Use of fresh garlic and garlic powder in duck sausages during refrigerated storage

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Abstract

Garlic is one of the most commonly used ingredients as a flavour enhancer for sausage. Four formulations of duck sausage were prepared by adding fresh garlic and garlic powder and compared to control with added butylated hydroxyanisole (BHA). The evaluation of moisture, protein, fat, pH, rancidity (TBA) and total plate count in this product was evaluated during 21 days refrigerated storage. Addition of FG and GP significantly (P < 0.05) affected all of the parameters. In this study, garlic was shown to be an effective antimicrobial during refrigerated storage with less inhibiting lipid oxidation that gives a higher rancidity value.

Keywords: additives, food preservation, TBA, TPC, Malaysia, Indonesia, *Allium sativum*.

Introduction

Duck sausage is one of the meat products that is particularly susceptible to oxidation due to high unsaturated fat content. The quality and safety of duck sausage is affected by oxidation of fats and lipids. Antioxidants are used to overcome these problems. Application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss [1]. The most common antioxidants used in food preservation are synthetic, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ), mainly due to their low cost and high stability [2]. However, consumers are concerned about the safety of synthetic food additives. Natural antioxidants, such as garlic, have proven to be more powerful and more potent than the synthetic antioxidants.

Garlic is one of the most commonly used ingredients as a flavour enhancer for sausage. In addition to flavouring food, garlic is appreciated for its medicinal properties. Garlic has a wide spectrum of actions; not only antibacterial, antiviral, antifungal and antiprotozoal, but also has
beneficial effects on the cardiovascular and immune systems [3]. During the last decade, the antimicrobial activity of garlic and garlic-derived organosulphur compounds was widely investigated against both food spoilage bacteria and food-borne pathogens [4, 5]. Besides its antimicrobial effects, garlic showed effective antioxidant activity in vivo and in vitro [6, 7]. Garlic-rich organosulphur compounds and their precursors (allicin, diallyl sulphide and diallyl trisulphide) are believed to play a key role in these biological effects [8]. Several components of garlic and garlic extracts possess antioxidant activity, which is concentration dependent [9], showing that garlic is an effective hydroxyl radical scavenger. Substances such as alliin, diallyl sulphide, allyl sulphide and propyl sulphide, account for the antioxidant effect. Additionally, garlic contains ascorbic acid and nitrates and nitrites [10].

The thiobarbituric acid (TBA) test is a common method to measure lipid oxidation in meat [11, 12] which determines malondialdehyde (MDA) content.

Principals from garlic have been shown to have antibacterial, antifungal, antiviral and antiprotozoal activities. They also modulate the cardiovascular and immune system as well as having antioxidant and anticancer properties [13]. Antioxidant and antimicrobial effects of four garlic-derived organosulphur compounds in ground beef not only enhanced colour and minimized lipid oxidation but also enhanced microbial safety of the product [1].

The antioxidant and antimicrobial effects of equivalent concentrations of fresh garlic (FG), garlic powder (GP) and garlic oil (GO) were investigated against lipid oxidation and microbial growth in raw chicken sausage during storage at 3°C [14]. The antioxidant activities were compared to that of a standard synthetic antioxidant; butylated hydroxyanisole (BHA). The result showed that addition of GO or BHA resulted in no significant difference in aerobic plate count when compared with control. Sensory analysis indicated that FG had a significantly stronger flavour than the other sausage formulations. The results suggest that fresh garlic and garlic powder, through their combined antioxidant and antimicrobial effects, are potentially useful in preserving meat products [14].

The objective of the present study was to investigate the antioxidant as well as the antimicrobial effectiveness of two garlic preparations, i.e. fresh and powder at various concentrations, in preserving duck sausage during refrigerated storage.

Materials and Methods

Raw material
Fresh garlic (FG) bulbs (Allium sativum, var. Chinese white garlic) and garlic powder (GP) were purchased from a local market. The dry skins of the bulb were removed before use; then the cloves were peeled and crushed finely by using a kitchen hand-held grater.

Preparation of duck sausage
Freshly obtained mechanically deboned duck meat (MDDM) was thawed at 10°C just before sausage manufacture. MDDM was cut into small pieces and mixed with all of the ingredients for about 5 min. The emulsion was formulated to contain either FG (50 g/kg), GP (15 g/kg), BHA (0.1 g/kg) and control (without garlic and BHA). All other ingredients were added in equal amount (g/kg) to the various formulations of sausage meat: 100g shattered ice, 7g palm oil, 20g salt (sodium chloride), 1.5g sodium polyphosphate, 1g monosodium glutamate, 1.5g sugar and 6g sausage seasoning (3g black pepper, 2g white pepper and 1g onion). The emulsion was stuffed into artificial casing separately, using a mechanical sausage-filler and linked manually and
steamed at 65°C 30 min and increasing to 90°C for 2 hours. Then the product was cool with water (10-15°C) for 2 min and then stored in a freezer for analysis.

**Proximate composition**
Moisture, protein and fat contents of duck sausages were determined according to standard AOAC [15] procedures. Analyses were conducted in triplicate; all reagents were of analytical grade. Moisture content was determined by drying the samples overnight at 105°C. Crude protein content was determined by Kjeldahl method. Crude lipid content was determined by soxhlet method.

**pH determination**
10g of sample was homogenised with 50 ml distilled water and pH value was measured by a digital pH-meter (HM-5S; TOA Electric Industrial Co. Ltd., Tokyo, Japan [14].

**TBA analysis**
TBA values were determined by the spectrophotometric method. Ground meat (10g) was homogenized with 49 ml of distilled water and 1 ml BHA in a Virtis homogenizer (The Virtis Co., Gardiner, NY) at 13,800 rpm (speed setting, 60) for 1 min. TBA solution: 0.02 M of TBA (Merck, Germany) in distilled water [16].

**Total plate count**
Sausage sample (10g) was homogenized with 90 ml of sterile peptone water (1 g/l) in a laboratory homogenizer (Lab. Blender, Stomacher Interscience) and serial dilutions were prepared, then 0.1 ml of each dilution was spread with a bent sterile glass rod on duplicate plates. After 48-h incubation at 25°C, colonies were counted and results were expressed as log10 CFU/g of sausage sample [17].

**Statistical analysis**
The data collected were analyzed using Statistical Package for Social Science (SPSS) version 16.00. Means of treatment showing significant differences (p<0.05) were subjected to Duncan’s Multiple Range Test.

**Results and Discussion**

**Proximate analysis**
The initial moisture, protein and fat ranged from 65.37±0.70 (BHA) to 66.83±0.17, 14.04±0.20 (Control) to 16.16±0.21 (FG) and 12.39±0.10 (BHA) to 13.13±0.32 (FG). The results showed that chemical composition of duck sausages was affected by the addition of fresh garlic and garlic powder. Moisture, protein and fat contents were significantly different ($P < 0.05$) and higher compared to the control and the BHA formulation after 21 days storage (Table 1). Sun, et al [18], found no differences ($P > 0.05$) from garlic treatment for fat and protein content on Chinese sausage, but there was a significant effect on moisture. Conversely, in this work the moisture and protein content were higher for treatments with fresh garlic and garlic powder compared to control and BHA formulation. This may be due to the fat content in sausages was significantly higher for all duck formulations with refined garlic because the moisture content decreased during storage. The fat content was not significantly different ($P > 0.05$) on the initial day and showed significant difference ($P < 0.05$) lowest on BHA than control, fresh garlic and garlic powder formulations until Day 21 storage period. Figure 1 shows the initial moisture, fat and protein contents.
Figure 1. Effect of adding garlic on moisture, protein and fat of duck sausage using fresh garlic and garlic powder.
**pH analysis**

Changes in the pH values of duck sausage during refrigerated storage are given in Fig. 2. The initial pH value ranged from 6.62 (in BHA sample) to 6.70 (in control sample). In all sausage formulations, storage had a significant ($P < 0.05$) decreasing effect on the pH values on 21 days of refrigerated storage in all formulations. After 21 days of storage significant difference was detected between pH of FG-formulated sausage and any of the other sausage formulations, which ranged from 6.40 (in BHA sample) to 6.49 (in Control sample). pH values significantly decreased from approximate pH 6.62 at day 0 to pH 4.7 at day 56 (Fig. 2a). This pH reduction was probably due to the fact that lactic acid bacteria were the predominant microorganisms in vacuum-packaged meat products, which produced lactic acid, which then reduced pH values of the products [19]. The pH values obtained in this study were higher than those found in sausage from spent duck where the pH values were found to be slightly lower (6.47) [19]. The results in this study being higher than those found in other work may be due to different raw material, compositions and processing conditions. Papadima and Bloukas [16], stated that in all sausage formulations, storage had a significant ($P<0.05$) effect on the pH values, which tended to increase with storage time. The pH was almost the same with research that used garlic in Chinese sausage [18]. This was different with other work where it was found that storage time had no effect ($P < 0.08$) on pH values of low-fat chicken sausage [20]. Similarly, other researchers found that the change of pH did not show significant microbiological difference [21]. The pH values of all treatments showed consistent change with storage time (Fig 2).

**TBA values of duck sausage**

TBARS for all the samples were determined up to 21 days of storage under accelerated conditions. Figure 2 shows the effect of different concentration of FG and GP on TBA in the duck sausage during storage at 4°C. There was a significant ($P<0.05$) effect of time on the concentration of TBARS per unit fat for the addition of fresh garlic and garlic powder compared to using BHA and control. TBARS were in the range of 0.39 µmol/g oil (in control sample) – 2.25 µmol/g oil (in FG 50 sample) for 0 day storage and 0.65 µmol/g oil (in control sample) - 0.896 µmol/g oil (in BHA sample) after 21 days of storage. The concentration of TBARS were low in BHA samples (Fig 2) and increased rapidly during the first 3 weeks of storage to reach a maximum of fat for the added fresh garlic and garlic powder, respectively. This result is the same as Aguirrezaábal, et al [10] who showed that added garlic increased the TBA values of chicken sausage during storage period. TBA value is routinely used as an index of lipid oxidation in meat products in storage [22], and the rancid flavour is initially detected in meat products between TBA values of 0.5 and 2.0 [23]. TBA values of duck sausage that used fresh garlic and garlic powder was higher than the control (without garlic and antioxidant) during storage period (Fig 2). This was a similar result to Gray and Pearson [24] who used linseed oil as an antioxidant and was higher (0.23 mg malonaldehyde/kg sample)) than the control (0.08 mg malonaldehyde/kg sample). The TBA in this study same as found by Sun et al [18] where sausages using fresh garlic and garlic powder had a higher TBA value than control.

**Total plate count of duck sausage**

A significant ($P < 0.05$) increase in total plate count (TPC) was observed in treatment and control up to the 7th day and thereafter (Fig 2). Initial TPC in the sausage was 3.03 log10 CFU/g to 3.69 log 10 CFU/g (Fig. 3); and during the first 7 days of storage the count in all sausage formulations remained below 6 log10 CFU/g which is the MPL (Maximal Permissible Limit) for TPC recommended by ICMSF [25]. At storage day 21, sausage samples formulated with either FG (50 g/kg) or GP (15 g/kg) maintained lower TPC (7.69 and 7.47 log10 CFU/g, respectively) compared with control (9.51 log 10 CFU/g) but still the lowest TPC was in samples with added BHA (7.16 log 10 CFU/g), while TPC in the only control formulated sample exceeded the limit of 7 log10
Figure 2. Effect of adding garlic on pH, TBA and TPC of duck sausage using fresh garlic and garlic powder.
CFU/g by 2.46, 2.23 and 1.12–1.64 logs, respectively. After 21 days storage, TPC of both FG- and GP formulated sausage were significantly lower than that of either the control or BHA-formulated samples. Moreover, GP-formulated samples showed significantly lower PC than GP formulation. However, addition of BHA resulted in slightly higher TPC when compared with control and garlic samples (Fig. 2).

The order of antimicrobial activity of the different materials added was BHA>GP> FG >Control. This observation did not support the findings of Sallam et al [14], who claimed that only fresh garlic preparation provided the full range of beneficial compounds. However, it was noted that GP showed slightly higher antimicrobial activity than FG. To date, although the antimicrobial activity of garlic and garlic derived organosulphur compounds were widely reported in culture media, few reports are available on its effect in meat products [1].

However, Sun et al [18] reported that garlic added to heat-dried Chinese style sausage did not produce significant differences in APC. Further studies are required to evaluate the antimicrobial activities of garlic added to sausage and other meat products in combination with heat treatment.

Conclusions

This study concluded that fresh garlic and garlic powder provide antioxidant and antimicrobial benefits to duck sausage during refrigerated storage (4°C). Among the garlic forms studied, fresh garlic at a concentration of 50 g/kg sausage demonstrated the most potent effect on TPC, but such increasing concentration also resulted in increased TBA values. Addition of fresh garlic and garlic powder produced significant antioxidant and antimicrobial effects and extended the shelf-life during refrigerated storage.

Acknowledgements

The authors acknowledge with gratitude the support given by Universiti Sains Malaysia (USM) and the Malaysian Ministry of Science, Technology and Innovation (MOSTI) through Science Fund research grant 305/PTEKIND/ 613502.

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