was trimmed of visible fat and connective tissue. Pork was ground through a 4 mm grinder plate (Super grinder-MK-G3, Matsushita Electric Industrial, Japan) before sausage manufacture. The ground meat was used to produce 6 different groups of emulsion-type sausages: control (no additives), GP1 (1% garlic

powder), GP3 (3% garlic powder), AT100 (100 IU of  $\alpha$ -tocopherol/kg of sausage), AT200 (200 IU of  $\alpha$ -tocopherol/kg of sausage), and GP1+AT100 (1% garlic powder+100 IU of  $\alpha$ -tocopherol/kg of sausage). The percentages of the basic components in the sausages were as follows: ground pork meat (60%), fat (20%), cornstarch (6%), sausage seasoning (3%, Taewon Food Industry CO., Ansan, South Korea), salt (1.5%), polyphosphate (0.25%), and ice water (10%). Emulsion-type sausages were manufactured as described by Kim *et al.* (2010). Briefly, all other ingredients were thoroughly mixed with the various formulations of sausage meat in the cutting chopper. While the emulsification was processing, ice water was added to absorb the generated heat and ensure that the emulsion held. The meat was cut to a very fine particle size, which encouraged protein extraction while chopping. When the emulsions were sufficiently formed by solubilizing the meat protein, fat was added. Then, the batter was mixed in an emulsifier (Model FP800, Kenwood Ltd., UK) for 5 min. The sausage mixture was tightly stuffed into polyvinylidene chloride casings 50 mm in diameter (Viskase Corporation, USA) that were divided into food-casing lengths of about 10 or 12 cm per unit. The sausage unit was heated at 75°C for 70 min in a cooking chamber, cooled immediately in ice water, and kept at 4°C for 0, 10, 20, and 30 d.

### Measurements

#### pH

The pH was determined according to the method described by Sallam *et al.* (2004). A 10 g sausage sample was cut into small pieces and homogenized in 90 mL of distilled water. A slurry was then made using a homogenizer and the pH was measured with a digital pH meter (Model 520A, Orion, USA).

#### Thiobarbituric-acid reactive substances (TBARS)

The presence of TBARS was assessed using the method of Witte *et al.* (1970) and was expressed as milligrams of malonaldehyde (MA) per kilogram of sausage. A 20 g sausage sample was added to 50 mL of 20% trichloroacetic acid (in 2 M phosphate solution) and homogenized in a blender. The solution was added to 50 mL of distilled water and then filtered through Whatman No. 1 filter paper. After adding 5 mL of the filtrate to 5 mL of 2-TBA reagent (0.005 M in water) in a test tube (50 mL), the test tubes were kept at room temperature in the dark for 15 h and the absorbance at 532 nm was measured with a Ultra-Violet/Visible (UV/VIS) spectrophotometer (UV-24D1 (PC)

5, Shimadzu, Japan)

### Residual nitrite (RN) contents

Residual nitrite content was evaluated using the AOAC method (2005) and is expressed as milligrams per kilogram of sausage. Briefly, a 5 g sausage sample was homogenized in 50 mL of distilled water and heated in a boiling water bath at 40°C for 10 min. The homogenized sample was combined with 5 mL of saturated HgCl solution and heated in a boiling water bath at 80°C for 2 h. After cooling, 1 mL of sulfanilamide was added to the tube containing 10 mL of supernatant and kept at room temperature for 15 min. The absorbance at 540 nm was read in an Ultra-Violet/Visible (UV/VIS) spectrophotometer (UV-24D; Shimadzu, Japan).

### Color

After the sausage samples were sliced and exposed to air for 30 min, color measurements [CIE L\* (lightness), a\* (redness), and b\* (yellowness)] were made using a tristimulus colorimeter (CR-300; Minolta, Japan) equipped with a D65 illuminant (diffuse illumination, 0° viewing angle and 2° observer). Results were recorded as the mean of these color measurements. L\*, a\*, and b\* values were measured on 3 different spots of each sample.

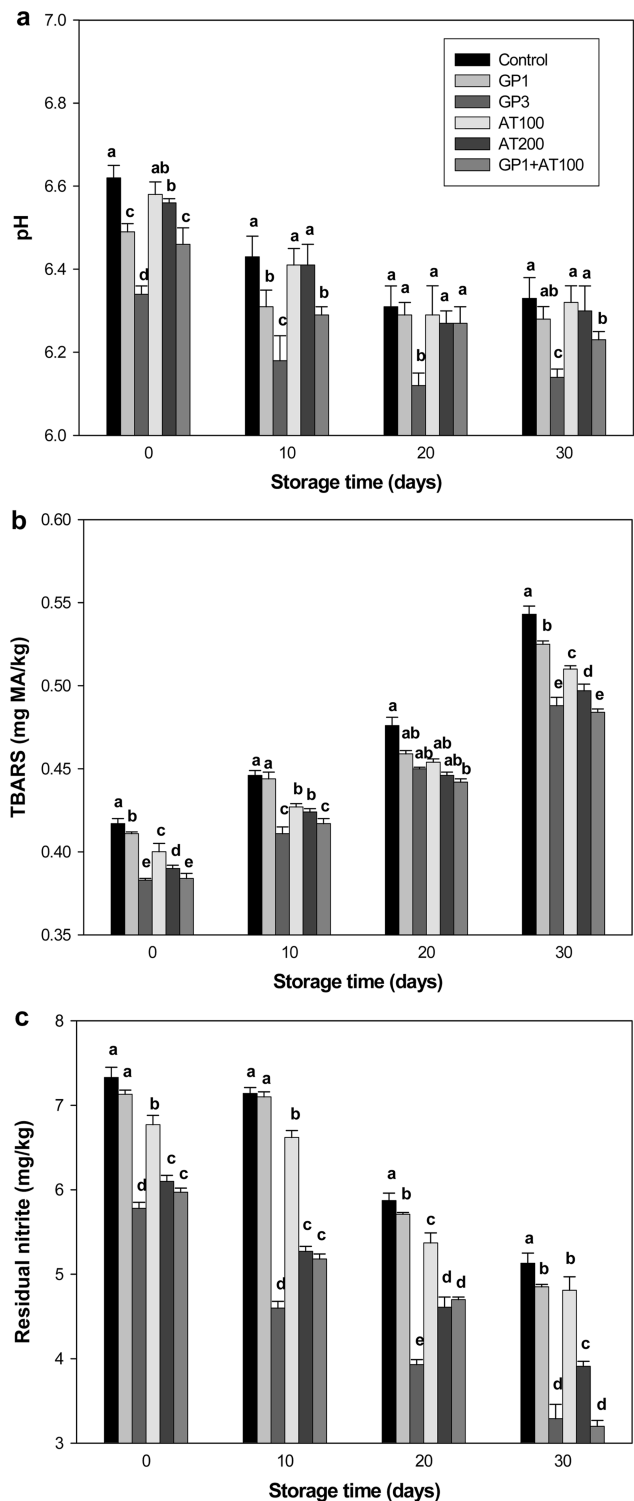
### Data analysis

All measurements were performed in triplicate, and data were analyzed by ANOVA using the GLM procedure in the SAS package program (SAS, 2002), which was used to determine the effects of the additives and storage time. Significant differences among the means were determined using Duncan's new multiple range test (Duncan, 1955) at a predetermined probability rate of 5%. The results of the statistical analysis are presented as the mean  $\pm$  standard errors in the figures.

## Results and Discussion

### pH, TBARS, and RN content in emulsion-type sausages

The effect of garlic powder and  $\alpha$ -tocopherol on pH, TBARS, and residual nitrite content in emulsion-type sausages during storage at 4°C is shown in Fig. 1. Fig. 1a shows that the pH was significantly affected ( $p < 0.05$ ) by storage time and the addition of garlic powder and/or  $\alpha$ -tocopherol. However, after 10, 20, and 30 d of storage, no remarkable difference was detected between the control sausages and those that included  $\alpha$ -tocopherol (AT100



**Fig. 1.** Effect of dried garlic powder and/or  $\alpha$ -tocopherol on (a) pH, (b) TBARS, and (c) residual nitrite in emulsion-type sausages during storage. Data shown are the means with the standard error represented by the vertical bars. <sup>a-c</sup>Indicates significant on different storage days. The treatments are as follows: GP1, 1% garlic powder; GP3, 3% garlic powder; AT100, 100 IU of  $\alpha$ -tocopherol/kg of sausage; AT200, 200 IU of  $\alpha$ -tocopherol/kg of sausage; GP1+AT100, 1% garlic powder + 100 IU of  $\alpha$ -tocopherol/kg of sausage

and AT200). Throughout the storage period, the pH values of all sausage formulations ranged from 6.62 to 6.12, and a slight reduction in pH was observed in all sausages as storage time increased. The lowest pH values were seen in the GP3 sausages, which ranged from 6.34 to 6.12, while the control sausages had the highest pH values, which ranged from 6.62 to 6.31. This reduction in the pH of emulsion-type sausages with added with garlic powder and/or  $\alpha$ -tocopherol could be due to the effectiveness of antioxidants being dependant on pH (Xiong *et al.*, 1993). Similar to the current study, Fista *et al.* (2004) observed a reduction in the pH of sausages supplemented with leek (*Allium porrum*) and onion (*Allium cepa*). Conversely, other studies have demonstrated that pH values are not significantly influenced by antioxidant supplementation (Aksu and Kaya, 2005). In another study, Sallam *et al.* (2004) reported that in all sausage formulations, storage had a significant ( $p < 0.05$ ) effect on pH, which tended to increase with storage time.

Fig. 1b shows that when garlic powder or  $\alpha$ -tocopherol was added alone or in combination, TBARS values were significantly lower than in the control, and these values increased with storage time ( $p < 0.05$ ). After 20 d of storage, no significant difference was noted between sausages treated with garlic powder (GP1 and GP3),  $\alpha$ -tocopherol (AT100 and AT 200), or both (GP1+AT100). TBARS values in GP 3 (ranging from 0.383 to 0.488 mg MA/kg) and GP1+AT100 (ranging from 0.384 to 0.484 mg MA/kg) sausages during storage were lower than those in the other formulations (GP1, AT 100, and AT 200). The control sausages had the highest TBARS values (from 0.417 to 0.543 mg MA/kg) during storage. This difference in TBARS values in the presence of garlic powder and/or  $\alpha$ -tocopherol may be associated with differences in their antioxidative activity. These results are in agreement with studies by Sallam *et al.* (2004) and Ismail *et al.* (2008) of garlic or a combination of  $\alpha$ -tocopherol and ascorbic acid added to chicken sausage and ground beef, respectively. Some authors have reported that in low concentrations, allicin is responsible for the antioxidative properties of garlic (Lawson, 1998). A study performed by Sammet *et al.* (2006) also reported that sausages with added  $\alpha$ -tocopherol had lower TBARS values (from 57.59 nM MDA/g at 0 wk to 72.37 nM MDA/g at 12 wk) than control group (CG) sausages (from 67.74 nM MDA/g at 0 wk to 73.51 nM MDA/g at 12 wk). However, Channon and Trout (2002) showed that  $\alpha$ -tocopherol (0–1,000 IU/kg) had little effect on lipid oxidation during frozen storage for 37 wk. Sun *et al.* (2000) sug-

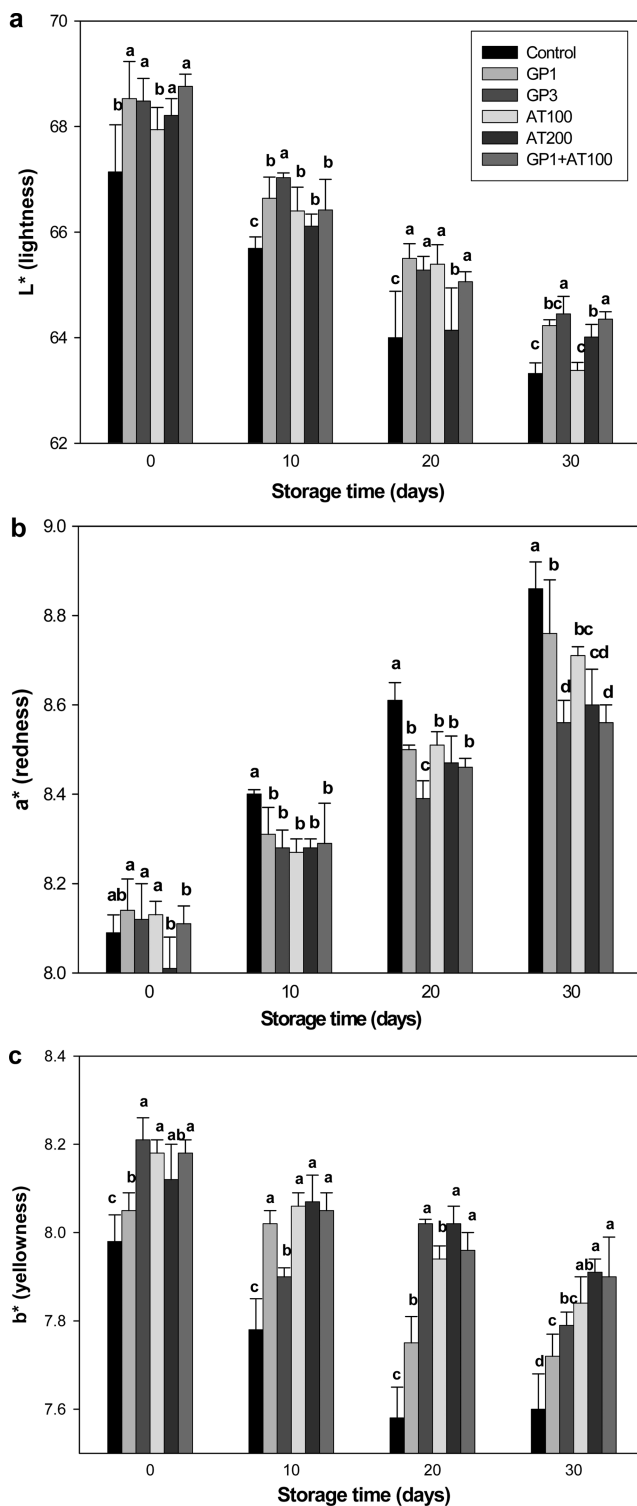
gested that the addition of garlic does not have any antioxidant effects in heat-dried Chinese-style sausages.

Fig. 1c shows that the residual nitrite content decreased significantly ( $p < 0.05$ ) in all sausages over the storage period. There were no statistically significant differences between the control sausages and those treated with 1% garlic powder (GP1) on storage day 0 and day 10, or with 200 IU  $\alpha$ -tocopherol (AT 200) or 1% garlic powder and 100 IU  $\alpha$ -tocopherol (GP1+AT100) on storage day 0, 10, and 20. During storage, residual nitrite content in emulsion-type sausages with garlic powder and/or  $\alpha$ -tocopherol varied from 7.30 to 3.20 mg/kg, while the highest residual nitrite content was found in the control group, ranging from 7.33 to 5.31 mg/kg. When compared with the control, the residual nitrite values decreased in the following order: GP3>GP1+AT100>AT200>AT100>GP1. These results clearly suggest that garlic powder and  $\alpha$ -tocopherol in emulsion-type sausages have antioxidant properties that decrease residual nitrite. Similar effects have been observed in fried bacon, where  $\alpha$ -tocopherol at 500 mg/kg reduced N-nitrosopyrrolidine (NPYR) levels (Reddy *et al.*, 1982).

In general, nitrite is an important food additive that suppresses the formation of pathogenic toxins and oxidative rancidity and maintains color stability. However, no real substitute for nitrite with the same level of effectiveness has yet been identified (Luecke, 1999). According to Thiemig *et al.* (2000), one reason that residual nitrite decreases rapidly in sausages is due to its high reactivity or the presence of substances such as myoglobin, lipids, and non-heme proteins. There are reports in the literature on the nitrite-scavenging effects of garlic extract at various pH levels (Kim *et al.*, 2002), which show that the important factors regulating nitrite-scavenging effects are pH and the concentration of garlic extract. On the basis of results, it appears that a decrease in residual nitrite is highly related to the reduction in pH of emulsion-type sausages supplemented with garlic powder and/or  $\alpha$ -tocopherol.

#### Color values in emulsion-type sausages

Fig. 2 shows the changes in the color of emulsion-type sausages supplemented with garlic powder and  $\alpha$ -tocopherol during storage. Overall, significant differences in L\* (lightness, Fig. 2a), a\* (redness, Fig. 2b), and b\* (yellowness, Fig. 2c) values were observed over time in all treatments ( $p < 0.05$ ). However, there were no statistically significant differences in L\*, a\*, and b\* values on storage day 0, 10, and 20 between sausages treated with garlic



**Fig. 2.** Effect of dried garlic powder and/or  $\alpha$ -tocopherol on (a) L\* (lightness), (b) a\* (redness), and (c) b\* (yellowness) in emulsion-type sausages during storage. Data shown are the means with the standard error represented by the vertical bars. <sup>a-d</sup>Indicates significant ( $p < 0.05$ ) differences on different storage days. The treatments are as follows: GP1, 1% garlic powder; GP3, 3% garlic powder; AT100, 100 IU of  $\alpha$ -tocopherol/kg of sausage; AT200, 200 IU of  $\alpha$ -tocopherol/kg of sausage; GP1+AT100, 1% garlic powder + 100 IU of  $\alpha$ -tocopherol/kg of sausage.

powder (GP1 and GP3),  $\alpha$ -tocopherol (AT100 and AT200), and in combination (GP1+AT100). The lowest L\* values (from 67.14 to 63.32) and b\* values (from 7.98 to 7.58) and the highest a\* values (from 8.09 to 8.86) were found in the control sausages. A decrease in L\* (from 68.76 to 63.32) and b\* (from 8.21 to 7.58) values and an increase in a\* values (from 8.01 to 8.86) were observed in all sausages with increasing storage time. In the present study, the increase in L\* values and the reduction in a\* values in treated sausages compared with the control groups may be explained in part by the effectiveness of each antioxidant added alone or combination. However, the mechanisms by which these products increase b\* values are not clear. For example, studies conducted by Genot *et al.* (1991) and Anton *et al.* (1993) suggested that the presence of antioxidant compounds in natural extracts could retard metmyoglobin formation, thereby decreasing L\* values. Our results are similar to those of Bekhit *et al.* (2003) and Aksu and Kaya (2005), which showed that antioxidants have an effect on the color stability of beef patties and kavurma, and all samples with added antioxidants were higher in lightness and yellowness than the controls. In a study on the use of natural extracts (rosemary, orange, and lemon) as antioxidants, Fernández-López *et al.* (2005) reported that lightness (L\*) increased and redness (a\*) decreased in all samples as storage time progressed. However, Lavella *et al.* (1995) found that internal cooked color, premature browning, and persistent redness were not influenced by vitamin E.

## Conclusions

In this study, we showed that the lipid and color stability and shelf life properties of emulsion-type sausages were maintained by application of the natural antioxidants garlic powder and  $\alpha$ -tocopherol either alone or in combination. Of all the antioxidant treatments tested, 3% garlic powder and 1% garlic powder+100 IU  $\alpha$ -tocopherol significantly reduced the pH of emulsion-type sausages, and resulted in the greatest reduction in TBARS and residual nitrites. However, further studies are still needed to investigate the antioxidative and antimicrobial activities of garlic powder and/or  $\alpha$ -tocopherol or to determine the optimal levels and the sensory properties that result from the inclusion of these products.

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