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Effect of Garlic (Allium Sativum) on Duck Sausage Quality during Refrigerated Storage

Muthia. D [#], Huda. N [*], Easa. A. M [#]

[#] *Program Study of Animal Husbandry, Agriculture Polytechnic,*
Andalas University, 26271 Payakumbuh, West Sumatera, Indonesia,
TEL: +62-0752-92004, FAX: +62-0752-7750220
E-mail: mutdw@yahoo.com

* *Fish and Meat Processing Laboratory,*
Food Technology Program, School of Industrial Technology,
Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia,
TEL: +604-6532112, FAX: +604-6573678
E-mail: nrlhd@usm.my

***Abstract*— The objective of this study was to compare the effects of adding natural antioxidant (garlic, fresh or powdered) or a synthetic antioxidant (butylated hydroxytoluene/ BHT) on the quality of duck sausage during 21 d of refrigerated storage. Proximate composition, pH, thiobarbituric acid (TBA), Aerobic plate counts (APC), and mold count were measured. Generally, all sample types showed decreased moisture content and pH and increased protein and fat contents over the course of the refrigerated storage period. While TBA values and APCs increased during the experimental period for all sample types, the increases were lower in the samples with garlic added due to the antioxidant effect of garlic. TBA values of duck sausage with fresh garlic or garlic powder added were higher than that of the control throughout the storage period. Fresh garlic and garlic powder were more effective in preventing microbial growth than without adding synthetic antioxidant but addition of BHT was slightly better. Overall, the addition of 50 g of fresh garlic per kg sausage was the best at reducing the TBA value among the six levels of garlic tested.**

***Keywords*— duck meat, garlic, storage, antioxidant, antimicrobial.**

I. INTRODUCTION

This Duck meat is the third most important type of poultry meat after chicken and turkey. Duck meat sausage is a new product that could expand the repertoire of duck meat products and orient consumers towards alternatives dishes. Therefore, a number of researchers have focused on developing more varieties of value-added products based on duck meat [1], [2], [3], [4].

Lipid oxidation in muscle tissue varies with animal species [4]. It is a process that can affect the quality of meat products. Thus, control and monitoring of this process during meat processing and storage are important, especially as the demand for precooked convenient meat products continues to increase. The demand for better quality duck sausage had led to the addition of antioxidants in the sausage formulation, as antioxidants inhibit both lipid oxidation and microbial growth and therefore prolong the shelf life of the product and prevent food-borne illnesses. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), currently are used in sausage formulations. However, questions about the safety of using synthetic antioxidants, which could have toxic or carcinogenic effects on health, have led to an increased interest in natural antioxidants, especially those from plants that also can be used as a preservative [6].

In this study, garlic was added to the duck sausage formulation as a natural antioxidant. Garlic is a common spice that has been used widely to control and minimize the lipid oxidation process and microbial growth in meat products [7], [8], [9], [10], [11], [12], [13]. The objective of this study was to compare the effects of garlic (fresh and powder form) to those of BHT on duck sausage during refrigerated storage.

II. Material and Methods

A. Sample preparation

Mechanically deboned duck meat was obtained from a local supplier (Fika Food Sdn Bhd, Penang, Malaysia), transported frozen to the laboratory, and stored at –18 oC to maintain its freshness. Before being used, the meat was thawed in a chiller at 5 oC for 1 h and then cut into small pieces (3 x 3 x 3 cm) using a meat bone saw machine (Powerline P79, Norwalk, CT, USA). Tapioca flour, palm oil, fresh garlic, spices, sugar, and salt were purchased from Tesco Stores (Penang, Malaysia), whereas egg white powder, garlic powder, and BHT were purchased from Dimeters Choice Trading (Penang, Malaysia). All chemicals used for analysis were of analytical grade. Fresh garlic was prepared as follows: Fresh garlic cloves (~1 kg) were peeled, washed, and blended using a blender machine (Pensonic, Penang, Malaysia) for 3 min until smooth; the blended garlic was kept chilled until it was added to the duck sausage formulation.

Eight different treatments were tested in this study (Table 1). Following [12], the levels of fresh garlic added to the duck sausage were 20, 30, and 50 g/kg (i.e., FG 20, FG 30, and FG 50) and for garlic powder the levels were 6, 9, and 15 g/kg (i.e., GP 6, GP 9, and GP 15). The BHT sample contained 200 ppm per kilogram sample. A control sample of duck sausage without garlic or BHT also was prepared.

Table 1 shows the formulations for the different types of duck sausage. Processing of the sausage was conducted following the method described by [1] with slight modification. The duck meat was chopped using a cutter mixer (Robot Coupe Blixer 3B, Burgundy, France) for 2 min, and all ingredients were then poured into the mixer and chopped for 3 min. The emulsions obtained were stuffed into synthetic casings (15 mm diameters) using a mechanical sausage stuffer and tied manually into 15 cm long lengths. The duck sausages were steamed at 65 °C for 30 min in a steamer (Kerres CS350, Beknang Germany), and the temperature was gradually raised to 85–90 °C for 2 h. Finally, the sausages were soaked in ice cubes for 2 min. The samples were placed into plastic containers, sealed with one layer of a semi-permeable film vacuum packaging, and stored at 4 °C. Storage conditions were recorded and sampling was conducted at 0, 7, 14, and 21 days of storage time.

TABLE 1.
FORMULATION OF DUCK SAUSAGE USING FRESH GARLIC, GARLIC POWDER, AND BHT BASED ON A 1000 G SAMPLE

Formulations (g)	Samples				
	CTR	**BHT**	**FG 20**	**FG 30**	**FG 50**
1.MDDM	650	650	650	650	650
2.Tapioca flour	40.0	40	40	40	40
3. Egg white powder	7.5	7.5	7.5	7.5	7.5
4. Cold water	137.7	137.7	137.7	137.7	137.7
5. Palm oil	70.0	70.0	70.0	70.0	70.0
6. Salt	25.0	25.0	25.0	25.0	25.0
7. Sugar	10.0	10.0	10.0	10.0	10.0
8. Monosodium glutamate	5.0	5.0	5.0	5.0	5.0
9. Spices	54.8	54.8	54.8	54.8	54.8
Total	**1000.0**	**1000.0**	**1000.0**	**1000.0**	**1000.0**

Continued

Formulations (g)	**Samples**		
	GP 6	**GP 9**	**GP 15**
1. MDDM	650	650	650
2. Tapioca flour	40	40	40
3. Egg white powder	7.5	7.5	7.5
4. Cold water	137.7	137.7	137.7
5. Palm oil	70.0	70.0	70.0
6. Salt	25.0	25.0	25.0
7. Sugar	10.0	10.0	10.0
8. Monosodium glutamate	5.0	5.0	5.0
9. Spices	54.8	54.8	54.8
Total	**1000.0**	**1000.0**	**1000.0**

Sample: MDDM = mechanically deboned duck meat; CTR = without any antioxidant; BHT = butylated hydroxytoluene added; FG 20 = 20 g fresh garlic added; FG 30 = 30g FG added; FG 50 = 50g FG added; GP 6 = 6g garlic powder added; GP 9 = 9g GP added; GP 15 = 15g GP added.

B. Proximate analysis

At each of the four sampling time points, moisture, protein, and fat contents of each sample type were determined in accordance with standard AOAC methods [14]. Moisture content was quantified by the oven drying method at 100–105 oC for 24 h. Protein content was determined using the Kjeldhal method, and fat content was determined using the Soxhlet method.

C. pH

At each time point, pH was measured following Sallam et al. (2004) with slight modification. For each treatment type, 10 g of a duck sausage sample were placed in a glass beaker and homogenized with 50 ml of deionised water using a homogenizer (T25 digital ULTRA-TURRAX®IKA®, Staufen, Germany). The pH value then was measured using a pH meter (Mettler Toledo delta 320, Switzerland). The pH value was measured three times for each sample.

D. TBA test

The oxidation of lipids, which leads to rancidity, is one of the most important changes that occur during food storage and production. During storage, autocatalytic mechanisms involving free radicals cause auto-oxidation of fatty acids. During this process, malondialdehyde (MDA) is produced, and this secondary oxidation product can be detected using 2-thiobarbituric acid (TBA). The TBA method is the most widely used test for measuring the extent of lipid oxidation in muscle foods.

In this study, the TBA content of the samples was determined using the distilled method with minor modifications [15], and the TBA value was reported in mg MDA/kg of sample. For each treatment type at each sampling time point, ~10 g of a sausage sample were homogenized with 25 ml of 20% trichloroacetic acid (TCA) solution (200 g/L of TCA in a 135 ml/L phosphoric acid solution) in a homogenizer (T25 digital ULTRA-TURRAX®) at 13,800 rpm for 30 s. The homogenized sample was filtered through Whatman paper, and 2 ml of the filtrate were added to 2 ml of 0.02 M aqueous TBA solution

(3 g/l) in a test tube. The test tube was incubated at 100 oC in the dark for 30 min and then cooled in tap water. The absorbance was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). Measurements were conducted in triplicate for each treatment type.

C. Aerobic plate counts (APCs)

APCs were performed following [12]. For each treatment type at each sampling time point, a duck sausage sample (10 g) was homogenized with 90 ml of sterile peptone water (1 g/l) in a Stomacher 400 (Colworth, London, U.K.). Serial dilutions were prepared. Next, 0.1 ml of each dilution was spread with a disposable spreader (SPL Labware, Milano, Italy) on triplicate plates containing Merck plate count agar. The agar plates were incubated at 25 oC for 48 h. The colonies were counted and the result was expressed as log10 colony forming units (CFU)/g of duck sausage sample.

D. Mold counts

Mold counts were performed following [16]. For each treatment type at each sampling time point, a duck sausage sample (25 g) was homogenized (Bag Mixer, Saint-Germain-En-Laye, France) with 225 ml of sterile peptone water solution (1g/l) in a stomacher 400 (Colworth, London, U.K.) for 1 min. Serial dilutions of the sample were prepared, and then 0.1 ml of each dilution was spread with a disposal spreader (SPL Labware, Milano, Italy) on triplicate plates containing Merck potato dextrose agar. The agar plates were incubated at 25 ºC for 4–5 d, and then the colonies were counted and the result was expressed as log10 CFU/g of duck sausage sample.

E. Statistical analysis

For each parameter measured (i.e., proximate composition, pH, TBA, APCs, and mold counts), mean values were compared for a given sample type over time and among the eight different sausage types over the course of the four sampling time points. The data collected were analysed using the statistical package for social science (SPSS Inc, Chicago, IL, USA) version 16.0. The means of treatments showing significant difference ($P < 0.05$) were subjected to analysis of variance (ANOVA).

III. Results and Discussion

A. Moisture, protein, and fat contents

Figure 1 shows the moisture, protein, and fat contents of the different sample types. The ranges of initial moisture, protein, and fat contents of the duck sausage samples were 65.37–66.83%, 14.06–16.16%, and 12.35–13.13%, respectively. Sausages with different types and quantities of garlic added, BHT added, and the control sample differed significantly from one another ($P < 0.05$) for moisture, protein, and fat contents during the 21 d of refrigerated storage.

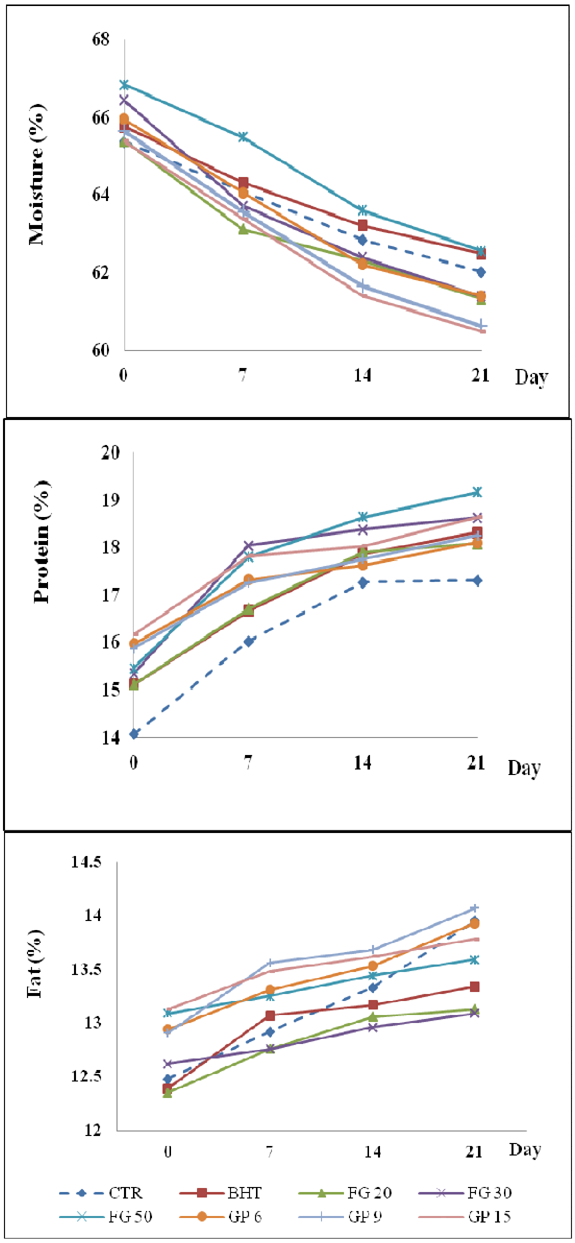

Fig 1. Moisture, protein, and fat contents of duck sausage with garlic added during 21 d of refrigerated storage. CTR = without any antioxidant; BHT = butylated hydroxytoluene added; FG 20 = 20 g fresh garlic added; FG 30 = 30g FG added; FG 50 = 50g FG added; GP 6 = 6g garlic powder added; GP 9 = 9g GP added; GP 15 = 15g GP added.

Samples with FG and GP added had significantly higher ($P < 0.05$) moisture, protein, and fat contents compared to the BHT added and control samples during the 21 d of storage time. Moisture content decreased progressively during refrigerated storage, whereas protein and fat contents progressively increased. The decreased moisture increased the total amount of solids, including protein and fat, in the sausages. [17] also found a significant effect of storage on the nutritional quality of meat and bone meal at ambient temperatures.

The moisture, protein, and fat contents found in this study were in the range reported for Malaysian commercial chicken sausages (i.e., 58.49–68.85%, 7.03–14.14%, and 4.91–18.48%, respectively) [4]. [9] previously reported that the addition of garlic to Chinese sausage prepared with frozen pork hams and backfat had no significant effect ($P > 0.05$) on fat and protein content but did have a significant effect ($P < 0.05$) on moisture content. [12] noted that the addition of different forms of garlic (fresh, powder, and oil) to chicken sausage did not significantly affect the moisture, protein, and fat contents of the product. Differences in the results may be due to differences in the type and amount of material used and in the sausage making technique.

B. pH

Figure 2 shows the changes in the pH value of the different types of duck sausage during the 21 d of refrigerated storage. Significant differences ($P < 0.05$) were observed in the pH values among all treatments. The initial pH range of the duck sausages was 6.30–6.68, but it changed to 6.40–6.52 by day 21. The sequence of pH values from high to low was Control > FG 20 > GP 6 > FG 30 > GP 9 > FG 50 > GP 15 > BHT, and the pH value in all samples consistently decreased over the 21 d storage period. This pH reduction likely was due to the presence of lactic acid produced by lactic acid bacteria, which are the predominant microorganisms found in vacuum-packaged meat products [18].

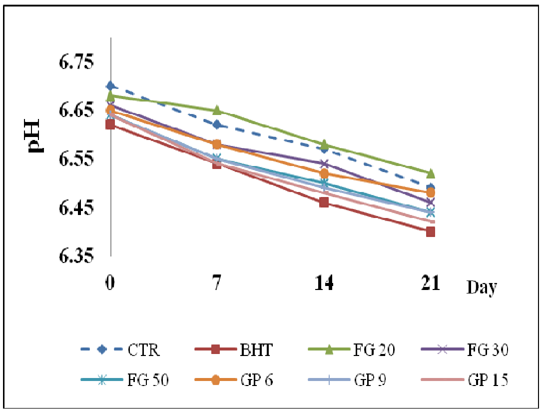

Fig 2. Effect of the addition of fresh garlic and garlic powder on the pH value of duck sausage during 21 d of refrigerated storage. CTR = without any antioxidant; BHT = butylated hydroxytoluene added; FG 20 = 20 g fresh garlic added; FG 30 = 30g FG added; FG 50 = 50g FG added; GP 6 = 6g garlic powder added; GP 9 = 9g GP added; GP 15 = 15g GP added.

Liu *et al.*, 2009 reported that the addition of garlic to Chinese sausage prepared from pork significantly decreased the pH values of the sausage during storage from approximately pH 6.3 at day 0 to pH 4.7 at day 56. Finding that the pH value of duck sausages decreased significantly during storage agrees well with results reported by [20], [9], [12], and [21]. [20] reported that the pH values of chicken sausage remained stable in the pH range of 6.4–6.6 during the first 10 d of refrigerated storage, but the values decreased significantly ($P < 0.05$) thereafter.

At day 14 of storage, the pH value of the control samples had decreased dramatically to 5.90. [22] stated that decreased pH values could decrease water holding capacity in stored duck sausage. [23] reported that increased storage time led to decreased ($P < 0.05$) pH values in the sausage formulations tested. Similarly, [24] noted that 14 d of storage time resulted in a decrease in pH ($P < 0.05$) for all traditional sausage treatments that contained leek and onion. In contrast, however, [26] found that storage time had no effect ($P < 0.05$) on the pH values of low-fat chicken sausage.

In this study, the pH values of the samples to which fresh garlic or garlic powder were added did not differ significantly throughout the storage period. The pH values of the sausages containing fresh garlic and garlic powder were the lowest, and the pH of the control sample was the highest. [1] reported the pH value of spent duck meat sausage to be 6.47. This value was lower than the pH value in this study, likely because of the different raw materials used: [1] used spent duck meat, whereas we used 3-month-old Peking duck. Thus, different raw materials, formulations, and processing conditions can affect the pH of the sausage.

The sequence of pH values from high to low was Control > FG 20 > GP 6 > FG 30 > GP 9 > FG 50 > GP 15 > BHT The sausage with 50 g/kg of fresh garlic (FG 50) added had the highest pH value and the sausage with 15 g/kg of garlic powder (GP 15) added had the lowest pH value at 21 d of storage compared to sausages with other garlic levels or BHT added and the control samples. [9] also found that the pH value of Chinese sausage was higher when fresh garlic was added than when garlic essential oil was added.

C. TBA

Figure 3 shows the TBA values of the different types of sausages. The range of TBA values (mg MDA/kg sample) of the duck sausages on the initial day of storage was 0.230 (BHT) to 0.282 (control). On day 7 the range was 0.288 (BHT) to 0.356 (GP 9), on day 14 it was 0.343 (BHT) to 0.459 (GP 15), and on day 21 it was in 0.391 (BHT) to 0.54 (control). Thus, the concentration of TBA was always lowest in the BHT samples and it was highest in the control sample at the beginning and end of the storage period, but the effect of additions of natural antioxidant such as garlic was better to reduce TBA values than control sample that addition of artificial antioxidant.

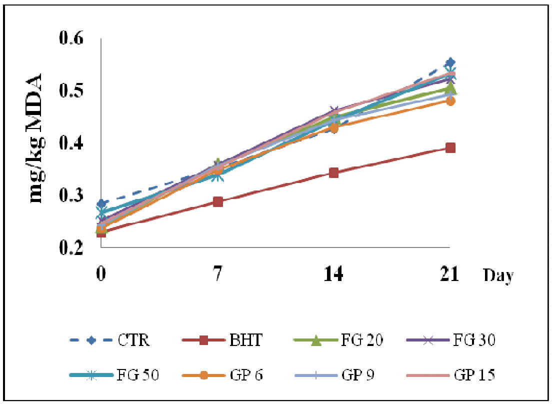

Fig. 3 Effect of the addition of fresh garlic and garlic powder on the thiobarbituric acid content of duck sausage during 21 d of refrigerated storage. CTR = without any antioxidant; BHT = butylated hydroxytoluene added; FG 20 = 20 g fresh garlic added; FG 30 = 30g FG added; FG 50 = 50g FG added; GP 6 = 6g garlic powder added; GP 9 = 9g GP added; GP 15 = 15g GP added.

Similarly, [6] found that the TBA value of chicken sausage was significantly higher in samples to which garlic was added compared to samples without garlic. Overall, the TBA values rapidly increased between the first to the last day of storage. The addition of fresh garlic at 50 g/kg of duck sausage had the most potent effect on reducing the TBA value compared to other levels and forms of garlic.

The TBA values in this study were not higher than 0.6 mg MDA/kg duck sausage, and Raharjo and Sofos (1993) stated that rancid flavour is initially detected in meat products at TBA values of 0.5 to 2.0 mg MDA/kg sample. Ansorena and Astiasar (2004) reported that using linseed oil as an antioxidant resulted in a higher TBA value (0.23 mg MDA/kg sample) compared to the control (0.08 mg MDA/kg sample). Fista (2004) found the TBA value was higher in sausages with leek and onion added (1.38 mg MDA/ kg sample) than in the control (0.39 mg MDA/kg sample). [12] reported that the TBA value of chicken sausages containing fresh garlic was lower (0.175 mg MDA/kg) than that in sausages containing garlic powder (0.187 mg MDA/kg) and those without garlic (0.278 mg MDA/kg). Thus, the addition of natural antioxidants appears to reduce the rancidity rate of sausage samples.

D. APCs and mold counts

The effect of the addition of garlic on the APCs of duck sausage is shown in Figure 4. The range of APCs of the duck sausages (log10 CFU/g) on day 0 was 3.25 (BHT) to 3.56 (GP 6), on day 7 it was 3.75 (BHT) to 5.33 (control), on day 14 it was 5.09 (BHT) to 7.03 (control), and on day 21 it was 7.16 (BHT) to 9.51 (control). The APCs increased significantly ($P < 0.05$) with increased storage time in all sausage types tested, and the addition of garlic had a significant ($P < 0.05$) effect on the APCs. Overall, the APCs were lowest in the BHT sample and highest in the control sample compared to the garlic-added samples at all time points (except on day 0, when GP6 had the highest value).

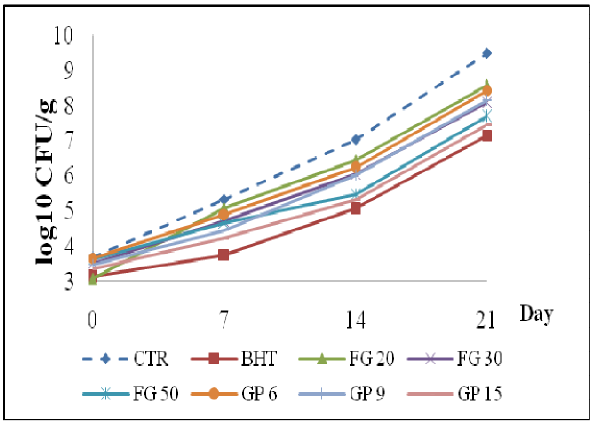

Fig 4. Effect of addition of fresh garlic and garlic powder on APC of duck sausage during 21 d of refrigerated storage. CTR = without any antioxidant; BHT = butylated hydroxytoluene added; FG 20 = 20 g fresh garlic added; FG 30 = 30g FG added; FG 50 = 50g FG added; GP 6 = 6g garlic powder added; GP 9 = 9g GP added; GP 15 = 15g GP added.

The maximum permissible level (MPL) recommended by the International Commission on Microbiological Specifications for APCs in all sausage products is below 7 log10 CFU/g (ICMSF, 2002). All duck sausage types except for the control sample remained safe for consumption until day 14 of storage because the values were below the MPL. After 21 d of storage, APCs of duck sausage followed the order BHT < FG 50 < GP 15 < FG 30 < GP 9 < GP 6 < FG 20 < Control. This result illustrates that the addition of FG 50 was more effective at controlling bacteria than addition of the other garlic types. This might be due to the antimicrobial action of fresh powder; it contains components such as allicin and diallyl sulphide, which are thought to be strong antibacterial agents. Verluyten et al. (2004) reported that garlic is the only spice that enhances specific bacteriocin production, resulting in higher bacteriocin activity in the cell-free culture supernatant. Furthermore, [30] found increased significantly ($P < 0.05$) on microbial counts in buffalo sausages during storage with using of vacuum-packaged at refrigerated temperature (4 ± 1 oC) compared to samples with CO2 and nitrogen packaging. [2] found that microbial counts of duck sausages increased significantly ($P < 0.01$) with the increase in storage period at ambient and chilling temperatures. [21] reported that coliform bacterial counts declined very quickly and that growth was totally inhibited within 7 d in more acidified sausages.

In contrast to garlic powder, [21] found that fresh garlic provided the full range of beneficial effects in meat products compared to garlic powder and garlic oil. Furthermore, [31] reported that garlic could differentially inhibit bacteria; lactic acid bacteria are the least sensitive microorganisms, and garlic can stimulate the growth of these bacteria by providing them with a carbohydrate source. Moreover, the addition of 1% and 3% garlic juice in emulsified sausage was shown to lead to decreased peroxide, TBA, residual nitrite, and total microbiological counts compared to those of the control during cold storage (Park and Kim, 2009).

Mold was not detected in any of the duck sausage samples at day 0, and at 7, 14, and 21 d of storage, no more than 10 mold colonies were found on the duck sausages containing any amount of fresh garlic or garlic powder or BHT. In the control samples at 14 and 21 d of storage, however, the mold count was 1.62 and 3.02 log10 CFU/g, respectively. Thus, the addition of garlic to duck sausage can effectively delay mold growth compared to sausages without garlic added.

IV. Conclusions

The addition of either fresh garlic or garlic powder affected the quality of duck sausage during refrigerated storage. In all sample types, moisture content and pH decreased and protein and fat contents, TBA values, and APCs increased during the 21 d of refrigerated storage. However, the presence of garlic reduced the increase in TBA values and APCs during storage. The TBA values of duck sausages containing fresh garlic and garlic powder were lower than that of the control throughout the storage period. Among the garlic forms studied, fresh garlic at 50 g/kg sausage had the most potent effect on reducing the TBA value. These results suggest that garlic, as a natural antioxidant and antimicrobial agent, could be used to extend the shelf life of duck sausage. Use of a natural product likely would be a more healthful choice than use of a synthetic agent.

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