

## Comparison of lipid changes in chicken frankfurters made by soybean and canola oils during storage

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### Summary

Two batches of frankfurters containing about 55% chicken meat with two different oils (including canola and soybean) were manufactured. Batch 1 included canola oil and Batch 2 included soybean oil in the products. Various analyses were performed to detect the lipid changes of both batches during storage. Fatty acid composition analysis using gas chromatography showed little change in the fatty acids of either batch over time. From a nutritional viewpoint, Batch 1 was more acceptable over time than Batch 2. Chemical analysis included oxidation reactions, such as peroxide and TBA (Thiobarbituric acid) value and free fatty acid analysis. Each chemical experiment had different trends at each time, but both batches had values lower than the maximum levels at all times. With regard to microbial analysis, psychrotrophic bacteria in both batches showed a decreasing order until day 30, and then growth increased. In Batch 1, a significant difference was observed only on day 1, but the differences were more significant in Batch 2, and there were significant differences on days 15, 30 and 45. Also, *Lactobacillus* counts were not significant after the cooking process until the end of storage (growth was not detectable after the cooking process). At the end of storage, we concluded that Batch 1 had a higher quality and storage stability than Batch 2 and canola oil could be a good substitution for soybean oil in meat products. The data were analyzed with GLM (repeated measures). Differences were reported as significant at  $P < 0.05$ . Also, the statistical software was SPSS ver. 11.5.

**Key words:** Lipid changes, Chicken frankfurter, Soybean oil, Canola oil, Refrigerated storage

### Introduction

Lipid oxidation is a main cause of spoilage in meat and meat products and affects many final-product properties, such as flavor, color, texture, and nutritional value. The rapid development of rancid flavor during storage is a major problem that the meat industry faces, especially because demands for meat and meat products have increased, and they are also produced as heated products and are stored during refrigerated storage. One type of rancidity is oxidative rancidity. The slow development

of rancidity in raw meats and fatty tissues occurs even during prolonged frozen storage. Oxidative stability of meats is related to the unsaturated level of their lipid fraction. Linolenic acid is one of the unsaturated fatty acids that acts as a precursor for EPA (eicosa pentaenoic acid) and DHA (docosa hexaenoic acid) fatty acids, which have an effective role in reducing body inflammations and in curing different diseases such as diabetes type II (Fernandez-Real *et al.*, 2003). Content of these unsaturated fatty acids in chicken meat is higher than in beef. On the other hand,

chicken meat is more sensitive than beef. This is because there is a higher level of phospholipids in chicken meat than there is in red meats. These fractions (phospholipids) are more sensitive to oxidation because of the close involvement of the cellular membrane. Chicken meat has superior nutritional value and is also preferred by consumers.

Stability of a product is dependent on the type of oil used. Weber *et al.* (2008) used various heating methods at different temperatures in their study on fish fillets. In frying the fillets, they used soybean and canola oils, and studied the fatty acid profile and other chemical factors. It was shown that canola oil was more acceptable than soybean oil. The reason may be due to the component of the two oils. Soybean oil has a higher content of polyunsaturated fatty acid, which causes an increase in the level and rate of oxidation (deMan, 1999).

Both chemical and microbial analyses were the key factors affecting the fatty acid profile of the products. Although chemical changes are more important in lipid fraction deteriorations, the growth of psychrotrophs and lactobacilli was also monitored because of producing lipases and oxidizing components such as  $H_2O_2$ , respectively (Visessanguan *et al.*, 2006). Due to the activity of these organisms, there is an opportunity to produce these by-products during refrigerated storage. These two by-products increase the free fatty acid content as well as the oxidation products (peroxide and TBARS) (Fennema, 1996). The first (lipase enzymes) decreases the value of analyzable fatty acids in gas chromatography (because gas chromatography measures all fatty acids except those that are in free form), and the second ( $H_2O_2$ ) changes the fatty acids structure and possibly converts them to each other (during the oxidation process, unsaturated fatty acids convert to saturated fatty acids).

The aim of this study was to follow and detect chemical and microbial changes in two types of chicken frankfurters (containing about 55% chicken meat) during refrigerated storage up to 45 days. In addition to the initial heating and time that the samples were exposed, oil type was

investigated as an interfering factor.

## Materials and Methods

### Formulation and sausage preparation

Chicken sausages were produced in two batches with canola and soya oils. The chicken was purchased from local markets. Nitrite and phosphate were purchased from the BASF Aktiengesell Schaft and Chemische Fabrika Budenheim Companies in Germany. Also, other constituents were prepared from local markets. The formulation of the sausage is shown in Table 1. After complete mixing of the components, one part of the mixture was wrapped in sterile foils and transferred to the laboratory, and the other samples were packaged in polyamide casings and cooked at 72°C for 2 h. Then, the samples were stored at +3°C until tests were performed.

**Table 1: Formulation of frankfurters**

Ingredients	%
Chicken thigh	24.85
Chicken breast	29.82
Water and ice	21.87
Oil	14.91
Salt	1.59
Phosphate	0.3
Nitrite	0.012
Red pepper	0.2
Garlic powder	0.5
Starch	2.98
Protein isolate	2.98
Sum	100

### Fatty acid profile

The lipid fractions of the samples were extracted using the method of Folch *et al.* (1957). About 20 g of the ground sample was mixed with 50 ml methanol and stirred for 30-40 min. 40 ml hexane was then added to the mixture (to separate the lipid phase from the methanol) and the mixture was mixed for 20 min. After complete mixing, the mixture was set into a motionless state to separate the two phases. The upper phase that contained hexane extracted lipids was separated and used for the methylation process. Methyl esters were prepared according to ISO 5509 (2000) using methanolic KOH (2 mol/lit KOH in methanol). Fatty acids methyl esters were analyzed using gas chromatography

(Younglin ACME 6000M, Korea) with a flame ionization detector and silica-capillary column Techno Kroma TR-CN 100 (60 m × 0.25 mm × 0.2 μm). Injector and detector temperatures were 250°C and 260°C, respectively. Carrier gas was hydrogen (0.2 ml/min). After injection of 1 μl of the sample and an 80:1 split ratio, the initial temperature was set at 150°C for 5 min and then increased at a rate of 5°C/min to 175°C. After 3 min, the temperature reached 190°C at 3°C/min again and was set at this final temperature for 15 min. Also, fatty-acids methyl esters standards were injected under the same conditions and the resulting peaks were analyzed. Fatty acids at each stage were reported as a percentage (with regard to total fatty acids).

### Microbial analysis

#### *Psychrotrophic bacteria*

Ten g of each sample was weighted aseptically in a flask containing 90 ml dilution fluid. Then, 1 ml of 0.1 prepared dilution was transferred to tubes containing 9 ml dilution fluid (ISO 6887-1, 1999). After this, 1 ml of 0.1 and 0.01 dilutions were inoculated on the surface of PCA culture media. After complete dispersion, petri dishes were placed in a refrigerator (+4°C) for 7-10 days (ISO 17410, 2001). All tests for each sample were done in triplicate.

#### *Lactobacillus*

In this test, MRS agar was used. For uncooked samples and the heated samples, 0.01 and 0.1 dilutions were used, respectively. Because of the micro-aerophilic nature of *Lactobacillus*, we applied the pour-plate technique. After inoculation, petri dishes were transferred to anaerobic jars and were incubated at 35-37°C for 48-72 h. Also, for more reliability, we placed a positive control (the petri dish that intentionally was inoculated with lactobacilli) and a negative control (the petri dish without any inoculation, as a blank) in each jar (Robinson *et al.*, 2000). All tests were done in triplicate.

### Chemical analysis

#### *Moisture content and pH*

Moisture content was analyzed

according to ISO 1442 (1997). Also, pH analysis was carried out directly using Metrom standard pH-meter (Egan *et al.*, 1981). Both tests were done in triplicate.

#### *Peroxide value*

Fat extraction from tissue was carried out using the method of Egan *et al.* (1981). About 50 g of the samples were weighted into 500 ml flasks. Then, 200 ml of chloroform in each flask was used. In order to complete the extraction, the flasks were shaken for 2 h. After this, the flask content was filtered and permeates were transferred into rotary flasks. Solvent evaporation was then carried out using rotary evaporation. After solvent evaporation, the weight of the residual oil was detected. In order to measure the peroxide according to AOAC (2005), the extracted oil was dissolved in 30 ml of acetic acid/chloroform (3:2 v/v) and 0.5 ml saturated potassium iodide was added and the mixture was shaken vigorously. After 1 min, 30 ml distilled water was added, and after complete mixing, the mixture was titrated with 0.01N Na<sub>2</sub>SO<sub>4</sub> until a light-yellow color appeared. 0.5 ml 1% starch indicator was then added and the color changed to dark blue. Titration continued until the blue color disappeared and the light color appeared. This test was done in triplicate for all samples.

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

Measuring of TBARS was carried out according to the method of Egan *et al.* (1981). Ten g of the sample was weighted and mixed with 50 ml distilled water. The content was transferred into a distillation flask with 47.5 ml distilled water. Then, 2.5 ml 4N HCl with antifoaming/antiboiling agents were added to the mixture and the flask was attached to distillation equipment. The mixture was boiled until 50 ml of distillate was collected. Following this, 5 ml of distillate and 5 ml of TBARS indicator were mixed into lidded test tubes and placed in boiling water bath for exactly 35 min. All of these stages were carried out for blank simultaneously. After 35 min heating, the tubes were cooled for 10 min and their absorbance measured at 538 nm in 1cm cells against blank.

**Table 2: Counts (log cfu/g) of two microbial groups in two batches of sausage during storage**

Microbial groups and batches		Time				
		Before cooking	After cooking	Day 15	Day 30	Day 45
Total psychrotrophic bacteria	Batch 1	4.68 ± 0.04 <sup>a</sup>	2.92 ± 0.12 <sup>b</sup>	2.4 ± 0.64 <sup>bc</sup>	1.95 ± 0.3 <sup>c</sup>	3.42 ± 0.47 <sup>abc</sup>
	Batch 2	4.63 ± 0.08 <sup>a</sup>	4.3 ± 0.13 <sup>a</sup>	2.44 ± 0.16 <sup>b</sup>	nd <sup>c</sup>	3.78 ± 0.56 <sup>ab</sup>
<i>Lactobacillus</i>	Batch 1	3.58 ± 0.24 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
	Batch 2	3.23 ± 0.14 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>

nd: not detectable. Values in the same row with different letters are significantly different (P<0.05)

TBARS (mg malondialdehyde/kg sausage) =  $7.8 \times A_{538 \text{ nm}}$

#### Free fatty acids (FFA)

Fat was extracted as described in the peroxide value section. Detection of free fatty acid in the extracted oil was carried out using Egan *et al.*'s method (1981), 25 ml of neutral alcohol was added and the mixture was titrated with 0.1N NaOH in the presence of phenol phetaleine.

$$\text{FFA (as oleic acid)} = \frac{V \times 0.0282 \times 100}{W}$$

Where,

V = volume of NaOH

W = weight of sample

#### Statistical analysis

The data from GC and chemical and microbial examinations were analyzed with GLM (repeated measures). Differences were reported as significant at P<0.05. Also, the statistical software was SPSS ver. 11.5.

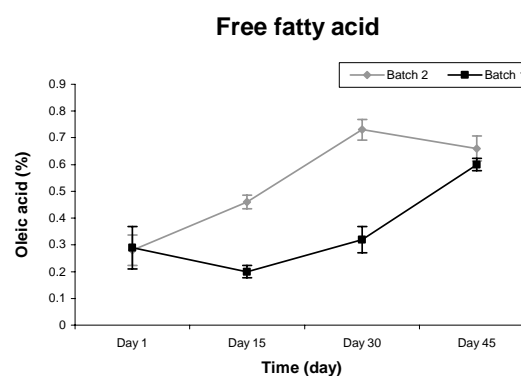
#### Results

Total psychrotrophic bacteria analyses are presented in Table 2. As presented, in both batches a decreasing order was observed until day 30, and then microbial growth increased. In Batch 1, a significant difference was observed only on day 1, but significant differences were more obvious in Batch 2, and there were significant differences on days 15, 30 and 45. There were significant differences between the psychrotrophs in the two batches on days 1 (P=0.01) and 30 (P<0.001). On day 1, Batch 2 had a higher growth than Batch 1. Also, as presented in Table 2, *Lactobacillus* growth was only observed in the before-heating stage.

Lipolytic changes of the sausages during the 45 days are depicted at Fig. 1. A higher value of FFA was observed in Batch 2 at all times, with the exception of day 1 when the

curves are overlapped. During storage, there were significant differences between the two batches on days 15 (P<0.001) and 30 (P<0.001).

The results of the FFA determination showed Batch 1 had no significant changes at any time, but Batch 2 had a significant increase on days 15 (P=0.035) and 30 (P=0.016).



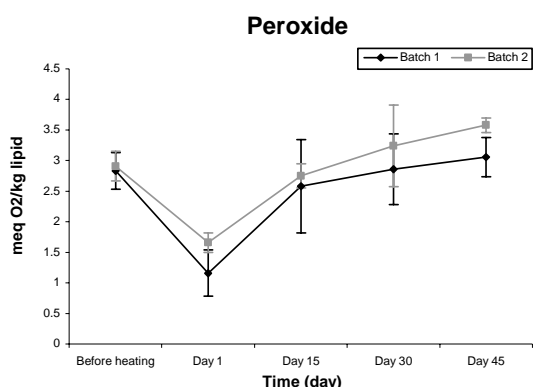
**Fig. 1: Changes of FFA in 2 batches with canola and soybean oil during 45 days of storage at 4°C**

Figure 2 shows the peroxide values of chicken sausages during refrigerated storage at 4°C. As shown, both sausages show exactly the same order at all times, and no significant differences were observed between the two samples, but Batch 2 had a consistently higher value than Batch 1.

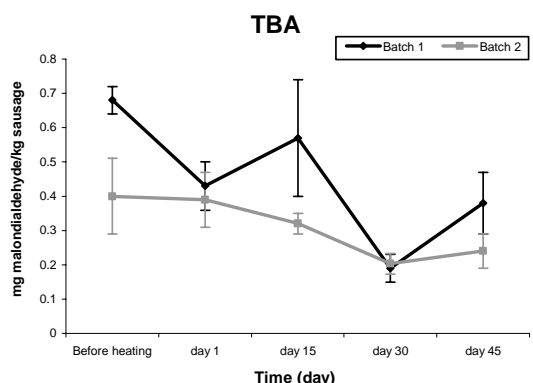
In Fig. 3, the TBA values of two batches are depicted. As observed in Fig. 3, except for day 30 when an overlap occurred, over the entire storage period Batch 1 had a higher TBA than Batch 2, although the differences were only significant on day 1 of storage (P=0.013) and not on the other days. The TBA value in Batch 1 showed no significant changes during this time, but had declining points on days 1 and 30. So we observed a sinusoidal manner in the TBA of Batch 1 instead of a constant increase or decrease over time. Besides, Batch 2 showed a slightly different situation in that the TBA

value decreased until day 30 and then increased.

Profiles of different fatty acids in the



**Fig. 2: Changes of peroxide in 2 batches with canola and soybean oil during 45 days of storage at 4°C**



**Fig. 3: Changes of TBA in 2 batches with canola and soybean oil during 45 days of storage at 4°C**

two batches of sausages are presented in Table 3. More than 95% of the total fatty acids that were analyzed during storage time were composed of palmitic, stearic, oleic, linoleic and linolenic acids, so we preferred to report the major fatty acids during storage. Comparison of the two batches revealed that almost all the fatty acids showed significant differences between the two batches at all times, but there were exceptions in  $\alpha$ -linolenic acid on days 1 and 45, palmitic acid in the before-heating stage and stearic acid on day 45 (in that no significant differences were observed at these times). With regard to each fatty acid change over time, Batch 2 showed a higher variation than Batch 1; we observed no significant differences in Batch 1 after cooking or during 45 days storage in all fatty acids, whereas in Batch 2 only oleic and linolenic acids did not show any significant differences and the other fatty acids had more variation. With respect to this, the palmitic acid value significantly increased on day 45. Stearic acid had a significant decrease on day 45. Linoleic acid showed no significant differences between each of the consequent stages, but its value increased from day 1 to day 45.

As observed in Table 3, Batch 2 had higher  $\omega 6/\omega 3$  and PUFA/SFA ratios than Batch 1. Also, the PUFA/SFA ratio in Batch 1 is not much lower than Batch 2, and both batches are within the acceptable range. On

**Table 3: Prominent fatty acids (%) in two batches of sausages during storage**

Fatty acids and nutritional ratios	Batch and stages									
	Batch 1					Batch 2				
	Before cooking	Day 1	Day 15	Day 30	Day 45	Before cooking	Day 1	Day 15	Day 30	Day 45
16:0	11.04 ±0.36 <sup>a</sup>	10.32 ±0.01 <sup>a</sup>	9.79 ±0.02 <sup>a</sup>	9.51 ±0.012 <sup>a</sup>	10.29 ±0.016 <sup>a</sup>	13.28 ±0.40 <sup>ab</sup>	15.95 ±0.08 <sup>ab</sup>	16.00 ±0.15 <sup>ab</sup>	14.19 ±0.001 <sup>a</sup>	15.61 ±0.01 <sup>b</sup>
18:0	4.48 ±0.22 <sup>a</sup>	4.66 ±0.011 <sup>a</sup>	4.98 ±0.02 <sup>a</sup>	4.72 ±0.023 <sup>a</sup>	4.56 ±0.14 <sup>a</sup>	5.68 ±0.03 <sup>a</sup>	5.31 ±0.001 <sup>a</sup>	5.65 ±0.13 <sup>a</sup>	5.61 ±0.01 <sup>a</sup>	4.76 ±0.006 <sup>b</sup>
18:1	55.92 ±1.04 <sup>a</sup>	55.49 ±0.01 <sup>a</sup>	55.99 ±0.10 <sup>a</sup>	55.26 ±0.01 <sup>a</sup>	55.09 ±0.014 <sup>a</sup>	31.45 ±0.073 <sup>a</sup>	30.80 ±0.13 <sup>a</sup>	31.15 ±0.085 <sup>a</sup>	31.47 ±0.05 <sup>a</sup>	30.18 ±0.20 <sup>a</sup>
18:2	22.03 ±0.85 <sup>a</sup>	22.86 ±0.01 <sup>a</sup>	22.92 ±0.01 <sup>a</sup>	22.64 ±0.04 <sup>a</sup>	22.44 ±0.52 <sup>a</sup>	42.88 ±0.18 <sup>ab</sup>	41.49 ±0.019 <sup>a</sup>	40.77 ±0.76 <sup>ab</sup>	41.97 ±0.02 <sup>ab</sup>	42.43 ±0.011 <sup>b</sup>
18:3	7.39 ±0.11 <sup>a</sup>	6.66 ±0.014 <sup>a</sup>	6.33 ±0.041 <sup>a</sup>	7.87 ±0.01 <sup>a</sup>	7.34 ±0.26 <sup>a</sup>	6.54 ±0.057 <sup>a</sup>	6.32 ±0.16 <sup>a</sup>	6.83 ±0.006 <sup>a</sup>	6.76 ±0.01 <sup>a</sup>	7.52 ±0.503 <sup>a</sup>
$\omega 6/\omega 3$	2.98	3.43	3.62	2.88	3.06	6.56	6.56	5.97	6.21	5.64
NHC/HC	5.47	5.83	6.23	6.31	6.8	2.8	2.26	2.3	2.61	2.24
PUFA/SFA	1.9	1.97	1.98	2.14	2.005	2.61	2.25	2.2	2.46	2.45

Values with different letters in the same row of each of the batches are significantly different (P<0.05)

the other hand, the NHC/HC ratio (non-hypercholesterolaemic / hypercholesterolaemic index) has a higher level in Batch 1, and approximately 2.5 times more than that of Batch 2.

## Discussion

### Microbial examinations

The microbial profiles of the sausages were analyzed for their effects on chemical reactions due to the production of by-products. Many bacteria, such as psychrotrophs, are commonly found in meat and meat products that have been reported to produce lipase at low temperatures. Lipase activity has been demonstrated for *Lactobacillus* spp. (Meyers *et al.*, 1996). The significant decrease after cooking (day 1) in both batches was due to the microorganism's instability to heat, which takes a relatively long time to show increase in growth during storage, so that non-detectable growth was observed on day 30 of storage in Batch 2. With regard to Table 4, Batch 2 has a higher moisture content and pH value than the other batch, and these factors contribute to the higher psychrotroph growth observed in Batch 2 on day 1. From the viewpoint of the effect of psychrotrophic lipases' role in free fatty acid (FFA) changes, we could not correlate microbial growth with FFA values; Thus, we deduce that microbial lipases did not affect lipolytic activity and that tissue lipases were probably the most effective in chemical profile.

**Table 4: Moisture content (%) and pH of sausages in two batches**

	Batch 1	Batch 2
Moisture	60.2 ± 0.16 <sup>a</sup>	61.79 ± 0.23 <sup>b</sup>
pH	6.3 ± 0.00	6.6 ± 0.00

Values in the same row with different letters are significantly different (P<0.05)

In the study of Yoon and Hwang (2008), *Lactobacillus* strains displayed optimum activity levels at neutral pH and at 35-37°C. Also, Cayré *et al.* (2003) reported that an increase in the storage temperature accelerated lactic acid bacteria (LAB) growth in vacuum-packed cooked meats so that the time necessary for LAB bacteria to reach the stationary phase was 8, 14 and 25

days for 15°C, 8°C, and 0°C, respectively. During the storage of Italian traditional fresh sausages at 2°C, a weak growth of LAB was reported by Kamdem *et al.* (2007). This was attributed to the negligible growth of Micrococcaceae, which can favor the LAB growth due to their ability to reduce the oxygen level. However, they analyzed fermented sausages that were inoculated with starter cultures. In comparison with our study, the non-detectable growth situation of *Lactobacillus* during the storage time is not unusual because there was no inoculation (the products were not inoculated). During the storage of bigeye snapper (Thai fermented fish mince) at +4°C, LAB counts increased slightly within the first 10 days, with a marked decrease on day 15 that continued until day 30. With this result, it was suggested that bacteria inhibited by lactic acid reproduced with sufficient exposure time (Riebroy *et al.*, 2007). With regard to peroxide changes after heating (day 1), during which significant decreases were observed in both batches (Fig. 2), these non-detectable growth states may be related to a peroxide decrease, of which the reduction of H<sub>2</sub>O<sub>2</sub> production by *Lactobacillus* was the main cause of decrease in peroxide value. Over time, after day 1, peroxide increases were observed, but *Lactobacillus* had non-detectable growth until the end of storage, so other factors (except with microbial growth) were effective in the oxidation reactions after day 1 until the end of storage.

### Free fatty acid analysis

The higher FFA content in Batch 2 may be due to its relatively higher moisture content than Batch 1 (Table 4), which contributes to lipolysis reactions. According to Naz *et al.* (2005) moisture content has an important role in hydrolysis. Also, according to this table, the lower pH in Batch 1 can affect the lipolysis and decrease its rate. Østerlie and Lerfall (2005) demonstrated that the hydrolytic activity of microorganisms decreases at lower pH. Molly *et al.* (1997) reported that lipolysis occurs by muscle and fat tissue enzymes and microorganisms do not increase the overall lipolytic activity of meats. We can verify this statement, because according to Table 2,

on day 30, Batch 1 showed a higher psychrotrophic bacteria growth than Batch 2, but in comparison, as mentioned before, lipolytic activity in Batch 2 was higher than Batch 1 at this time (day 30). Also, non-detectable growth of *Lactobacillus* was observed after heating (day 1) until the end of storage. The normal FFA content of both batches at a lower level of the upper limit (1.1% as oleic acid) (Egan *et al.*, 1981) and desirable stability in rancid flavor and taste over time was due to the sausages components and packaging and storage condition. According to Stahnke (1995), nitrite can affect lipolytic activity, which may be due to its antimicrobial effect and inhibition of enzyme liberation by microorganisms.

### **Peroxide value**

The reason for the higher peroxide value in Batch 2 is probably related to product properties such as pH. According to Table 4, Batch 2 has a higher pH value than Batch 1. Increasing the pH toward neutral point causes a favorable condition for oxidation. The effect of pH on the oxidation rate of micellar solutions was investigated in the study of Xie *et al.* (2007). According to this study, the amount of hydroperoxide increased more rapidly at pH = 6.8 than pH = 3. In addition to this, a more rapid increase of MDA (malondialdehyde) was observed at pH = 6.8 than pH = 3. This difference between two pH values might be due to the difference in the availability of endogenous metals, because in their presence hydroperoxides decompose easily. At pH = 6, metals precipitate onto the micelles and are in closer contact with lipids. The effect of cooking in our study can be considered from two viewpoints. Firstly, heating caused a reduction in peroxide on day 1 in both batches ( $P < 0.05$ ), which was due to oxidation compounds decomposition. Secondly, it causes a higher oxidation rate, as observed in an increasing trend after day 1 in both samples, especially on day 15 in Batch 2 ( $P = 0.03$ ). Most oxidized products are found in foods that are subjected to heating process. This effect is due to protein denaturation and the loss of antioxidant enzyme activity and/or the release of iron from metallo-protein, the disruption of cell

membranes (bringing PUFA (poly unsaturated fatty acids) into contact with pro-oxidant), the transformation of myoglobin from an antioxidant to a pro-oxidant, and thermal decomposition of hydroperoxide. Additionally, oxidation is initiated in susceptible membrane-bound phospholipids that contain large amounts of PUFA. Thus, because poultry meat has a higher ratio of PUFA/SFA than other meats, this process becomes important in poultry meat products (Hur *et al.*, 2007). On the other hand, enzymatic activity, such as lipoxygenase, can contribute to oxidation. This enzyme may be from various origins. Lipoxygenase is known to be widely distributed in plants, animals, and microorganisms that oxygenate PUFA and convert them to hydroperoxide (Banerjee, 2006). As the main source of lipoxygenase is soybean seed, another reason for the higher peroxide in Batch 2 may be their component, especially oxidizing enzymes.

### **Thiobarbituric acid**

The lower TBA in Batch 2 is probably due to the higher antioxidant content (polyphenolic compounds) in soybean oil in comparison with canola oil. In Batch 1, decline points on days 1 and 30 were probably due to the MDA reaction with textural components such as proteins. However, TBA in Batch 2 increased on days 15 and 45, as observed in the study of Fanco *et al.* (2002), when TBA increased during the storage of pork sausages. Of course, the antioxidant effect of nitrite and other compounds such as garlic and phosphate in sausages cannot be ignored. The sinusoidal manner in the TBA of Batch 1 instead of a constant increase or decrease during time may be related to changes of peroxide. Until day 15, both factors (TBA and peroxide) had a similar trend, but after that a different manner was observed. TBA is one of the products derived from peroxide decomposition, and has the potential for reaction with other components (deMan, 1999). On day 30, TBA value decreased due to its reaction with tissue components such as protein. At the end of storage, a simultaneous increase of TBA and peroxide was probably due to the partial decomposition of peroxide beside its

formation, which resulted in an increase in TBA. The significant decrease on day 30 ( $P=0.021$ ) and the increase on day 45 ( $P=0.02$ ) in Batch 2 were in agreement with the study of Salgado *et al.* (2005) who observed a decrease in TBA of chorizo until day 14 and then an increase until the end of storage. In contrast, during the refrigerated storage of fermented fish mince, the TBA value increased continually due to long chain polyunsaturated fatty acids in fish that are very susceptible to oxidation (Riebroy *et al.*, 2007).

### Fatty acid profile

Two key fatty acids in the batches were oleic acid in Batch 1 and linoleic acid in Batch 2, and these were the most abundant among all. No significant differences of these fatty acids in either batch were in agreement with Røra *et al.* (2003). In contrast, fish that had been fed on a soybean oil diet was characterized by high levels of oleic and linoleic acids, and no significant effect of the time (5 and 15 days) or temperature ( $+4^{\circ}\text{C}$ ) during storage was observed on the fatty acid composition of salmon fillets; but the quantity of  $\omega 3$  fatty acids seemed to be lower in the package of fillets.

The same result was observed in the study of Ahn *et al.* (1996). In their study, the value of monounsaturated fatty acids decreased in various pork products prepared from pigs fed on a linseed diet, and in comparison, the value of linoleic acid increased. On the one hand, the higher value of PUFA in Batch 2, in addition to its higher linoleic acid content, could be due to higher  $\alpha$ -tocopherol content in soya than in canola (Kilcast and Subramaniam, 2000). It has been reported that dietary content affects the fatty acid composition of muscle mitochondria and the higher concentration of PUFA observed in groups with vitamin E supplementation (as an antioxidant) than the control (Nuernberg *et al.*, 2002).

The potential nutritional properties of sausages were also studied. The value of  $\omega 6/\omega 3$  in Batch 2 is higher than the maximum recommended range that the detects by Pereira *et al.* (2000). This scientists stated that the maximum level for  $\omega 6/\omega 3$  intake must be up to 4.0. Thus,

according to the recommended value, Batch 1 has a more acceptable  $\omega 6/\omega 3$  than Batch 2. In other words, NHC/HC ratio can be a good factor for the comparison of two batches with two different oils. As the NHC/HC ratio increases, fewer coronary and heart diseases occur. Also, chicken sausages containing about 55% chicken meat were produced (which is considered a better source of desired nutritional factors than beef). It seems that oil substitution in sausages (canola instead of soya) may increase their desirability so they can tolerate different deteriorations and show better properties in the final product. Oleic acid has a positive role in health. As Lee *et al.* (1998) reported, oleic acid is considered a hypolipidaemic factor. As mentioned before, the oleic acid value is relatively high in Batch 1, such that it comprised more than 55% of total fatty acids.

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