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# Effect of various levels of rosemary or Chinese mahogany on the quality of fresh chicken sausage during refrigerated storage

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ABSTRACT

The purpose of this study was to evaluate the effect of rosemary or Chinese mahogany, at levels of 500, 1000 and 1500 ppm, of the phenolic compounds, on the quality of fresh chicken sausage stored at 4 °C for 14 days. The results showed that sausages with addition of Chinese mahogany or rosemary underwent less pH value reduction. The intense colour of Chinese mahogany or rosemary resulted in samples with lower *L* values and higher *a* values. Samples with more Chinese mahogany or rosemary added had higher total phenolic compounds. Lower TBA (thiobarbituric acid) and VBN (volatile basic nitrogen) values, and lower total plate counts were observed for the samples with Chinese mahogany or rosemary added. Samples with Chinese mahogany added had higher overall acceptance than had samples with rosemary added. Some volatile compounds, including alcohols, acids, esters, aldehydes, ethers and phenolic compounds, were isolated from the samples and identified.



## 1. Introduction

Sausage is one of the oldest known forms of processed meat products and is very popular in many areas. Fresh sausages, e.g. fresh pork sausage, country-style pork sausage, fresh kielbasa (Polish), Korr (Swedish), Italian sausage, bratwurst, bockwusrt, chorizo (fresh) and thuringer (fresh), are some common examples (Romans, Costello, Carlson, Greaser, & Jones, 1994). The cited authors indicate that fresh sausage is a sausage "made from selected cuts of fresh meat (not cooked or cured) and must be stored in a refrigerated (or frozen) state prior to being consumed." Therefore, adding "curing agents" (mainly nitrites and nitrates) to a formula, or not, is the major criterion used to judge whether the product belongs to "fresh sausage" or cured sausage. Also, raw materials of fresh sausage should not be cooked. No typical thermal treatments, such as drying, smoking or cooking, should be applied when making fresh sausages.

Lipid oxidation, resulting in rancidity, is one of the most important quality defects of meat or meat product during storage. Antioxidants can retard lipid rancidity in foods and prolong product shelf life. Since consumers have concerns regarding synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG), natural antioxidants may be applied in foods (Aruoma, Halliwell, Aeschbach, & Löligers, 1992). Many herbs and spices contain phenolic compounds, which have some antioxidative properties.

Rosemary (*Rosmarinus officinalis* L.), like other aromatic herbs and spices, which has been planted in many areas and used in Mediterranean and other cuisine, is not only used to improve or modify flavours of foods, but also to provide some functionality. For example, its extract has been widely used as an antioxidant in the food industries. Carnosol, carnosic acid and rosmarinic acid have been identified as major constituents that contribute to the antioxidant activity of rosemary (Aruoma et al., 1992). Utilising DPPH and ABTS radical-scavenging assays, and the ferric thiocyanate test, Erkan, Ayranci, and Ayranci (2008) pointed out that rosemary extract had a higher phenolic content than had blackseed (*Nigella sativa* L.) essential oil, thus leading to a higher antioxidant activity. Many reports have indicated that rosemary extracts can retard lipid oxidation and prolong the shelf life of meat products (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007; Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007; Sebranek, Sewalt, Robbins, & Houser, 2005). In addition, rosemary extracts have been shown to have some antimicrobial effect (Angioni et al., 2004).

Chinese mahogany, also known as *Toona sinensis* Roem, is a perennial tree that has become widely grown in Taiwan and China (Edmonds & Staniforth, 1998). Its leaves have a special aroma and are often consumed in Taiwan. Several reports regarding the medical uses of this plant, such as for treatment of enteritis, dysentery and itch (in the practice of oriental medicine) and for anticancer and hypoglycaemic effects have also appeared (Edmonds & Staniforth, 1998). Hseu et al. (2008) reported that *T. sinensis* aqueous extracts, at levels up to 100 μg/ml, showed some antioxidant activities, including the scavenging of free and superoxide anion

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radicals, reducing power and metal chelation. Methanol extracts of *T. sinensis* also demonstrated strong DPPH radical-scavenging activities and inhibitory effects on lipid peroxidation Cho et al. (2003). Similarly, some potent antioxidative components in the young leaves and shoots of *T. sinensis* let to a promising healthy-promoting food (Wang, Yang, & Zhang, 2007). Even though, *T. sinensis* was reported to have some antimicrobial activity (Shi, 2003), limited information regarding the antimicrobial effect of this plant is available.

Therefore, the aim of this study was to compare the effects of rosemary or Chinese mahogany on the quality of fresh chicken sausage during refrigerated storage. We also wanted to identify the volatile compounds from Chinese mahogany, rosemary and sausages with rosemary or Chinese mahogany added.

## 2. Materials and methods

### 2.1. Rosemary and Chinese mahogany preparation

Rosemary, which was obtained from a local spice company in Taiwan, was comminuted with a grinder (DIAX 600, Heidolph, Germany) into approximately 2 mm lengths, and stored in a moisture-proof cabinet (BK236, Bossmen, Taiwan). Chinese mahogany leaves, which were obtained from a local farm in Pingtung, Taiwan, were dried in an oven at 60 °C for 8 h, ground with the same grinder to approximately 2 mm in length, and then stored in the moisture-proof cabinet.

A pre-measurement of the total phenol contents in rosemary and Chinese mahogany was first conducted according to the Folin–Ciocalteu method of Tsau (2006) and is described briefly as follows. Two grams of ground rosemary or Chinese mahogany were mixed with 100 ml of distilled water, boiled and extracted for 20 min, cooled rapidly, and filtered. The filtered liquid was combined with phenol reagent (Sigma) and saturated $Na_2CO_3$ (Union Chemical Works Ltd., Hsinchu, Taiwan), vortexed, and then held for 1 h. The optical density values were determined using a spectrophotometer (U3210, Hitachi, Japan) at 700 nm wavelength. A standard curve was prepared with gallic acid added and regression determined as $Y = 2.35X - 0.0472$, where $Y$ represents OD (optic density) and $X$ represents the concentration of the total phenol contents of the solution (mg/ml). Total phenol contents of rosemary or Chinese mahogany were determined according to the formula: Total phenol content (mg/g) = $(X \times 10 \times 100)/2 \times 1000$. Based on the preliminary test results, 395 and 82 mg/g total phenol contents were determined for the Chinese mahogany and rosemary, respectively, in this study. Therefore, amounts of 1.265, 2.530, or 3.795 g of ground Chinese mahogany were added to 1 kg of sausage mixtures, respectively, in order to have 500, 1000 or 1500 ppm of phenolic compounds, respectively. Similarly, amounts of 6.1, 12.2, or 18.3 g of ground rosemary were added to 1 kg of sausage mixtures, respectively, in order to have 500, 1000, or 1500 ppm of phenolic compounds, respectively.

### 2.2. Sausage preparation

Fresh chicken tenderloin, chicken skin, pork backfat and salted natural pork casing were purchased from local markets in Nantou, Taiwan. Chicken meat, chicken skin and pork backfat were first frozen at −20 °C and then ground. Chicken meat was ground through a 9 mm plate, whereas chicken skin and pork backfat were ground through a 6 mm plate. Ground chicken (75%) was mixed thoroughly with salt (1.8%) and polyphosphates (0.15%) with a mixer (DITO, BM10) for 1.5 min, and then other spices and seasonings were added, including 0.5% sugar, 0.3% monosodium glutamate, 0.1% white pepper powder, 0.075% nutmeg powder, 0.03% parsley powder, 0.03% thyme powder, 0.03% onion powder, pre-assigned amounts of ground Chinese mahogany leaves or rosemary and non-lean tissue (25%, in which the ground chicken skin:ground pork backfat ratio = 1:2), then mixed for another 1.5 min. The mixtures were cured at 4 °C for 16 h, and then stuffed (Stuffer, Dick D-73779, Germany) into pork casings which were soaked in water prior to use. Raw sausages were manually linked, packed in a tray with PVC film and stored at 4 °C.

### 2.3. Proximate composition and pH

Samples were first ground (with a grinder, 31BL91, Blender, USA). Proximate compositions of samples including moisture, crude fat, crude protein and ash contents, were measured according to the AOAC (1990) method. Crude fat was measured using a fat extractor (Sotec System HT 1043 Extraction Unit, Tecator Co. Sweden). Crude protein was measured using the Kjeldahl method using a digester (Model 2006, Foss tecator, Sweden) and a distillation unit (Model 2100, Foss tecator, Sweden). Ten gram samples were blended with 90 ml of distilled water in a polyethylene bag for 1 min using a stomacher (Stomacher 400, Seward Ltd., England) at high speed for 2 min, and then the pH of the mixture was measured using a pH meter (Micro-Computer pH meter, Model 6210, Taiwan).

### 2.4. Instrumental colour measurement

Ground samples were placed in a measuring container, and then the Hunter *L* (lightness), *a (redness)* and *b* (yellowness) values of samples were measured with a colour meter (Spectrophotometer, Model TC1, Tokyo Co., Ltd., Japan). A standard plate, with "*Y*" = 86.53, "*X*" = 82.45, and "*Z*" = 91.28, was used as a reference.

### 2.5. Total phenol contents in products

Fifty grams of ground sausage samples were mixed with 100 ml of distilled water, boiled and extracted for 20 min, cooled rapidly, filtered, and then put through the same method as described in Section 2.1 to determine the total phenol contents in products.

### 2.6. Thiobarbituric acid (TBA) values and volatile basic nitrogen (VBN)

TBA values of the samples were determined according the methods described by Faustman, Specht, Malkus, and Kinsman (1992). TBA value was expressed as mg malonaldehyde/kg of meat. Volatile basic nitrogen was determined according to CNS (1982) by the Conway micropipette diffusion method.

### 2.7. Microbial evaluation

At a specified sample time, sausages were aseptically removed from the bags. Ten gram samples were placed in a sterile bag containing 90 ml of sterile water and homogenised with a stomacher (Stomacher blender, Model 400, Seward) for 2 min. Serial dilutions were then made. Plate count agar (PCA, Merck) was used for enumeration of total plate count, and the pour plate method was used for enumeration of bacteria. Total microflora were incubated at 37 °C for 48 h. Microbial counts in this study were expressed as $\log_{10}$ colony forming units (CFU) per gram of sample.

### 2.8. Sensory evaluation

At days 0, 7 and 14, during storage, sausages were first cooked on a grill at 160 °C for 15 min, cooled at room temperature (approximately 25 °C), sliced (approximately 0.25–0.30 cm thickness), and then served to a sensory panel which consisted of 12

meat science-majored faculty and students. Sensory attributes, including colour, aroma, off-odour, flavour and overall acceptance were determined using 1–7 point hedonic scale, with 1, 4 and 7 representing extremely dislike, neither like nor dislike and extremely like, respectively, for the attributes.

### 2.9. Volatile compounds analysis

Volatile compounds were analysed according to the methods of Wang, Liu, and Chen (1998), and are described briefly as follows: 500 g of ground sausage samples was treated with 1 l of saturated sodium chlorine solution, homogenised for 30 s, transferred to a 5 l round-bottom flask attached to a Likens–Nickerson apparatus, and extracted for 4 h. A mixture of pentane (Merck) and diethyl ether (Merck) at 1:1 ratio (v/v) was used as an extracting solvent. After adding anhydrous sodium sulphate (Merck) to the extracted solution, extracted solution was filtered (Whatman No. 1), and condensed to 1–2 ml. The Likens–Nickerson concentrates were analysed by injecting 0.2 μl into a gas chromatograph (Model 5890 II, Hewlett–Packard, Palo Alto, CA, USA) coupled to a gas chromatograph–mass spectrometer (GC–MS, Hewlett–Packard, USA). The GC was equipped with a capillary fused silica column (CP-Wax 52 CB, 60 m × 0.25 mm i.d., Chrompack Inc., The Netherlands). Carrier gas was hydrogen (1.0 ml $min^{-1}$ flow rate) and the column temperature was initially maintained at 40 °C for 5 min and subsequently programmed from 40 to 250 °C at a rate of 5 °C $min^{-1}$. The mass spectra were obtained by electron impact at 70 eV. Identification of the volatiles was based on comparison of the spectra with the spectra of the Wiley Spectrum Library.

### 2.10. Statistical analyses

Data were analysed using the general linear model (GLM) of Statistical Analysis System's Procedures (SAS Institute Inc., Cary, NC) with a 5% level of significance. Means were separated using the Duncan's new multiple range test.

## 3. Results and discussion

### 3.1. Proximate composition and pH

The contents of moisture, crude protein, crude fat and ash, of the fresh chicken sausages, were 59.8–61.8%, 15.7–16.4%, 20.1–22.4% and 2.70–2.95%, respectively. Three samples for each replicate and, totally, three replicates, were analysed for the proximate composition in this study. The pH values of the sausage remained stable and were approximately 6.4–6.6 during the first 10 days of refrigerated storage, and significantly ($P < 0.05$) decreased thereafter. This pH reduction was probably due to the fact that some existing oxygen inside the package might trigger fat oxidation, thus resulting in the decrease of pH values. At day 14, the pH value of the control samples decreased dramatically to 5.90, whereas, in the samples treated with rosemary or Chinese mahogany, it ranged from 6.00 to 6.14. A comparatively smaller pH reduction was observed for the samples with more rosemary or Chinese mahogany added.

### 3.2. Instrumental colour measurement

Fig. 1 illustrates the *L* and *a* colour value changes of the samples during storage. It shows that addition of rosemary or Chinese mahogany significantly decreased sample *L* values and increased sample *a* values. The more rosemary or Chinese mahogany that was added into the formula, the lower were the *L* values and the higher were the *a* values of the sausages. The *a* values of the control samples decreased during refrigerated storage while those of the samples with either rosemary or Chinese mahogany added increased. More intense colours of rosemary or Chinese mahogany itself led the sausages to have lower *L* values and higher *a* values, which resulted in darker colours. A possible browning reaction, occurring in the rosemary or Chinese mahogany added, might also contribute to this colour change. Polyphenol oxidases (PPOs), which are widespread enzymes in many plants, are responsible for browning in plants (Dogan & Dogan, 2004). In the presence of oxygen, the cited authors explained that these PPOs would oxidise plant phenolic compounds to corresponding quinones, which then condense to form darkened compounds.

Sebranek et al. (2005) reported that frozen pork sausages with 2500 ppm rosemary extract added had better red colour retention than did BHA/BHT-treated sausages during 84 days of storage. In the same study, they also reported that there was no significant difference of the $a*$ values between BHA/BHT treated, rosemary extract treated and controls for the refrigerated sausages, while a significant difference of colour loss occurred during 14 days of refrigerated storage. In contrast, Georgantelis and Blekas, et al. (2007) reported that $a*$ values of both rosemary extract treated and control beef burgers decreased during frozen storage for 180 days and addition of rosemary extract improved colour stability when compared to the controls. In the same study, they reported that a combination of chitosan and rosemary extract significantly improved sample colour stability and red colour retention. Georgantelis and Blekas, et al. (2007) explained that many factors, such as differences in the oxidation pattern of oxymyoglobin under conditions of reduced enzymatic activity, storage temperature, packaging method, muscle type and light intensity and differences in the meat species studied, might contribute to the variations in rosemary colour retention efficiency between different studies. In addition, types of antioxidative components added, for example, dried rosemary but not extract in this study, could also influence its efficiency.

### 3.3. Phenolic compounds in sausages

In this study, a higher content of total phenolics in Chinese mahogany, of 395 mg/g was observed, when compared to 65.0 mg/g, as reported by Chen, Lin, and Hsieh (2007), and 139 mg/g, reported by Yang et al. (2006). This deviation was probably due to various measuring conditions (i.e. absorbance measured at 700 or 750 nm) applied in these studies. In addition, drying conditions of Chinese mahogany leaves might not be the same in these studies (60 °C for 8 h in this study, but not reported by Chen et al. (2007). The phenolic compounds in sausages decreased with storage time for all the treatments (Fig. 2). This reduction of phenolic compounds in sausages during storage is in agreement with other studies. Daood, Vinkler, Markus, Hebshi, and Biacs (1996) reported that some antioxidative compounds, existing in spices, such as α-tocopherol, ascorbic acid and β-carotene, would lose their antioxidative ability dramatically during drying and storage. A dramatic decrease in the concentration of antioxidant compounds and loss of active antioxidation performance was observed during the late stage of storage, which resulted in an increase of fat oxidation. This phenomenon of progressive lipid oxidation, due to loss of protection by antioxidants after storage for a period of time, was also indicated by the dramatic increase of TBA values. The samples treated with Chinese mahogany showed comparatively higher total phenolic compound residuals at the end of storage when compared to the samples treated with rosemary at the same level. This more advanced protection against oxidation might be the reason that the samples with Chinese mahogany added had comparatively lower TBA values.

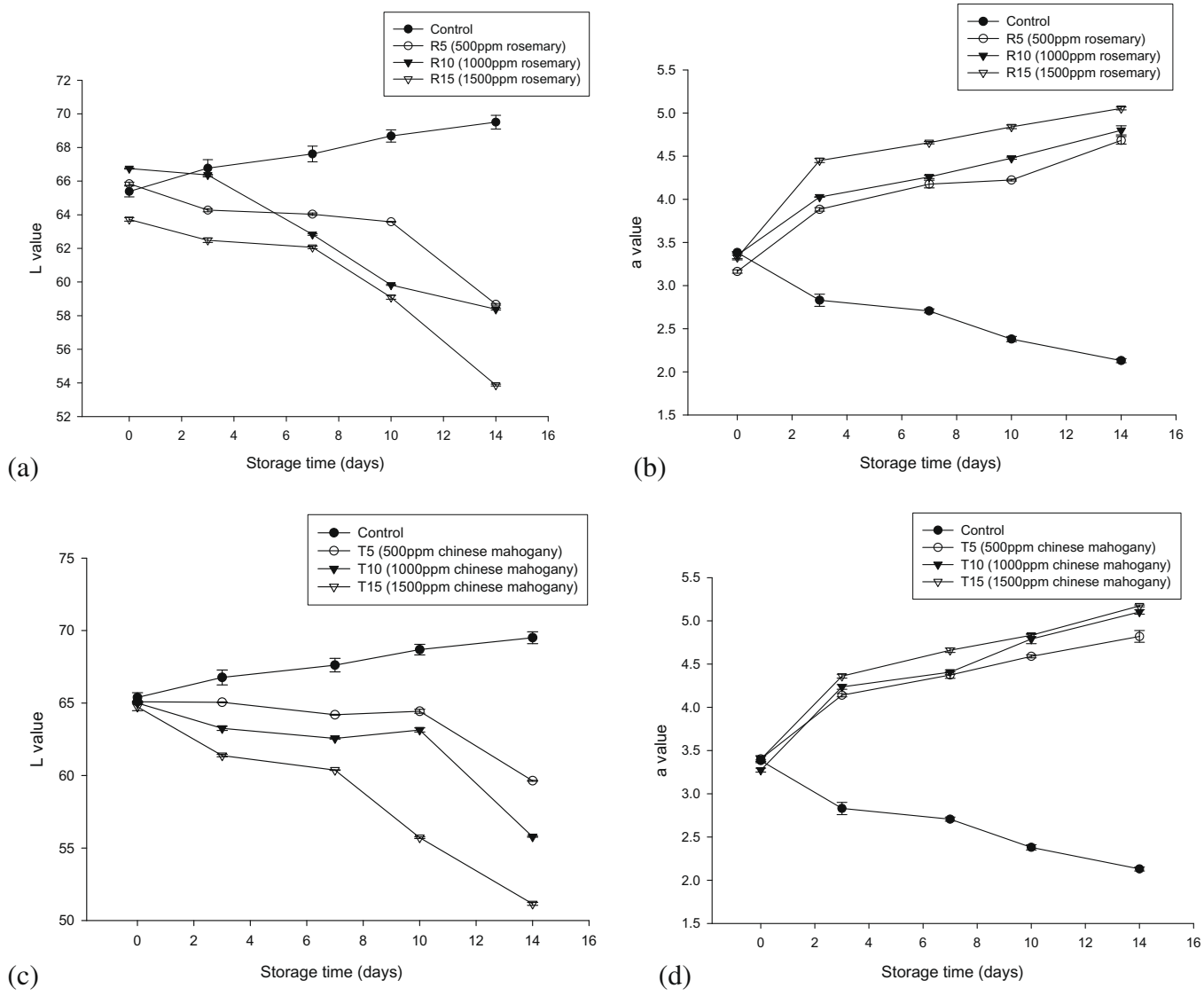


**Fig. 1.** Changes of (a) *L* values, (b) *a* values of fresh chicken sausages with addition of rosemary, (c) *L* values and (d) *a* values of fresh chicken sausages with Chinese mahogany during storage at 4 °C.

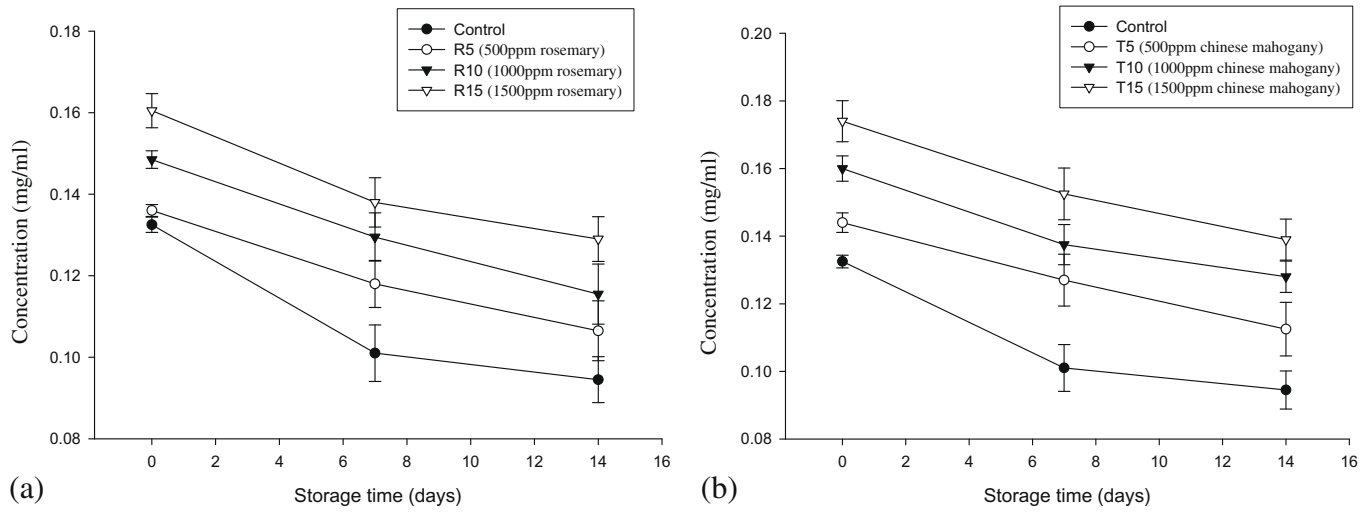


**Fig. 2.** Changes of residual amounts of phenolic compounds of fresh chicken sausages with addition of (a) rosemary or (b) Chinese mahogany during storage at 4 °C.

### 3.4. TBA and VBN

TBA values, which are indicators of lipid oxidation, are shown in Fig. 3. The TBA values of the sausage samples increased with refrigerated storage time during the first 3 days, indicating that lipid oxidation had occurred during this stage. This increase of TBA values of fresh sausages during storage has also been reported by other researchers. In this study, TBA values increased up to a maximum point at day 3, and then decreased gradually, and this tendency agreed with a study reported by Gokalp, Ockerman, Plimpton, and Harper (1983). Kuo, Dresel, Huang, and Ockerman (1987) explained that, during storage, malonaldehyde, which is an intermediate by-product during lipid oxidation, is further oxidised to other organic acids and alcohols that can not react with TBA agent. This might be the reason why the TBA values increased then decreased.

In this study, the TBA values of the samples with rosemary or Chinese mahogany added were lower than those of the controls. At day 3, the TBA values of the controls increased from 1.15 to 1.54 mg malonaldehyde/kg, while the TBA values of the Chinese mahogany treated samples were 1.30, 1.21, and 1.17 mg malonaldehyde/kg for T5 (500 ppm Chinese mahogany), T10 (1000 ppm Chinese mahogany), and T15 (1500 ppm Chinese mahogany) samples, respectively, and were significantly lower than the controls. Similarly, samples with rosemary added had significantly lower TBA values of 1.42, 1.40 and 1.25 malonaldehyde/kg for R5 (500 ppm rosemary), R10 (1000 ppm rosemary) and R15 (1500 ppm rosemary) samples, respectively. During storage, between day 3 and 14, the TBA values decreased for all treatments. The TBA value of the controls decreased to 1.10 mg malonaldehyde/kg at day 14, while the TBA values of the samples with rosemary added ranged from 1.00 to 1.05 mg malonaldehyde/kg for R5, R10, R15, and from 0.93 to 1.01 mg malonaldehyde/kg for T5, T10, and T15 samples, respectively. These decreases of TBA values were probably due to both the rosemary and Chinese mahogany, which are reported to have some antioxidative ability, and to retard fat oxidation. The results in this study demonstrate that the addition of rosemary or Chinese mahogany inhibited the lipid oxidation of fresh chicken sausages, and agree with other studies. In agreement with our findings, the positive effects of use of rosemary to prevent lipid oxidation have been well documented. Addition of 1000 ppm of rosemary extract was as effective as a combination of 100 ppm BHA plus 100 ppm BHT to maintain low TBARS values of precooked-frozen pork sausage (Sebranek et al., 2005). Yu, Scanlin, Wilson, and Schmidt (2002) reported that water-soluble rosemary extracts significantly decreased TBARS values of cooked turkey during 13 days of storage at 4 °C when compared to the controls, and higher levels of extracts added at 100, 250, or 500 ppm, were more effective in delaying lipid oxidation. The authors explained that free radical-scavenging and transition metal-chelating activities of water-soluble rosemary extract might contribute to the inhibitory effects on lipid peroxidation in cooked samples. Carnosol has been recognised as a major antioxidant in rosemary (Wei & Ho, 2006). Similarly, some phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which break free radical chain reactions by donation hydrogen atoms, have been reported to be associated with the antioxidant activity of rosemary extracts (Aruoma et al., 1992).

Hseu et al. (2008) reported that *T. sinensis* aqueous extracts, at 25–100 μg/ml, had some effective antioxidant activities, including the scavenging of free and superoxide anion radicals, reducing power, and metal chelation. Methanol extracts of *T. sinensis* have also demonstrated strong DPPH radical-scavenging activities and inhibitory effects on lipid peroxidation (Cho et al., 2003). Similarly, some potent antioxidative components in the young leaves and shoots lead *T. sinensis* to become a promising healthy-promoting food (Wang et al., 2007). Chen et al. (2007) reported that triterpenes and phenolic compounds, isolated from *Toona* species, were responsible for its antioxidant activity. In the DPPH radical-scavenging assay, Wang et al. (2007) reported that the 80% acetone extract of Chinese toon (the fresh young leaves and shoots of *T. sinensis*) had considerable antioxidant activity. Furthermore, the authors reported that gallic acid and its derivatives, gallotannins and flavonoids, were the main constituents contributing to its antioxidant ability. The antioxidative compounds contained in rosemary and Chinese mahogany might contribute to the antioxidative activity, thus retarding the fat oxidation of products during storage.

Increase amounts of volatile basic nitrogen (VBN), which is the result of decomposition of protein during storage by microorganisms, can be an index of meat product freshness. Fig. 4 illustrates that VBN values of the sausage samples increased as expected when storage time increased. In this study, VBN values, as well as the microbial counts, increased significantly during storage. In addition, samples that had more rosemary or Chinese mahogany added had significantly ($P < 0.05$) lower VBN values. At day 14, the control samples had significantly higher VBN value of 30.7 mg%, compared with those of the samples with rosemary added (24.4, 23.9 and 21.4 mg% for the R5, R10 and R15 samples,

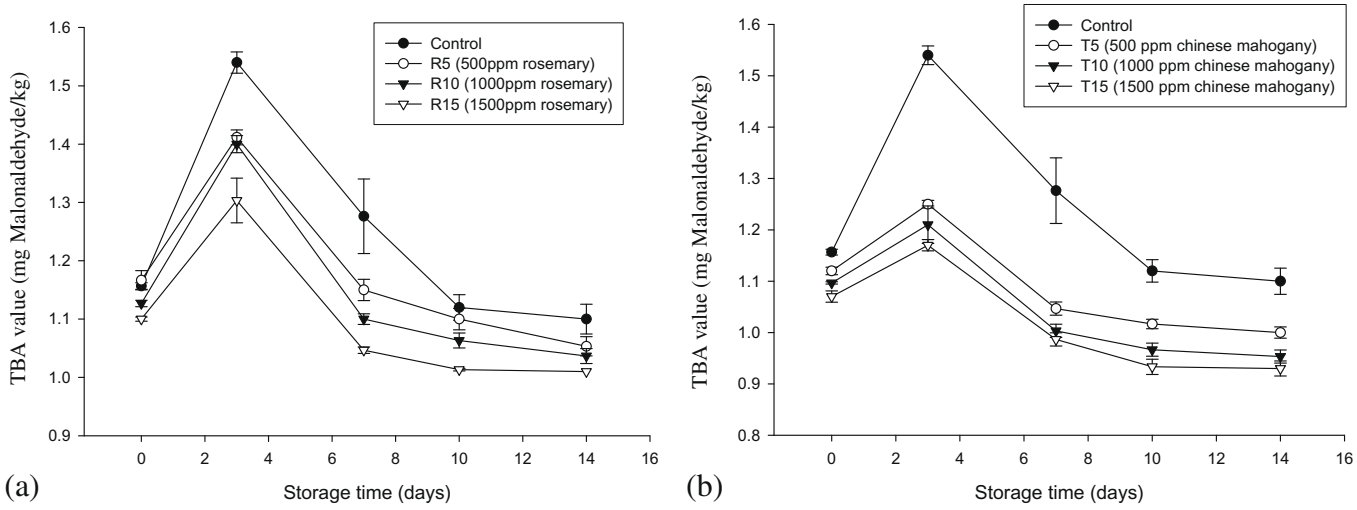


**Fig. 3.** Changes of TBA values of fresh chicken sausages with addition of (a) rosemary or (b) Chinese mahogany during storage at 4 °C.

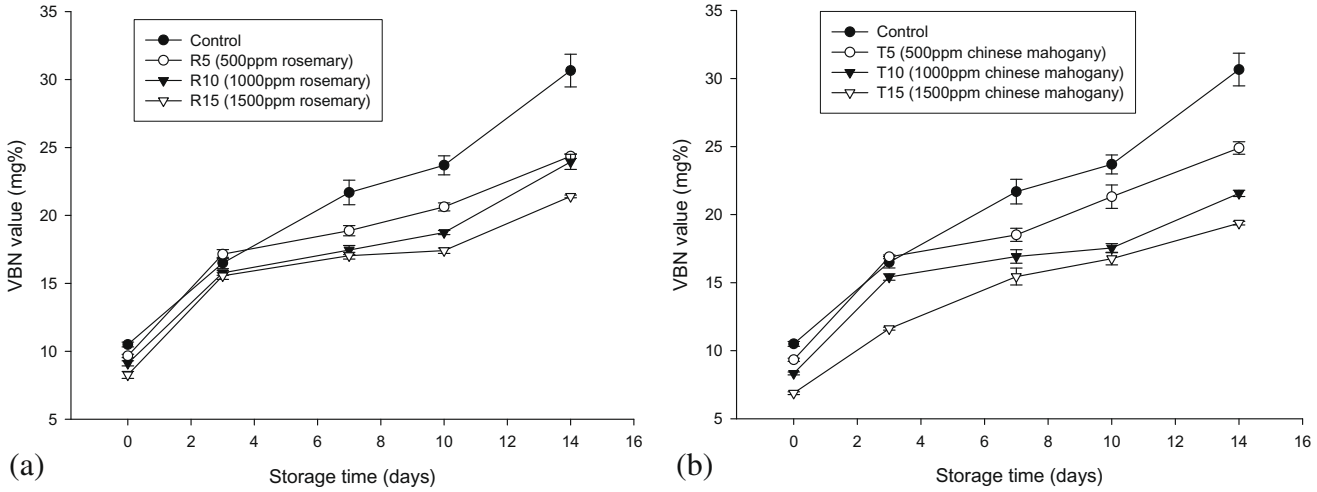


**Fig. 4.** Changes of VBN values of fresh chicken sausages with addition of (a) rosemary or (b) Chinese mahogany during storage at 4 °C.

respectively). Similarly, the samples with Chinese mahogany added had significantly lower VBN values (24.9, 21.6 and 19.4 mg% for the T5, T10 and T15 samples, respectively). The more rosemary or Chinese mahogany that was added to sausages, the lower were the VBN values of the samples observed. In this study, lower VBN values might suggest smaller bacterial populations for the samples with more rosemary or Chinese mahogany added, which is in agreement with the microbial counts shown in this study. These decreases of sample VBN values were probably because rosemary or Chinese mahogany contained some antimicrobial compounds. Carnosol and ursolic acid, which were the two antioxidant compounds extracted from rosemary, were demonstrated to inhibit the growth of 6 strains of food-associated bacteria and yeasts (Collins & Charles, 1987). Less microbial growth in sausages, due to the addition of rosemary or Chinese mahogany to the formula, thus led to less protein decomposition and lower VBN values.

### 3.5. Microbial qualities

The total plate counts of all samples increased during refrigerated storage (Fig. 5). Those with rosemary or Chinese mahogany added had comparatively lower microbial counts. At day 14, the total plate counts of sausages with the addition of rosemary at the levels of 500 ppm (R5), 1000 ppm (R10) and 1500 ppm (R15) were 5.99, 5.72 and 5.55 log CFU/g, respectively, which were significantly ($P < 0.05$) lower than that of the controls (6.08 log CFU/g). Similarly, total plate counts of 5.75, 5.59 and 5.30 log CFU/g were obtained for the sausages with 500 ppm (T5), 1000 ppm (T10) and 1500 ppm (T15) of Chinese mahogany added, respectively. In addition, samples with Chinese mahogany added had comparatively lower microbial counts than had the rosemary-treated sausages in this study. This difference was probably due to the total phenol content of Chinese mahogany which was higher than that of the rosemary (395 mg/g vs. 82 mg/g). Such antimicrobial effects due to the addition of rosemary or Chinese mahogany were also reported by other researchers.

Georgantelis and Ambrosiadis, et al. (2007), reported that the fresh pork sausages with addition of rosemary extract at level of 260 mg/kg had significantly lower ($P < 0.05$) Enterobacteriaceae, pseudomonad and yeast/mould counts than had the controls. Addition of carnosol, which was isolated from rosemary at levels of 50 μg/ml and 150 μg/ml, could significantly inhibit *Staphylococcus aureus* and *Escherichia coli* (Collins & Charles, 1987). Rosemary extracts have been demonstrated to have some inhibitory effects on the selected lactic acid bacteria and *Listeria* in an agar diffusion test (Fernández-Lopez, Zhi, Aleson-Carbonell, Pérez-Alvarez, &

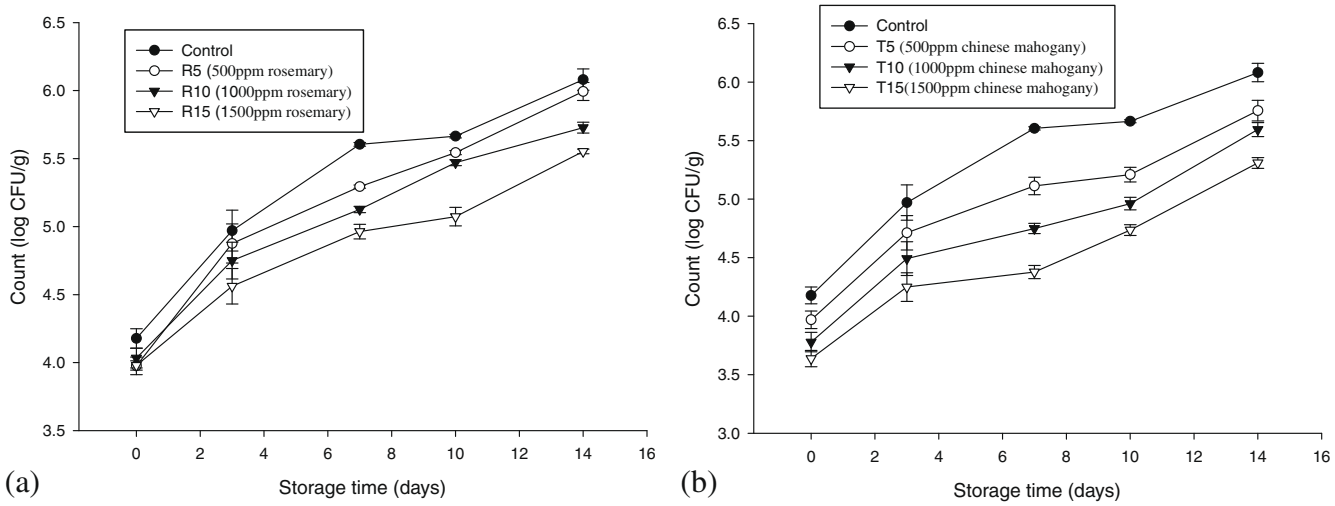


**Fig. 5.** Changes of total plate counts of fresh chicken sausages with addition of (a) rosemary or (b) Chinese mahogany during storage at 4 °C.

Kuri, 2005). Some non-polar components, such as phenolic diterpenes, which were isolated from rosemary, were indicated to be responsible for its antimicrobial properties, especially towards Gram-positive bacteria (Fernández-Lopez et al., 2005). Furthermore, when in combination with factors which can disturb cell membrane integrity and/or its permeability, such as lowered pH values, increased NaCl concentrations, combined with chitosan, phenolic diterpenes could further be used to inhibit Gram-negative bacteria (Georgantelis and Blekas, et al., 2007).

### 3.6. Sensory evaluation

Results of the sensory evaluation of the sausage during the 14-day storage period are presented in Table 1. A 7-point hedonic scale test was used in this study, with scores 1, 4 and 7, representing extremely dislike, neither like nor dislike, and extremely like, respectively. At day 0, colour scores of each treatment were less than 5, among which R15 (1500 ppm rosemary added) had the lowest colour score. In addition, the R15 (1500 ppm) colour score was significantly lower than those of R5 (500 ppm) and R10 (1000 ppm). No significant difference ($P > 0.05$) of aroma between treatments was observed. However, there was a significantly higher off-odour of 3.33 for the R15 (rosemary 1500 ppm added) than of 1.92 for the control sample. This higher off-odour of the samples might be due to a too condensed aroma due to higher level addition of rosemary. Samples with Chinese mahogany added had significantly higher flavour and overall acceptance scores than had the samples with rosemary added. At day 7, control samples had significantly higher colour scores than had those of the other treatments. This result was probably because colours of the samples with either rosemary or Chinese mahogany added became darker, which could have decreased sensory preference. On the other hand, samples with rosemary or Chinese mahogany added tend to have higher aroma scores, while the controls tended to have higher off-odours, even without significance. Addition of rosemary or Chinese mahogany seemed, not only to enhance the aroma of samples, but also to retard off-odour produced by fat oxidation. Samples with Chinese mahogany added (T5, T10 and T15) tended to have higher overall acceptance than did rosemary-added samples (R5, R10 and R15). At day 14, samples with 1500 ppm Chinese mahogany (T15) had significantly lower colour scores because of the darker colour of Chinese mahogany after being stored for a long time. This less preferred darker colour could also be evaluated subjectively by the lower *L* and higher *a* colour values. At the same level of addition, samples with Chinese mahogany had higher total overall acceptance.

### 3.7. Volatile component analysis

Flavour is one of the most important factors influencing the eating quality of meat and consumer decisions. Less aroma is sensed when meat is uncooked, while a complex series of thermally induced reactions occur between non-volatile components of lean and fatty tissues during cooking (Mottram, 1998). These thermally induced reactions and some volatile compounds formed during cooking thus contribute to specific aroma and flavour of meats (Mottram, 1998). In addition, not only enhancing colours, addition of some seasonings or spices, such as rosemary or Chinese mahogany, used in this study, also provides some unique aromas and flavours for meat products. Some volatile compounds, including esters (etheyl acetate), alcohols (ethanol) and acids (formic acid and acetic acid), were identified in fresh chicken sausage. Alcohols (ethanol, 1,8-cineole, L-terpineol, α-terpineol, mytenol, and ρ-cymen-8-ol), ketones (acetone and camphor), an ether (ε-anethole), alkenes (α-pinene, camphene, β-pinene, myrcene, and verbenene), acids (formic acid and acetic acid), and esters (etheyl acetate and methyl eugenol) and phenols (estragol, thymol and carvacrol), were identified from the fresh chicken sausage with 1500 ppm rosemary added. Alkenes (α-pinene, β-pinene, limonene, phellandrene and anethole), alcohols (ethanol, linalool, L-terpineol and α-terpineol), acids (formic acid and acetic acid), esters (etheyl acetate and ethyldicanoate), phenol (2-acetylfuran), and ethers (safrole and myristicin), were identified in the fresh chicken sausage with 1500 ppm Chinese mahogany added.

Unsaturated fatty acids are more readily oxidised than are saturated fatty acids. Therefore, a comparatively much higher proportion of unsaturated fatty acids leads phospholipids to become a more important source of volatile compounds during cooking than

**Table 1**
Sensory evaluation of fresh chicken sausages with addition of rosemary or Chinese mahogany during storage at 4 °C.

| Characteristics[1] | Treatment[2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | R5 | R10 | R15 | T5 | T10 | T15 |
| | Day 0 | | | | | | |
| Colour | 4.42 ± 0.23$^{ab}$ | 4.75 ± 0.28$^{a}$ | 4.58 ± 0.34$^{a}$ | 3.75 ± 0.30$^{b}$ | 4.75 ± 0.30$^{a}$ | 4.50 ± 0.15$^{ab}$ | 4.33 ± 0.14$^{ab}$ |
| Aroma | 4.33 ± 0.26$^{a}$ | 4.17 ± 0.30$^{a}$ | 3.83 ± 0.32$^{a}$ | 3.83 ± 0.34$^{a}$ | 4.67 ± 0.40$^{a}$ | 4.58 ± 0.26$^{a}$ | 4.50 ± 0.29$^{a}$ |
| Off-odour | 1.92 ± 0.29$^{b}$ | 1.92 ± 0.29$^{b}$ | 3.00 ± 0.43$^{ab}$ | 3.33 ± 0.56$^{a}$ | 2.08 ± 0.29$^{ab}$ | 2.58 ± 0.34$^{ab}$ | 2.58 ± 0.54$^{ab}$ |
| Flavour | 4.12 ± 0.31$^{ab}$ | 4.33 ± 0.26$^{ab}$ | 3.58 ± 0.34$^{b}$ | 3.75 ± 0.30$^{b}$ | 5.08 ± 0.26$^{a}$ | 4.83 ± 0.30$^{a}$ | 5.00 ± 0.25$^{a}$ |
| Overall acceptance | 4.83 ± 0.24$^{ab}$ | 4.42 ± 0.29$^{bc}$ | 3.83 ± 0.30$^{cd}$ | 3.17 ± 0.24$^{d}$ | 5.25 ± 0.25$^{a}$ | 5.00 ± 0.21$^{ab}$ | 5.08 ± 0.19$^{ab}$ |
| *Day 7* | | | | | | | |
| Colour | 5.50 ± 0.31$^{a}$ | 4.42 ± 0.26$^{bc}$ | 3.75 ± 0.18$^{cd}$ | 3.25 ± 0.18$^{d}$ | 5.00 ± 0.25$^{ab}$ | 4.17 ± 0.21$^{c}$ | 4.00 ± 0.12$^{c}$ |
| Aroma | 3.58 ± 0.29$^{b}$ | 4.00 ± 0.28$^{ab}$ | 4.25 ± 0.22$^{ab}$ | 4.25 ± 0.37$^{ab}$ | 4.83 ± 0.24$^{a}$ | 4.00 ± 0.25$^{ab}$ | 4.42 ± 0.23$^{ab}$ |
| Off-odour | 3.33 ± 0.38$^{a}$ | 2.75 ± 0.35$^{a}$ | 2.75 ± 0.43$^{a}$ | 2.25 ± 0.33$^{a}$ | 2.50 ± 0.38$^{a}$ | 2.25 ± 0.37$^{a}$ | 2.42 ± 0.36$^{a}$ |
| Flavour | 4.25 ± 0.25$^{ab}$ | 4.33 ± 0.22$^{ab}$ | 3.75 ± 0.22$^{bc}$ | 3.33 ± 0.22$^{c}$ | 4.75 ± 0.30$^{a}$ | 5.00 ± 0.33$^{a}$ | 4.67 ± 0.36$^{a}$ |
| Overall acceptance | 4.42 ± 0.26$^{bc}$ | 4.50 ± 0.19$^{bc}$ | 3.58 ± 0.19$^{d}$ | 3.83 ± 0.24$^{cd}$ | 5.17 ± 0.24$^{ab}$ | 4.92 ± 0.38$^{ab}$ | 5.33 ± 0.31$^{a}$ |
| *Day 14* | | | | | | | |
| Colour | 5.75 ± 0.39$^{a}$ | 4.83 ± 0.39$^{ab}$ | 4.00 ± 0.28$^{b}$ | 4.08 ± 0.36$^{b}$ | 4.83 ± 0.30$^{ab}$ | 4.00 ± 0.39$^{b}$ | 2.58 ± 0.26$^{c}$ |
| Aroma | 3.33 ± 0.53$^{a}$ | 3.58 ± 0.34$^{a}$ | 3.75 ± 0.39$^{a}$ | 3.75 ± 0.51$^{a}$ | 4.25 ± 0.48$^{a}$ | 4.25 ± 0.39$^{a}$ | 4.25 ± 0.58$^{a}$ |
| Off-odour | 4.17 ± 0.66$^{a}$ | 3.83 ± 0.47$^{a}$ | 4.00 ± 0.52$^{a}$ | 3.42 ± 0.62$^{a}$ | 3.58 ± 0.45$^{a}$ | 3.50 ± 0.42$^{a}$ | 3.00 ± 0.52$^{a}$ |
| Flavour | 4.33 ± 0.28$^{a}$ | 2.50 ± 0.31$^{ab}$ | 2.25 ± 0.28$^{bc}$ | 1.92 ± 0.31$^{c}$ | 3.83 ± 0.46$^{a}$ | 3.92 ± 0.48$^{a}$ | 3.17 ± 0.52$^{a}$ |
| Overall acceptance | 4.58 ± 0.42$^{a}$ | 2.25 ± 0.39$^{b}$ | 1.83 ± 0.30$^{b}$ | 1.92 ± 0.31$^{b}$ | 4.17 ± 0.37$^{a}$ | 4.33 ± 0.45$^{a}$ | 3.50 ± 0.36$^{a}$ |

Mean ± SD. $n = 12$.
$^{a-d}$Means within the same row for the same test day with different superscripts are significantly different ($P < 0.05$).
[1] A 7-point hedonic scale test, 1 = extremely dislike, 4 = neither like nor dislike and 7 = extremely like.
[2] C = control, R5 = 500 ppm rosemary, R10 = 1000 ppm rosemary, R15 = 1500 ppm rosemary, T5 = 500 ppm Chinese mahogany, T10 = 1000 ppm Chinese mahogany and T15 = 1500 ppm Chinese mahogany.

triglycerides (Mottram, 1998). In addition to verbenone, which was the major component (35.4%), Mata et al. (2007) identified several components, including α-terpineol (7.2%), camphor (5.5%), 4-terpineol (3.9%), 1,8-cineole (3.1%), caryophyllene oxide (2.4%), β-caryophyllene (2.3%), borneol (2.1%), α-bisabolool (2.1%), linalool (1.9%) and α-humulene (1.2%), in the essential oil of rosemary. Carvalho, Moura, Rosa, and Meireles (2005) explained that the chemical compositions of rosemary volatile oil and extracts varied widely, probably because of the difference in agricultural conditions of cultivation and the extraction technique used.

## 4. Conclusion

This study showed that smaller pH reduction was observed for the samples with more rosemary or Chinese mahogany added. Addition of rosemary or Chinese mahogany significantly decreased sample *L* values and increased sample *a* values. The phenolic compounds in sausages decreased during storage. Addition of rosemary or Chinese mahogany resulted in lower TBA, VBN values and microbial counts of the samples. Samples with addition of Chinese mahogany had significantly higher overall acceptance. The main volatile compounds of fresh chicken sausage were alcohols, acids, esters, aldehydes, ethers and phenolic compounds. In conclusion, Chinese mahogany and rosemary, applied in this study, have been demonstrated to improve meat products quality and function as a promising natural ingredient applied to food.

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