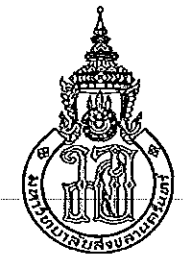

Compositions and Effect of Heating Conditions on Lipid Oxidation of Meat from Different Chicken Breeds

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ชื่อวิทยานิพนธ์	องค์ประกอบและผลของสภาวะในการให้ความร้อนต่อการเกิดออกซิเดชันของไขมันของเนื้อจากไก่ต่างพันธุ์
ผู้เขียน	นางสาวจุฑาภรณ์ ลิวา
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บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีของเนื้อไก่ต่างพันธุ์และความสัมพันธ์ต่อการเกิดออกซิเดชันของไขมันในเนื้อไก่ในระหว่างการเก็บรักษาแบบแช่เย็น โดยใช้เนื้อไก่ส่วนหน้าอกและสะโพกจากไก่ 3 พันธุ์ คือ ไก่กระทง ไก่ไข่ปลดระวางและไก่พื้นเมืองเก็บรักษาแบบมีหนังและไม่มีหนังในถุงพลาสติกแบบซิปล็อคที่ 4 °C นาน 9 วัน พบว่าเนื้อส่วนสะโพกมีปริมาณไขมัน ไมโอโกลบิน เหล็กที่อยู่ในรูปฮีมและเหล็กที่ไม่อยู่ในรูปฮีมสูงซึ่งสัมพันธ์กับการเกิดออกซิเดชันของไขมันที่สูงกว่าเมื่อเปรียบเทียบกับในเนื้อส่วนหน้าอก ปริมาณเหล็กที่อยู่ในรูปฮีมลดลงในขณะที่ปริมาณเหล็กที่ไม่อยู่ในรูปฮีมสูงขึ้นเมื่อผ่านการเก็บรักษาแสดงให้เห็นว่าเกิดการเสียสภาพของเหล็กที่อยู่ในรูปฮีม การเพิ่มขึ้นของการเกิดออกซิเดชันของไขมันในเนื้อไก่เมื่อผ่านการเก็บรักษานาน 6 วันในแต่ละตัวอย่างมีความเกี่ยวข้องกับการเพิ่มขึ้นของปริมาณเหล็กที่ไม่อยู่ในรูปฮีม ไขมัน การเกิดเมทไมโอโกลบิน กรดไขมันอิสระและกรดไขมันไม่อิ่มตัวชนิดหนึ่งพันธะคู่ซึ่งเกี่ยวข้องกับการลดลงของปริมาณความชื้น เหล็กที่อยู่ในรูปฮีม ไมโอโกลบิน ไตรกลีเซอไรด์ ฟอสโฟไลปิด กรดไขมันอิ่มตัวและกรดไขมันไม่อิ่มตัวชนิดหลายพันธะคู่ จากการศึกษาพบว่าไม่มีความแตกต่างระหว่างองค์ประกอบทางเคมีและการเกิดออกซิเดชันของไขมันในเนื้อไก่ที่ผ่านการเก็บรักษาแบบมีหนังและไม่มีหนัง ในช่วงการเก็บรักษา 6 วันแรกพบว่าเนื้อไก่กระทงส่วนสะโพกมีการเกิดออกซิเดชันของไขมันสูงที่สุดซึ่งมีความสัมพันธ์กับปริมาณไมโอโกลบิน ฟอสโฟไลปิด กรดไขมันอิสระและกรดไขมันชนิดไม่อิ่มตัวหลายพันธะคู่ที่สูงที่สุดเมื่อเทียบกับเนื้อจากไก่พันธุ์อื่นที่ศึกษา

การศึกษาผลของวิธีการให้ความร้อน คือ ต้ม นึ่ง ทอด ย่างและอบด้วยไมโครเวฟต่อองค์ประกอบทางเคมีและกรดไขมันและการเกิดออกซิเดชันของไขมันในเนื้อไก่กระทงส่วนสะโพกในระหว่างการเก็บรักษาที่ 4 °C นาน 15 วัน พบว่าเนื้อที่ผ่านการทอดมีการสูญเสียน้ำหนักหลังให้ความร้อนสูงที่สุดและเนื้อผ่านการย่างมีการสูญเสียน้ำหนักหลังให้ความร้อนต่ำที่สุด เนื้อไก่ที่ผ่านการให้ความร้อนมีปริมาณไมโอโกลบิน ความชื้นและเหล็กที่อยู่ในรูปฮีมลดลงส่วนปริมาณเหล็กที่ไม่อยู่ในรูปฮีมและปริมาณไขมันและการเกิดเมทไมโอโกลบินเพิ่มขึ้นเมื่อเปรียบเทียบกับในเนื้อสด ปริมาณรงควัตถุที่อยู่ในรูปฮีมที่สูงที่สุดโดยเฉพาะเหล็กที่ไม่อยู่ในรูปฮีมมีความสัมพันธ์กับการเกิดออกซิเดชันของไขมันในเนื้อไก่ที่สูงที่สุดพบในเนื้อไก่ที่ผ่านการนึ่ง การเพิ่มขึ้นของการ

เกิดออกซิเดชันของไขมันในเนื้อไก่ส่วนสะโพกที่ผ่านการให้ความร้อนเมื่อผ่านระยะเวลาการเก็บรักษานาน 10 วันมีความสัมพันธ์อย่างมากกับการเพิ่มขึ้นของปริมาณไขมัน การเกิดเมทไมโอโกลบิน ปริมาณเหล็กที่ไม่อยู่ในรูปฮีม กรดไขมันอิสระและผลิตภัณฑ์จากการเกิดออกซิเดชันของคอเลสเตอรอล (Cholesterol Oxidation Products; COPs) ร่วมกับการลดลงของปริมาณความชื้น ไมโอโกลบิน เหล็กที่อยู่ในรูปฮีม ไตรกลีเซอไรด์และฟอสโฟไลปิด

การศึกษาผลของอุณหภูมิในการให้ความร้อนต่อองค์ประกอบทางเคมีและกรดไขมันและการเกิดออกซิเดชันของไขมันในเนื้อไก่กระทงส่วนสะโพกในระหว่างการเก็บรักษาที่ 4 °C นาน 15 วัน การสูญเสียน้ำและไขมันหลังให้ความร้อนพบสูงที่สุดในเนื้อที่ผ่านการนึ่งและใช้ไมโครเวฟที่อุณหภูมิสุดท้ายของเนื้อที่ 90 °C เมื่อเปรียบเทียบกับในเนื้อที่ผ่านการให้ความร้อนด้วยวิธีการเดียวกันจนได้อุณหภูมิ 80 และ 70 °C ตามลำดับ นอกจากนี้เนื้อไก่ที่ผ่านการให้ความร้อนแบบนึ่งและใช้ไมโครเวฟที่อุณหภูมิสุดท้ายเท่ากับ 70 °C นั้นมีการเปลี่ยนแปลงปริมาณรงควัตถุที่อยู่ในรูปฮีม มากกว่าที่อุณหภูมิ 80 และ 90 °C เมื่อเปรียบเทียบในวิธีการให้ความร้อนเดียวกัน ส่วนการเปลี่ยนแปลงสัดส่วนของไขมัน องค์ประกอบของกรดไขมัน การเกิดออกซิเดชันของไขมันและคอเลสเตอรอลในเนื้อที่ผ่านการนึ่งที่อุณหภูมิสุดท้ายเท่ากับ 90 °C และใช้ไมโครเวฟที่อุณหภูมิสุดท้ายเท่ากับ 70 °C สูงกว่าที่อุณหภูมิอื่นเมื่อเปรียบเทียบในวิธีการให้ความร้อนเดียวกัน การเพิ่มขึ้นของการเกิดออกซิเดชันของไขมันในเนื้อไก่ที่ผ่านการให้ความร้อนแบบนึ่งและใช้ไมโครเวฟที่อุณหภูมิสุดท้ายในเนื้อเท่ากับ 70, 80 และ 90 °C นั้นมีความสัมพันธ์อย่างมากกับปริมาณไขมัน การเกิดเมทไมโอโกลบิน ปริมาณเหล็กที่ไม่อยู่ในรูปฮีม กรดไขมันอิสระและ COPs ร่วมกับการลดลงของปริมาณความชื้น ไมโอโกลบิน เหล็กที่อยู่ในรูปฮีม ไตรกลีเซอไรด์และฟอสโฟไลปิด

Thesis Title	Compositions and Effect of Heating Conditions on Lipid Oxidation of Meat from Different Chicken Breeds
Author	Miss Jutaporn Liwa
Major Program	Food Science and Technology
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ABSTRACT

Chemical compositions of chicken meat from different breeds and their correlation to lipid oxidation of chicken meat during refrigerated storage were investigated. Breast and thigh meat from three chicken breeds; broilers, spent hens and Thai indigenous chickens, were stored with and without skin in sealed plastic bag at 4 °C for 9 days. Thigh meat contained higher lipid, myoglobin, heme and non-heme iron relating to more lipid oxidation compared to whole breast meat were observed. The heme iron content decreased in a coincidence with an increase in non-heme iron content, suggesting to the destruction of the heme protein. The increase in lipid oxidation with extended storage time from 0 to 6 days in each meat sample associated with the increase in non-heme iron, lipid content, metmyoglobin formation, free fatty acid and monounsaturated fatty acids (MUFAs) and relating to the decrease in moisture, heme iron, myoglobin, triglyceride, phospholipids content, saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) of chicken meat. No significant difference in chemical compositions and lipid oxidation of chicken meat between storage with and without skin was observed. During 6 days storage, lipid oxidation of broiler thigh meat was the highest among those meat from the other breeds ($P<0.05$) relating to the highest in myoglobin, phospholipids, free fatty acid content and unsaturated fatty acid proportion.

The effects of cooking methods (boil, steam, fry, grill and microwave) on chemical and fatty acid compositions and lipid oxidation in broiler thigh meat during refrigerated storage at 4 °C for 15 days were investigated. The highest cooking loss was found in fried samples and the lowest were found in grilled samples. The decrease in myoglobin, moisture and heme iron content of cooked meat was in coincidental with the increase in non-heme iron and lipid content and metmyoglobin formation compared to those in raw meat. The highest content in heme pigment of meat especially non-heme iron

content which coincided with the highest lipid oxidation were found in steamed meat. The increase in lipid oxidation with extended storage time to 10 days in cooked broiler thigh meat had high correlation to the increase in lipid content, metmyoglobin formation, non-heme iron, free fatty acid, and total cholesterol oxidation products (COPs) coincided with the decrease in moisture content, myoglobin content, heme iron content, triglyceride, phospholipids, saturated fatty acid.

The effect of heating temperatures on chemical and fatty acid compositions and lipid oxidation of broiler thigh meat during refrigerated storage at 4 °C for 15 days were studied. The highest water and lipid loss were found in steamed and microwaved meat at 90 °C end point temperatures when compared with those at 80 and 70 °C, respectively. Cooked meat at 70 °C end point temperatures had a higher change in heme pigment while the highest changes in lipid fractions, fatty acid compositions, lipid and cholesterol oxidation was observed in steamed meat at 90 °C and microwaved meat at 70 °C than those of other end point temperature when compared within cooking method. The increase in lipid oxidation with extended storage time to 10 days of steamed and microwaved thigh meat at 70, 80 and 90 °C end point temperatures had high correlation to lipid content, metmyoglobin formation, non-heme iron, free fatty acid, and total COPs coincided with the decrease in content of moisture, myoglobin, heme iron, triglyceride and phospholipids.

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Jutaporn Liwa

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CHAPTER 1
INTRODUCTION

There are many chicken breeds in Thailand. Among broilers, spent hens and Thai indigenous chicken are commercially produced for meat consumption in Thailand. Breeds of chickens have been genetically developed for commercialization. Broiler raising in Thailand has been continuously developed. Thailand's broiler meat production is grown by 6-8 percent per annum in 2008 and 2009, mainly due to continued increase export demand (Figure 1) (Official USDA data, 2009). Thai native chicken are the cross breeds of Thai male indigenous fighting cocks and female broilers (Intarapichet *et al.*, 2008). In general, they are the so-called Gai Baan Thai, meaning Thai domestic chickens. Thai indigenous chicken in Southern part reared under extensive production system where chicken were allowed to scavenge on natural food around the homestead during the day and supplement with the concentrated feed in the evening when they came back to roost and sheltered at night. Among Thai consumers, the meat of the Thai indigenous chicken is more preferable and recognized as lean, tasty, not so tough and chewy, and has higher economic values compared to commercial broiler meats. To promote Gai Baan Thai as a commercial product for exporting, the Livestock Development Department and the Exporting Promotion Department have been working closely with the Thailand Research Fund to develop new hybrid breeds with higher meat quality (Intarapichet *et al.*, 2008). Spent hen is mostly under utilized and used in low priced mince product at the end of egg laying cycle. Broiler, Thai indigenous chickens and spent hen are commercially produced for consumption but those breeds are different in growth rate, feeds or supplements and method of production. However, those chickens are consumed at approximately the same commercial live weight. Meat obtained from different chicken breeds, ages and muscle types results difference in their properties quality characteristics and change during storage.

Although numerous studies on breeding selection, methods of raising, dietary and growth performance, and body composition of the Thai indigenous chickens have been widely conducted (Ratanasetakul *et al.*, 1987; Bansidhi *et al.*, 1988; Theerapanturat *et al.*, 1988; Choprakarn *et al.*, 1988b; Chotesangasa, 2001; Bunchasak and Kittichonnthawat, 2003) including Thai indigenous hens and their egg characteristics (Choprakarn *et al.*, 1988a), little is known on differences of carcass and nutrient composition and lipid oxidation of meat of these chickens. To gain quality and safety information for effective marketing of these chicken meats, it is necessary to know their chemical and lipid compositions and especially

lipid oxidation. Moreover, the previous researches on these chicken meats focused on thermal processing influences texture, protein changes, cooking yield, and other important quality factors, such as juiciness, color, and flavor, which are associated with palatability and consumer acceptance of the final product (Xiong *et al.*, 1990; Kijowski and Mast, 1988; Califano *et al.*, 1997; Fletcher, 1999; Murphy and Marks, 2000; Lee *et al.*, 2000; Beltran *et al.*, 2003; Cortinas *et al.*, 2004; Wattanachant *et al.*, 2004; Wattanachant *et al.*, 2005)

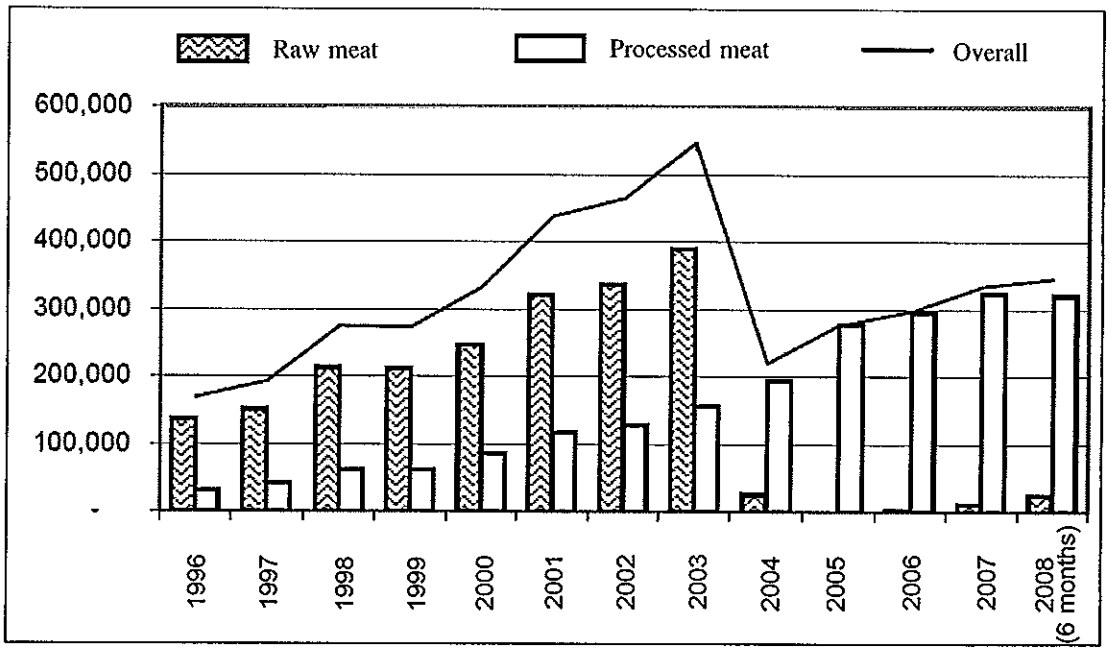


Figure 1 Thailand's exports of chicken meat during 1996-2008 (6 months).
Source: Thai Broiler Processing Exporters Association (2008).

Poultry meat is very sensitive to the development of oxidative rancidity because of its higher content in polyunsaturated fatty acids (PUFAs) (Igene *et al.*, 1980). During nonfrozen storage, raw chicken meat undergoes several changes that can reduce its quality (Kanatt *et al.*, 1998). A major cause of such quality deterioration is lipid peroxidation (St. Angelo, 1996). Oxidation of lipids results in unacceptable odors and flavors and can also decrease the nutritional quality as the lipids oxidize and react with other constituents such as proteins, carbohydrates and vitamins (Labuza *et al.*, 1969). Numerous studies have been devoted to oxidative changes in poultry meat showing as increase in the amount of Thiobarbituric acid reactive substance (TBARS) during refrigerated storage and cooking (Sharma *et al.*, 1982). When cell are injured, such as in muscle foods after slaughtering, oxidative processes are favoured, and traces of $O^{-}_{2}\cdot$ and H_2O_2, indicating lipid peroxides, are formed (Igene *et al.*, 1980). Moreover, lipolysis is suspected to promote lipid oxidation

because free fatty acids (FFAs) are often regarded as more sensitive to oxidation than esterified ones (Alasnier *et al.*, 2000). In breast and thigh chicken muscles, free fatty acids amount and oxidation level increased simultaneously during refrigerated storage suggesting that lipolysis could promote lipid oxidation (Sklan *et al.*, 1983). However, the previous study was performed during 60 days of frozen storage, limit data are available on lipolysis during the first days of refrigerated storage covering the delay during which carcasses are cold-stored in the supermarket both fresh and cooked chicken meat. Therefore, this study aimed to evaluation the chemical compositions and effect of heating conditions on lipids oxidation of different breeds chicken meat from different during refrigerated storage.

Literature Reviews

The chicken is one of the most common and widespread domestic animals. With a population of more than 24 billion in 2003 (Firefly Encyclopedia of Birds, 2003), there are more chickens in the world than any other bird. Humans keep chickens primarily as a source of food, with both their meat and their eggs consumed. Chickens in nature may live for five to eleven years depending on the breed. In commercial intensive farming, a meat chicken generally lives only six weeks before slaughter (The poultry guide, 2007). A free range or organic meat chicken will usually be slaughtered at about 14 weeks. Hens of special laying breeds may produce as many as 300 eggs a year. After 12 months, the hen's egg-laying ability starts to decline, and commercial laying hens are then slaughtered and used in baby foods, pet foods, pies and other processed foods (Animals Australia, 2007).

1. Chemical composition of chicken meat

Within poultry, there are two types of muscle; white and dark muscle. The different colors are based on the different locations and uses of the muscles. Dark muscles occur in the legs, which are used to support the weight of the animals while they move (Greaser, 1986). These muscles are designed to develop endurance for long-term use and contain a large amount of myoglobin, allowing the muscle to use oxygen more efficiently for aerobic respiration (Haard, 1992). In contrast the white muscle, generally found within the breasts of the birds, are used for quick bursts of power which requires little of the meat-darkening myoglobin (Greaser, 1986). Note that this holds for ground-based bird like chickens and turkeys-birds which use their chest muscles for sustained flight (such as geese and ducks) have dark muscle throughout their bodies.

Chemical composition of the muscles contains water (fluid medium of the body), proteins (structure and metabolic reactions in body), lipids (sources of energy, cell membrane structure and function, and metabolic functions-vitamins and hormones), carbohydrates (low level in body, mainly glycogen in muscle and liver) in variable amounts upon several factors such as breeds, muscle types and form in which they are present as shown in Table 1. Wattanachant *et al.* (2004) reported *pectoralis major* and *biceps femoris* muscles from Thai indigenous chicken muscles contained significantly higher protein but lower fat than those of broiler muscles (Table 2). However, raw and cooked chicken meats are difference in chemical compositions as shown in Table 3. The effect of heat on specific muscle components is one of the most important yet least understood aspects of meat research, the application of heat to meat has been conducted for the development of

characteristic color, odor, flavor and texture but conversely heat treatment had effect on off-flavor in meat due to heating can be stimulate lipid oxidation in meat (Greaser, 1986).

Table 1 Chemical composition of typical adult mammalian muscle

Components	% Wet weight
Water	75.0
Protein	19.0
Sarcoplasmic	5.5
Myofibrillar	11.5
Connective tissue	2.0
Lipid	2.5
Carbohydrate	1.2
Soluble non protein substances	2.3
Nitrogenous substances	1.65
Minerals	0.85
Total soluble phosphorus	0.20
Potassium	0.35
Sodium	0.05
Magnesium	0.02
Calcium, Zinc, trace metals	0.23
Vitamins	Trace

Source: Adapted from Lawrie (1991).

Table 2 Chemical composition of meat from Thai indigenous chicken, broiler and spent hen

Breed	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
[1]Thai indigenous	74.88 ± 0.61	22.05 ± 0.62	0.37 ± 0.14	1.03 ± 0.04
[1]Broiler	74.87 ± 0.46	20.59 ± 0.26	0.68 ± 0.06	1.10 ± 0.01
[2]Spent hen	67.46 ± 3.13	24.36 ± 2.81	7.15 ± 0.09	1.04 ± 0.09

Source: [1] Adapted from Wattanachant *et al.* (2004).

[2] Adapted from Lee *et al.* (2003).

Table 3 Chemical composition of raw and broiled chicken meat

Chicken type	Percentage (%)			
	Protein	Moisture	Fat	Ash
Spent hens				
Raw	20.6	73.7	4.7	1.0
Broiled	28.0	63.4	6.3	1.2
Broilers				
Raw	23.4	73.7	1.9	1.0
Broiled	31.6	63.8	3.4	1.2

Source: Forrest *et al.* (1975).

2. Lipids

Lipids are a class of hydrocarbon-containing organic compounds (Wikipedia, 2006). The term lipid is used to denote fats and fatlike substances. Lipids are usually defined as food components that are insoluble in water and soluble in organic fat solvents (Pomeranz, 1985) or soluble in nonpolar organic solvents such as ether, chloroform, benzene, hexane and intermediate polar organic solvents such as alcohol and acetone (Gurr *et al.*, 2002). In living organisms, lipids are used for energy storage, serve as structural components of cell membranes, and are important hormones or contain essential fatty acids. Although the term lipid is often used as a synonym for fat, the latter is in fact a subgroup of lipids called triglycerides (Wikipedia, 2006). The subunit of lipid structures is fatty acids.

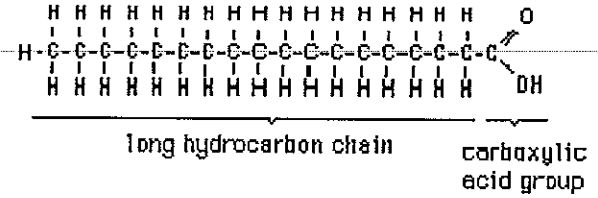
2.1 Fatty acids

Chemically, fatty acids can be described as long-chain monocarboxylic acids and have a general structure of $CH_3(CH_2)_nCOOH$. The length of the chain usually ranges from 12 to 24, always with an even number of carbons (Gunstone and Norris, 1983). When the carbon chain contains no double bonds, it is called saturated fatty acid and if it contains one or more such bonds, it is unsaturated fatty acid.

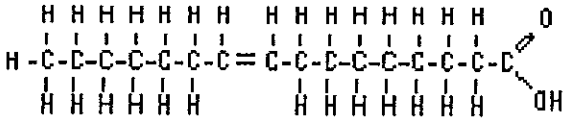
2.1.1 Saturated fatty acids

Saturated fatty acids do not contain any double bonds or other functional groups along the chain (Figure 2(a)). The term "saturated" refers to hydrogen, in that all carbons (apart from the carboxylic acid [-COOH] group) contain as many hydrogen as possible. Saturated fatty acids form straight chains and, as a result, can be packed together very tightly, allowing living organisms to store chemical energy very densely. The fatty tissues of animals contain large amounts of long-chain saturated fatty acids. In IUPAC

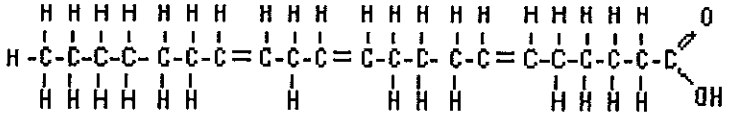
nomenclature, fatty acids have an [-oic acid] suffix. In common nomenclature, the suffix is usually -ic (Wikipedia, 2006).



(a) Saturated fatty acid (SFA)



(a) Monounsaturated fatty acid (MUFA)



(c) Polyunsaturated fatty acid (PUFA)

Figure 2 Structure of saturated fatty acid and unsaturated fatty acid (mono- and poly- type).
Source: Wikipedia, the free encyclopedia (2006).

2.1.2 Unsaturated fatty acids

Unsaturated fatty acids are of similar form, except that one or more alkenyl functional groups exist along the chain, with each alkene substituting a singly-bonded "-CH_2-CH_2-" part of the chain with a doubly-bonded "-CH=CH-" portion (that is, a carbon double bonded to another carbon) (Wikipedia, 2006). If there is one or more double bonds in the fatty acid, it is no longer considered saturated, rather it makes it mono- or polyunsaturated (Figure 2 (b) and (c), respectively) (Gunstone and Norris, 1983). The presence of double bonds generally reduces the melting point of fatty acids (Gurr *et al.*, 2002). Furthermore, unsaturated fatty acids can occur either in *cis* or *trans* geometric isomers. In most naturally occurring fatty acids, the double bonds are in the *cis* configuration (Wikipedia, 2006). In other words, the omega (ω) end contains 3 hydrogen (CH_3-) and each carbon within the chain contains 2 hydrogen (Gunstone and Norris, 1983).

The two next carbon atoms in the chain that are bound to either side of the double bond can occur in a *cis* or *trans* configuration.

- *cis* configuration

A *cis* configuration means that adjacent carbon atoms are on the same side of the double bond. The rigidity of the double bond freezes its conformation and, in the case of the *cis* isomer, causes the chain to bend and restricts the conformational freedom of the fatty acid. The more double bonds the chain has in the *cis* configuration, the less flexibility it has. When a chain has many *cis* bonds, it becomes quite curved in its most accessible conformations. For example, oleic acid, with one double bond, has a kink in it, whereas linoleic acid, with two double bonds, has a more pronounced bend. Alpha-linolenic acid, with three double bonds, favors a hooked shape. The effect of this is that, in restricted environments, such as when fatty acids are part of a phospholipid in a lipid bilayer, or triglycerides in lipid droplets, *cis* bonds limit the ability of fatty acids to be closely packed, and therefore could affect the melting temperature of the membrane or of the fat.

- *trans* configuration

A *trans* configuration, by contrast, means that the next two carbon atoms are bound to opposite sides of the double bond as shown in Figure 3. As a result, they do not cause the chain to bend much, and their shape is similar to straight saturated fatty acids. In most naturally, occurring unsaturated fatty acids, each double bond has 3*n* carbon atoms after it, for some n, and all are *cis* bonds. Most fatty acids in the *trans* configuration (*trans* fats) are not found in nature and are the result of human processing (e.g., hydrogenation).

The differences in geometry between the various types of unsaturated fatty acids, as well as between saturated and unsaturated fatty acids, play an important role in biological processes, and in the construction of biological structures (cell membranes) (Wong, 1989).

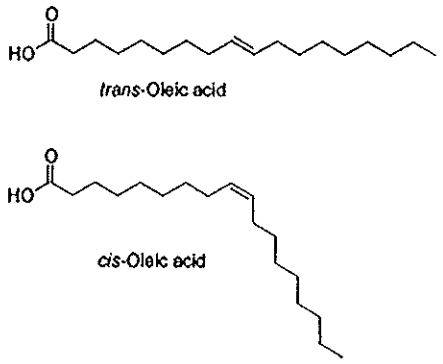


Figure 3 Comparison of the *trans*-isomer and the *cis*-isomer of oleic acid.
Source: Wikipedia, the free encyclopedia (2006).

3. Lipid in muscle foods

Foods vary widely in their lipid content and composition. In muscle lipids; avian, aquatic and mammalian tissues vary widely in quantity and composition. Muscle lipids are associated with, and to a large extent govern, the material's processing and end product properties (Pomeranz, 1985).

Table 4 Different of species and muscles on intramuscular neutral lipids and phospholipids

Species	Muscle	Content (% of total lipid)		
		Lipid	Neutral lipids	Phospholipids
Chicken	White	1.0	52	48
	Dark	2.5	79	21
Turkey	White	1.0	29	71
	Dark	3.5	74	26
Fish (Sucker)	White	1.5	76	24
	Dark	6.2	93	7
Beef	L.dorsi	2.6	78	22
	L.dorsi	7.7	92	8
	L.dorsi	12.7	95	5
Pork	L. dorsi	4.6	79	21
	Psoas majpr	3.1	63	37
Lamb	L. dorsi	5.7	83	10
	Semitendinosus	3.8	79	17

Source: Allen and Foegeding (1981).

The phospholipids and cholesterol muscle lipids are essential because of their role in the structure of the muscle cell and its organelles and their function. The neutral lipids provide fatty acids for energy metabolism and contribute to the characteristics of the meat (Pomeranz, 1985). Most of the phospholipids and cholesterol is membrane-associated. The neutral lipid (triglyceride) is present as microscopic droplets within the muscle cell or in fat cells. As these cells become more numerous, the fat becomes more visible in the muscle cross-section, exhibiting the phenomenon called marbling or intramuscular fat (Pomeranz, 1985). The content and composition of muscle cells differ within an animal depending on the muscle function (Allen and Foegeding, 1981). Within a species, light meat contains fewer lipids than dark meat as shown in Table 5 (Pomeranz, 1985). Neutral lipid of white and dark

chicken meat had a higher content of monounsaturated fatty acid than polyunsaturated fatty acid while phospholipids of both muscles had a higher content of polyunsaturated fatty acid more than monounsaturated fatty acid as shown in Table 5. Natural fats are composed principally of the glycerol esters of the straight chain carboxylic acids having an even number of carbon atoms. Triglycerides predominate in meat fats although small amount of monoglyceride and diglycerides may be present (Price and Schweigert, 1971). Figure 4 show the complex of lipid molecule in cell membrane muscle. The major compositions of muscle lipids included triglyceride, phospholipid, free fatty acids and cholesterol.

Table 5 Fatty acid compositions of chicken meat lipid with different muscle and type of lipid

Muscle	Lipid	Content (% of total fatty acids)			
		Monoenoic	Dienoic	Trienoic	>Trienoic
White	Neutral lipid	41.0	25.5	1.6	0.5
White	Phospholipid	18.9	19.1	2.7	23.7
Dark	Neutral lipid	42.2	25.5	1.7	0.5
Dark	Phospholipid	16.9	21.5	2.1	24.1

Source: Pomeranz (1985).

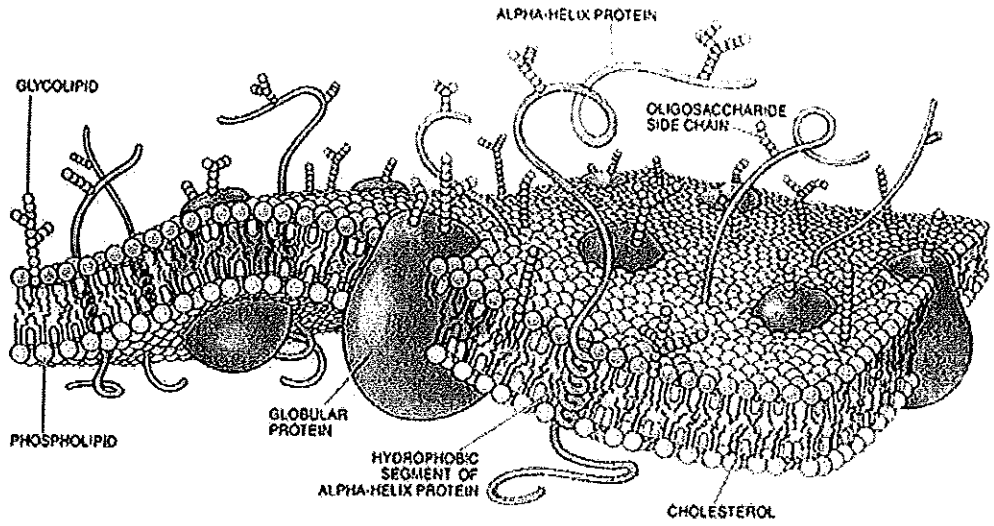


Figure 4 Diagram of complex of lipid molecules in cell membrane muscle.

Source: Desert Vista High School Biology (2008).

3.1 Triglycerides

Glycerides are lipids possessing a glycerol (propan-1, 2, 3-triol) (Gunstone and Norris, 1983) core structure with one or more fatty acyl groups, which are fatty acid-derived chains attached to the glycerol backbone by ester linkages. Glycerides with three acyl groups (triglycerides or neutral fats) are the main storage form of fat in animals and plants (Wikipedia, 2006). Glyceride hydrolysis products: glycolipid contains a sugar residue, a phospholipid furnishes phosphoric acid on hydrolysis, a sphingolipid is based on sphingosine or related long chain amine and a sulpholipid contains a sulphate group (Gunstone and Norris, 1983).

Triglyceride (also known as triacylglycerol or triacylglyceride) is glyceride in which the glycerol is esterified with three fatty acids (Figure 5). It is the main constituent of vegetable oil and animal fats (Wong, 1989). The chemical formula is $CH_2COOR\text{-}CHCOOR'\text{-}CH_2\text{-}COOR''$, where R, R', and R" are long alkyl chains. The three fatty acids RCOOH, R'COOH and R"COOH can be all different, all the same, or only two the same (Wikipedia, 2006).

(a) (b)

Figure 5 General structures of (a) glycerol and (b) triglyceride.
Source: Michael (2008).

Natural fatty acids found in plants and animals are typically composed only of even numbers of carbon atoms, due to the way they are bio-synthesized from acetyl CoA (Wikipedia, 2006). Chain lengths of the fatty acids in naturally occurring triglycerides can be from 5 to 28 carbon atoms, but 17 and 19 are most common. Shorter chain lengths may be found in some substances (butyric acid in butter) (Gunstone and Norris, 1983). Most natural fats contain a complex mixture of individual triglycerides; because of this, they melt over a broad range of temperatures.

3.2 Phospholipids

An important type of glyceride-based molecule found in biological membranes, such as the cell's plasma membrane and the intracellular membranes of

organelles, are the phosphoglycerides or glycerophospholipids (Gunstone and Norris, 1983). These are phospholipids that contain a glycerol core linked to two fatty acid-derived tails by ester or, more rarely, ether linkages and to one head group by a phosphate ester linkage as shown in Figure 6. The head groups of the phospholipids found in biological membranes are phosphatidylcholine (lecithin), phosphatidyl-ethanolamine, phosphatidylserine and phosphatidylinositol (Wikipedia, 2006). These phospholipids are subjected to a variety of reactions in the cell: for instance, polar head groups or fatty acid tails can be released from specific phospholipids through enzyme-catalyze hydrolysis to generate second messengers involved in signal transduction (Gurr *et al.*, 2002). In the case of phosphatidylinositol, the head group can be enzymatically modified by the addition of one to three phosphate molecules, and this constitutes another mechanism of cell signaling. While phospholipids are the major component of biological membranes, other non-glyceride lipid components like sphingolipids and sterols (such as cholesterol in animal cell membranes) are also found in biological membranes (Wikipedia, 2000).

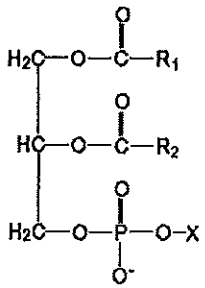


Figure 6 General structures of phospholipids. R_1, R_2 are representated to alkyl group and X can be a number of different substituents.

Source: Michael (2008).

3.3 Free fatty acids

Fatty acids can be bound or attached to other molecules, such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as free fatty acids. The uncombined fatty acids or free fatty acids may come from the breakdown of a triglyceride into its components (fatty acids and glycerol) (Wikipedia, 2006).

3.4 Cholesterol

Cholesterol is a monounsaturated lipid with a double bond on carbon-5 (Figure 7) found in the cell membranes of all body tissues, and transported in the blood plasma of all animals (Nawar, 1996). Lesser amounts of cholesterol are also found in plant membranes. Cholesterol plays a central role in many biochemical processes, but is best

known for the association of cardiovascular disease with various lipoprotein cholesterol transport patterns and high levels of cholesterol in the blood (Wikipedia, 2006).

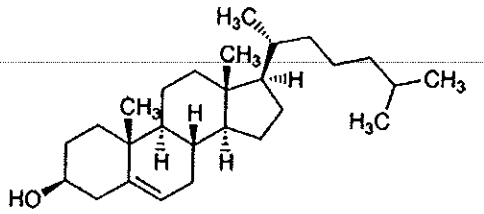


Figure 7 Cholesterol structure.

Source: Wikipedia, the free encyclopedia (2006).

Oxidation of lipids is a major cause of deterioration in the quality of meat and meat products affects many characteristics such as flavor, color, texture and nutritive value. Rapid development of rancid flavors during storage is a major problem facing meat industry, especially with increased demand for precooked meat items. Warmed-over flavor defined as rapid onset of rancidity in cooked meats during refrigerated storage (Gunstone and Norris, 1983).

4. Lipid oxidation

The oxidation of unsaturated lipids has been one of the most extensively studied because of related to the deterioration of foods, the production of both desirable (e.g., flavor, color) and undesirable breakdown products (e.g., toxic dimers). Lipid oxidation is a complex process whereby polyunsaturated fatty acids are degraded via formation of free radicals, causing flavor, texture, color and nutritional deterioration of foodstuffs (Gray, 1978) as shown in Figure 8.

Lipid oxidation in food systems is a detrimental process. It deteriorates the sensory quality and nutritive value of a product, poses a health hazard, and presents a number of analytical problems. The complex process of food lipid oxidative changes is interpreted in terms of an oxidation mechanism derived from model studies, predominantly involving a single fatty acid. Lipid oxidation in foods is assumed to proceed along a free radical route (autoxidation), photooxidation route, and/or lipoxygenase route. The oxidation mechanism is basically explained by involving free-radical reactions, while the photooxidation and lipoxygenase routes differ from it at the initiation stage only. For this reason, they can be treated as different forms of free radical reaction initiation.

4.1 Autoxidation

The major non-enzymatic oxidation process is a radical chain reaction involving initiation, propagation and termination steps (Gunstone and Norris, 1983). Oxidation is initiated by radicals present in living organisms (e.g., hydroperoxide HO_2•; hydroxide •OH; peroxide ROO•; alcoxyl RO• and alkyl L•) or by thermal or photochemical homolytic cleavage of an R-H bond. The oxidation activation energy and reaction rate at this stage depend on the type of initiator and the number of unsaturated bonds in the substrate. The dissociation energy of the C-H bonds in saturated fatty acid depend on the length of the fatty acid carbon chain and is similar in fatty acid, their esters, and in triacyglycerols (TAG) (Litwinienko *et al.*, 1999). In unsaturated acids, the weakest C-H bond is found in the bis-allylic position, the activation energy there amounting to 75 kcal/mol versus allylic and methylene hydrogens 88 and 100 kcal/mol, respectively (Simic *et al.*, 1992).

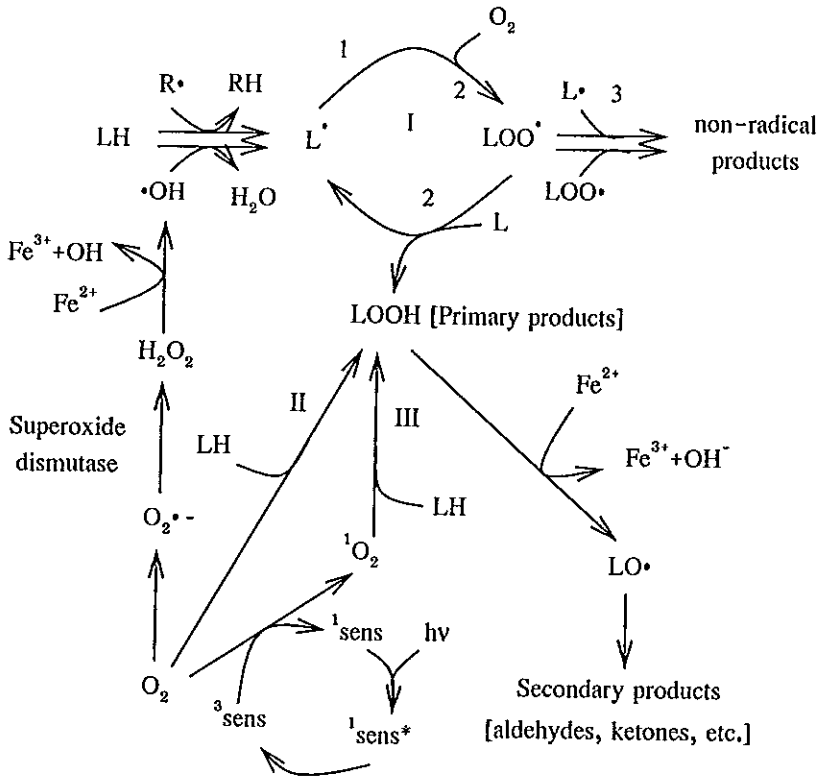


Figure 8 Lipid oxidation of unsaturated fatty acid (3 mechanisms).

I : Autoxidation (1 : initiation, 2 : propagation, 3 : termination).

II : Enzymatic oxidation.

III : Photoxidation.

Source: Carlsen *et al.* (2005).

4.1.1 Initiation

In the presence of an initiator (In), unsaturated lipids (LH) lose hydrogen to form a lipid radical (L•) following as:

$$LH \xrightarrow{\text{initiator}} L\bullet + H\bullet$$

4.1.2 Propagation

The alkyl radical lipid (L•) reacts with molecular oxygen to form peroxyl radical (LOO•). Peroxyl radical abstract hydrogen from another molecule of unsaturated lipid (LH) to form hydroxyperoxides (LOOH) and a new lipid radical (L•) following as:

$$L\bullet + O_2 \longrightarrow LOO\bullet$$
$$LOO\bullet + LH \longrightarrow LOOH + L\bullet$$

4.1.3 Termination

The peroxyl radicals react with each other to form non-radical products following as:

$$LOO\bullet + L\bullet \longrightarrow LOOL$$
$$L\bullet + L\bullet \longrightarrow LL$$
$$LOO\bullet + LOO\bullet \longrightarrow LOOL + O_2$$

} non-radical products

4.2 Enzymatic oxidation

Lipoxygenase (LOX)-catalyzed lipid oxidation differs from the free radical reaction by the formation of hydroperoxides at a certain position of the chain of, most often, a free fatty acid. Lipoxygenases use molecular oxygen to catalyze the stereo- and regiospecific oxygenation of polyunsaturated fatty acid with 1-*cis*, 4-*cis*-pentadiene moieties. LOX react enzymatically with more than one methylene carbon on the substrate molecule to yield double oxygenation sites (German *et al.*, 1992). The newly formed fatty acid peroxy free radical removes hydrogen from another unsaturated FA molecule to form a conjugated hydroperoxy diene. LOX forms a high-energy (radical) intermediate complex with the substrate; this complex is capable of initiating the oxidation of lipids and other compounds (e.g., carotenoids, chlorophyll, tocopherols, thiol compounds, and protein), which can themselves interact with the enzyme substrate complex as well (Hammer, 1993; Hultin, 1994). They are responsible for the off flavor in frozen vegetables (Ganthavorn *et al.*, 1991), lipid oxidation in

cereal products, rapeseed, pea, avocado, and for the beany and bitter flavor. Grinding of grains accelerates lipolysis, thus enhancing LOX activity (Frankel, 1998).

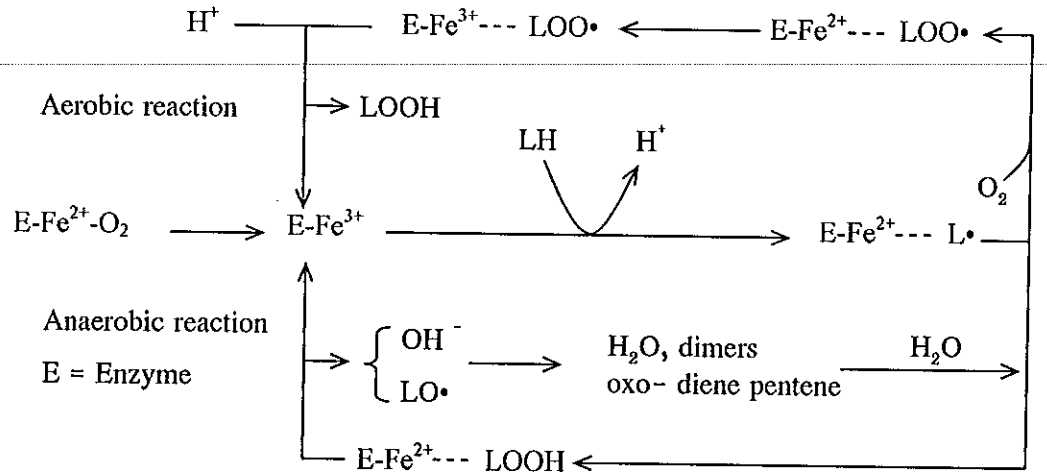


Figure 9 Enzymatic oxidation of lipid under aerobic and anaerobic reaction condition. **Source:** Gunstone and Norris (1983).

4.3 Photooxidation

Photooxidation involves the formation of hydroperoxides in a direct reaction of singlet oxygen addition to unsaturated lipids, without radical formation as shown in Figure 10 and 11. The singlet oxygen $^1O_2$ emerges during a reaction of sensitizers (e.g., chlorophyll, hemoglobin, myoglobin, and riboflavin) with atmospheric oxygen. Photosensitizations also can occured *in vivo* (Halliwell *et al.*, 1995). The singlet oxygen is 1450 times more reactive than molecular oxygen. It is inserted at the end carbon of a double bond, which is shifted to an allylic position in the *trans* configuration. The resulting hydroperoxides have an allylic *trans* double bond, which renders them different from hydroperoxides formed during autoxidation. Hydroperoxides formed during photooxidation are more easily cyclized than hydroperoxy epidioxides (Frankel, 1998). In addition, light, particularly ultraviolet light may be involved in initiation of the classical free radical oxidation of lipids and catalyze other stages of the process. In the presence of light energy-activated riboflavin, which is a sensitizer, a lipid radical can form, while oxygen gives rise to superoxide radical anion O_2•.

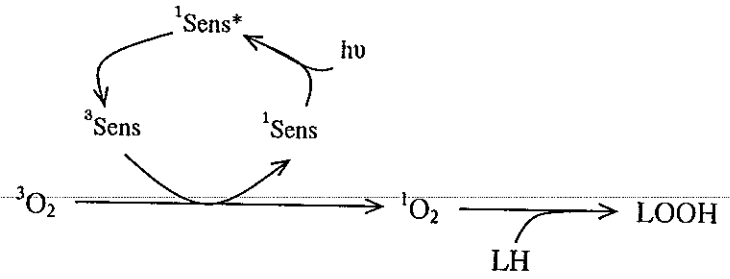


Figure 10 Photosensitized oxidation of lipid stimulating with oxygen.
Source: Adapted from Frankel (1998).

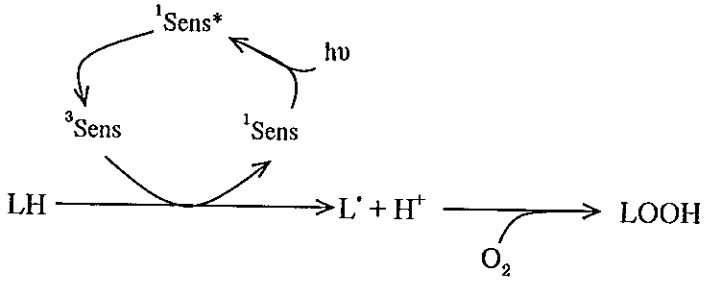


Figure 11 Photosensitized oxidation of lipid stimulating with fatty acid.
Source: Adapted from Frankel (1998).

5. Cholesterol oxidation products (COPs)

Cholesterol has been exploited with great advantage to detect any oxidation process in cell membranes. In contrast with unsaturated fatty acids, cholesterol exists as a single molecular species, its oxidation products are thus much less complicated to isolate and characterize (Smith, 1981). Cholesterol may undergo autoxidation and photo-oxidation, when cholesterol esters are oxidized; the structure and the yield of the formed oxysterols depend on the type of oxidation, the physical state of the substrate and the fatty acid species.

The cholesterol oxidation products (COPs) are group of sterols that are similar in structure to cholesterol but contain an additional hydroxy, ketone or epoxide group on the sterol nucleus and/or a hydroxyl group on the side chain of their molecules as shown in Figure 12. The hydroperoxides of polyunsaturated fatty acid formed during lipid oxidation may be necessary to initiate cholesterol oxidation (Park and Addis, 1986). The amount of COPs in foods could frequently reach 1% of total cholesterol and occasionally 10% or more. Animal products are a complex food with a highly structured nutritional composition and major source of cholesterol in the diet. It becomes edible and more digestible when it is subjected to cooking. However, heat treatment can lead to undesirable modifications, such as the loss of the nutritional value of meats mainly due to lipid oxidation and changes in some components of the cholesterol. The loss of the nutritional value of meats mainly due to lipid

oxidation and changes in some components included cholesterol. The degree of oxide formation is related to processing temperature, heating time, storage conditions, level of activator present, packaging and most of the oxides found in foods were subjected to processing conditions or exposure to heat (Paniangvait *et al.*, 1995). The most predominant oxidized cholesterol detected was 7-ketocholesterol; 20-hydroxy cholesterol; 25-hydroxycholesterol; 7α-hydroxycholesterol; 5,6α-epoxide; 7β-hydroxycholesterol; 5,6β-epoxide and triol (Paniangvait *et al.*, 1995). COPs have been known to be more injurious to arterial cells than pure cholesterol and are more directly connected to the development of atherosclerosis and coronary heart disease (Kowale *et al.*, 1996).

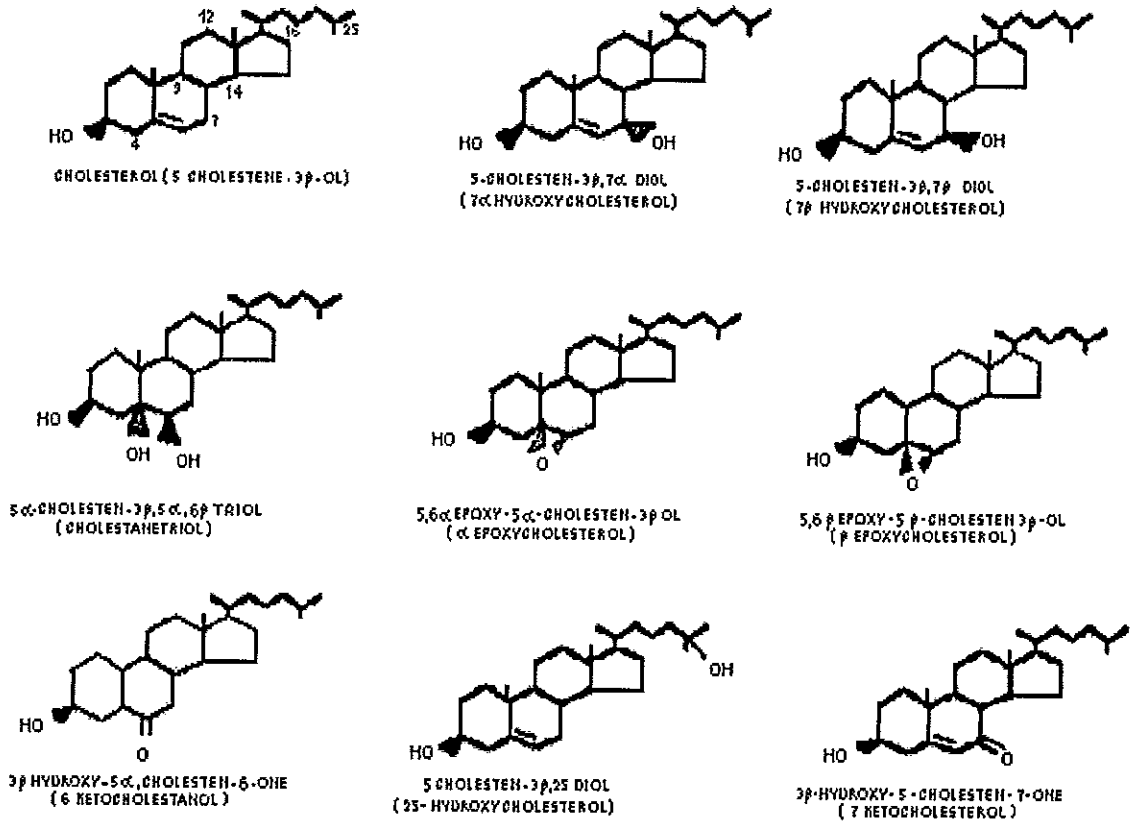


Figure 12 Schematic of main cholesterol oxidation products.
Source: Valenzuela *et al.* (2003).

6. Factors influencing lipid oxidation in meat

Lipid oxidation is affected by numerous internal and external factors such as fatty acid composition and content and activity of pro- and antioxidants, irradiation, temperature, oxygen pressure, surface area in contact with oxygen, and water activity (a_w). Lipid oxidation can be initiated, inhibited or altered by many factors including metal,

enzymes, antioxidants, temperature, light and pH (Wong, 1989), but in the most case usually point to metal and temperature as external factors while fatty acid composition as internal factor.

6.1 Metals

Hydroperoxides formed at the propagation stage of the free radical oxidation, as well as those produced by photooxidation and enzyme-catalyzed oxidation, can disintegrate and yield alcoxyl, alkyl, and peroxyl radicals, which reinitiate the oxidation of unsaturated fatty acids. Hydropesroxide decomposition may be triggered by temperature and/or light, but most important in this respect is the activity of transition metals, mainly iron and copper (See in equation 1 and 2).

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO\bullet + OH^{-} \text{ (fast)} \qquad (1)$$

$$Fe^{3+} + LOOH \rightarrow Fe^{2+} + LOO\bullet + H^{+} \text{ (slow)} \qquad (2)$$

The Fe^{2+} ions are more reactive than Fe^{3+} ions and decompose hydrogen peroxide over 100 times faster (Girotti, 1998). Iron occurs in human and animal bodies, in up to 90% in a bound form in: hemoglobin, myoglobin, cytochromes, the storage protein ferritin and hemosiderin, the iron transport proteins, transferrins, and as prosthetic groups of enzymes. A small amount of iron occurs in a free form, that is, primarily as low-molecular weight (LMW) iron. It colud be complex with organic phosphates, inorganic phosphates, amino acids (histidine, glycine, cysteine), and organic acids (citric acid) (Decker and Hultin, 1994). LMW iron contributes between 2.5 and 3.8% to the total iron content in muscle tissue of lamb, pork, and chicken. Dark muscles of chicken, turkey, and mackerel contain twice as much LMW iron and more ferritin than light muscles (Kanner, 1992). LMW iron acts as a catalyst. Protein-bound Fe and Cu are minimally catalytic in oxidation. Ascorbate, NAD(P)H, thiol compounds, reduced glutathione, cysteine, and protein thiol groups release iron, which can catalyze the Fenton reaction (Kanner, 1994; Hultin, 1994).

The rate of oxidation in the presence of metals is higher at lower pH than at neutral pH for Fe^{3+} and for Fe^{2+} (Koppenol, 1994). The effects of pH on the catalytic action of transition metals in emulsion systems are not so clear cut (Decker, 1990a). Food products contain less copper than iron; copper is mainly bound to protein as in ceruloplasmin. Copper ions are also chelated by albumin; in mammalian and avian skeletal muscles and brain, they are chelated by carnosine, anserine, and other histidine dipeptides. Cu^{1+} ions are more reactive than Fe^{2+} ions and decompose hydrogen peroxide to produce hydroxyl radicals at a rate over 50 times higher than Fe^{2+} ions (Decker, 1990b). However, the mechanism of the prooxidative

effects of copper is most likely a mechanism other than that for iron, which is the reason why prevention of that catalysis in food systems requires a different strategy (Hultin, 1994).

Concerning oxidation promoters in animal foodstuffs, iron is thought to have high catalytic activity (Rhee and Ziprin, 1987). Non-heme iron is considered the most important oxidation promoter in meat systems and, therefore, knowledge of the proportions of the chemical forms of iron is of great importance (Kanner *et al.*, 1991). An increase in the amount of non-heme iron as a result of thermal processes on meat systems has been shown (Lombardi-Boccia *et al.*, 2002; Schricker *et al.*, 1982). Miller *et al.* (1994a, 1994b), suggested cooking is not as important as the subsequent refrigerated storage of cooked meats for the release of non-heme iron from myoglobin. The increase of non-heme iron in meats and fish is considered to be a reflection of the decrease of heme iron as a consequence of the breakdown of the heme molecule during cooking or storage (Gomez-Basauri and Regenstein, 1992a, 1992b; Miller *et al.*, 1994a, 1994b) and this has been linked to the oxidative deterioration of the porphyrin ring of myoglobin (Schricker and Miller, 1983). Lipid and protein oxidation result to the release of iron from heme molecule and color deterioration during refrigerated storage of liver pate (Kristensen and Purslow, 2001).

6.2 Myoglobin

Myoglobin is an extremely compact heme protein (Molecular Weight ~ 17,800), found primarily in cardiac and red skeletal muscles (myoglobin and haemoglobin compose in sarvolemme, sarcoplastic reticulum, sarcoplasm that is sarcoplasmic protein which is dissolved in water or salt solution dilute less than 50 mM), functions in the storage of oxygen and facilitates the transport of oxygen to the mitochondria for oxidative phosphorylation (Greaser, 1986). The protein myoglobin is found only in skeletal, cardiac, and smooth muscles. The function of myoglobin is to store and transport oxygen in the muscle cell. The protein is soluble at low ionic strength and can contribute up to 95 % of the red pigment of muscle. The content of myoglobin in skeletal muscle will vary depending on the metabolic profile of the muscle, animal species and age of the animal (Greaser, 1986). The pectoralis muscle from chicken contains myoglobin concentration less than 1 mg per gram of muscle, and muscles from older cattle can have myoglobin concentration in the range 10-20 mg per gram muscle (Rhee and Ziprin, 1987).

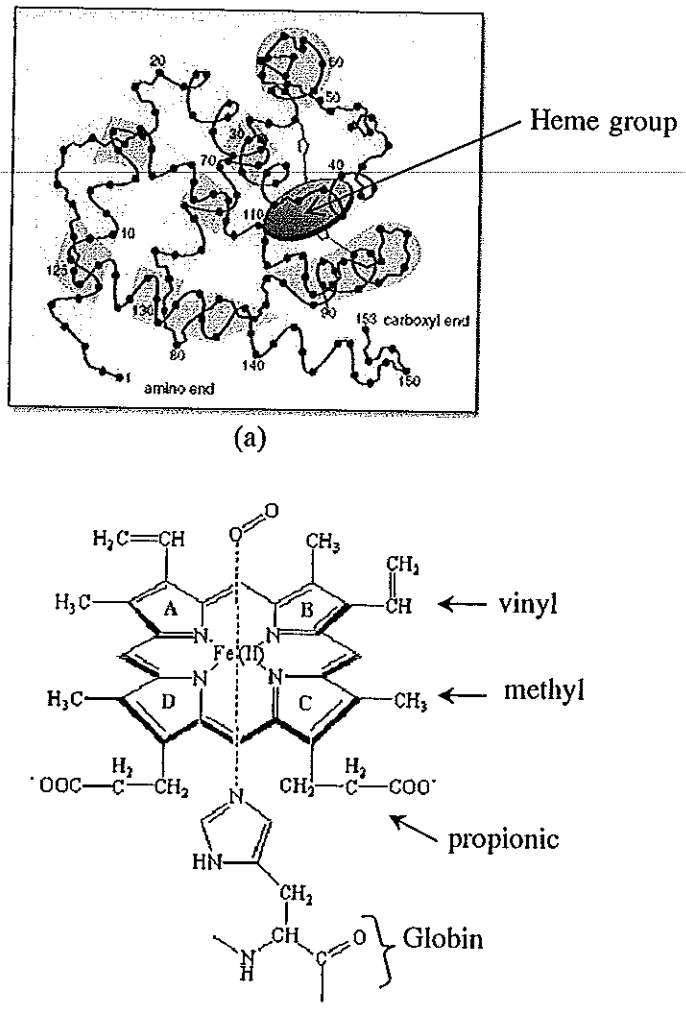


Figure 13 A schematic view of myoglobin showing heme group and globin protein (a), a closeup of heme showing iron (Fe^{2+}) coordinated to four nitrogens of the porphyrin ring plus a histidine residue in the globin protein (b).

Source: Wikipedia, the free encyclopedia (2006).

Myoglobin consists of a single polypeptide chain of about 153 amino acids as shown in Figure 13 (Schricker and Miller, 1983). The main chain peptide groups are rigid planar units, and the carbonyl group (C=O) of each is trans to the N-H; therefore, all peptide bonds are in planar-trans conformation. The peptide group is planar because the carbon-nitrogen bond has partial double-bond character. As a result, rotation about the C-N bond is restricted (Gomez-Basauri and Regenstein, 1992a). The interior consists almost entirely of nonpolar residues including leucine, valine, methionine, and phenylalanine. Polar residues such as aspartate, glutamate, lysine, and arginine are absent from the interior protein surface.

In fact, two histidines are the only polar residues which possible to play an integral role in the binding of heme oxygen. The outside of the protein has both polar and nonpolar residues (Rhee and Ziprin, 1987).

The structure of myoglobin suggests that the oxygen-carrying heme group is buried inside the protein portion of this molecule, which keeps pairs of hemes group from coming too close together (Schricker and Miller, 1983). This is important, because these proteins need to bind O_2 reversibly and the Fe (II) heme, by itself, cannot do this. When there is no globin to protect the heme, it reacts with oxygen to form an oxidized Fe (III) atom instead of an Fe(II)-O_2 complex (Decker, 2001). At the center of the heme group is the iron +2 metal ion. The nitrogen atoms bind to the iron ion through what are called coordinate covalent bonds (Rhee and Ziprin, 1987). The oxygen molecule will ultimately bind to this iron ion also using a coordinate covalent bond. In the heme complex, the coordination positions are directed to the four porphyrin nitrogens, and in the fifth and the sixth positions, to histidine F8 (proximal histidine) and is closely associated with a second histidine E7 (distal histidine) and O_2 or H_2O, respectively (Schricker and Miller, 1983).

Table 6 Heme and nonheme iron, total iron and percent heme iron content of raw and cooked animal products (mg/100 g of edible portion)

Product	Cooking time (min)	Heme iron	Non-heme iron	Total iron
Beef loin, raw	-	1.37 ± 0.6	0.77 ± 0.3	2.17 ± 0.3
Beef loin, boiled	10	1.17 ± 0.5	1.37 ± 0.5	2.47 ± 0.5
Chicken breast, raw	-	0.17 ± 0.0	0.37 ± 0.1	0.47 ± 0.0
Chicken breast, boiled	5	0.17 ± 0.0	0.37 ± 0.1	0.47 ± 0.0
Chicken drumsticks, raw	-	0.37 ± 0.1	0.67 ± 0.1	0.97 ± 0.1
Chicken drumsticks, boiled	18-20	0.37 ± 0.1	1.07 ± 0.2	1.27 ± 0.2
Pork loin, raw	-	1.37 ± 0.6	0.77 ± 0.3	2.17 ± 0.3
Pork loin, boiled	5-7	1.17 ± 0.5	1.37 ± 0.5	2.47 ± 0.5

Source: Adapt from Kongkachuichai *et al.* (2002).

In this study, called the iron ion in heme group which both bind and not bind with globin chain is heme iron and called iron ion released from heme group to free iron form is non-heme iron as shown in Figure 13. Dietary iron presents in food in two forms as heme and nonheme iron. Heme iron is that derived from hemoglobin and nonheme iron is all the

other compounds present in a food. Although plant materials contain only nonheme iron, animal products contain both heme and nonheme iron. Heme iron is considered to be nutritionally important as it is higher in bioavailability (>15%) than nonheme iron (<5%) (Kalpalathika *et al.*, 1991). However, the cooking process involved can decrease the amount of heme iron concentration in food as shown in Table 6 (Clark *et al.*, 1997).

6.3 Fatty acids composition

There is an increasing the interested in the lipid composition of edible meat and fat of domestic animals because of its relationship with human health, particularly with cardiovascular illnesses (Hu and Willett, 2002). Most of previous studies point to the effect of diet supplemented with advantage fatty acids such fatty acid omega-3, -6 or CLA (Conjugated Linoleic Acid) on oxidation stability in poultry meat and other meat (Keefe *et al.*, 1995; Lauridsen *et al.*, 1997; Hulan *et al.*, 1998; Sklan *et al.*; 1983; Marion and Woodroof, 1963). Fatty acid compositions in meat are very might be influenced from animal types, breeds, diet, age, gender, environment, etc. It is well known that the quantity and composition of the fatty acids found in monogastrics is directly influenced by the composition of the diet (Bernardini *et al.*, 1999; Oliver *et al.*, 1997). In poultry, diets for fast growing broilers are generally rich in polyunsaturated fatty acids and the degree of unsaturation in carcass fat is thereby increased. Furthermore, there are some evidences indicated that dietary oils can alter the fatty acid composition of mitochondrial and microsomal lipids (Tahin *et al.*, 1981; Asghar *et al.*, 1990). However, increasing the degree of unsaturation of the muscle membranes by dietary manipulation increases the peroxidizability of tissues and muscle foods (Igene *et al.*, 1980; Pikul *et al.*, 1984). While Zaninia *et al.* (2006) reported that under refrigeration, the lipid oxidation of the breast and the thigh meat was minimized by the synergistic effect between CLA and the fat source which under this storage condition the CLA was more efficient in reducing lipid oxidation. However, there still have limit correlation between fatty acid compositions in chicken meat which commercially consumed in Thailand and their oxidative stability. In the fact that chicken from different breeds feed with different diets might leading to the difference in fatty acid composition of their meat and probably related to different lipid oxidation.

6.4 Heating

Cooking processes are important because of their effect on nutrient loss (Kongkachuichai *et al.*, 2002). Also results in the release of heme-bound iron and in forming other polymers with proteins; those polymers enhance the catalytic effect of iron. This is also true with respect to the thermal inactivation of enzymes that contain metals acting as prosthetic groups (e.g., lipoxygenases and peroxidases). These enzymes, even after

denaturation, are capable of catalyzing oxidation. On the other hand, heating does not release iron from ferritin, but does enhance its reduction (Kanner, 1992).

Results of several study showed that heme iron content in foods decreased during the cooking process, while nonheme iron increased. The increase of nonheme iron was derived from the alteration of hemoglobin and myoglobin structures. These findings are consistent with several observations (Ahn *et al.*, 1993; Wang and Lin, 1994). Cooking processes reduce the amount of heme iron, which is known to be the better absorbed form; however, heating processes are necessary for food safety and human digestibility. Comparison was made among the means of percent heme iron values of animal products in this study with previously published research (Cook and Monsen, 1976; Carpenter and Clark, 1995). Cook and Monsen (1976) reported that percent heme iron content ranged from 30 to 40% in raw pork and from 50 to 60% in raw beef. Kalpalathika *et al.* (1991), Carpenter and Mahoney (1992) and Carpenter and Clark (1995) reported that percent heme iron content in raw beef and chicken muscles was approximately 61% in beef and 25% in chicken muscle. Similarly, the analyzed data in this study showed that the means percent heme iron were found to be 66% for raw and 45% for cooked beef, 30-40% for raw and 30% for cooked pork, 33 for raw and 25% for cooked meat chicken, 16-50% for cooked shellfish, and 33-40% for raw fish and 14-38% for cooked fish. From above data indicated that heat treatment and temperature influenced on lipid oxidation in meat.

6.4.1 Heating method

Kowale *et al.* (1996) reported that cooking (broiling and pressure cooking) of mutton meat significantly increased the lipid oxidation (as thiobarbituric acid value: TBA value) during storage, included cholesterol oxidation products; 7-α-hydroxy cholesterol, 19-hydroxycholesterol, 7-ketocholesterol, cholesterol-α-epoxide and cholesterol-β-epoxide. Agreement with Rao (1996) who obtained the same results from the same cooking methods on buffalo meat. Both researches found that the increase in TBA values in broiled meat was more pronounced compared to in that of pressure-cooked meat. Results clearly indicated that even frozen storage of cooked meat did not prevent the development of cholesterol oxidation products. However, the values were below the threshold level for rancidity development (1-2 mg MDA/kg meat (Watts, 1962)). While Rodriguez-Estrada *et al.* (1997) studied on regarding of cholesterol oxidation in ground beef hamburger and founded that the combination of roasting and microwave heating caused more oxidation than the other treatments (boiling; barbecue and frying pan). In addition, the result of Kanatt *et al.* (1998) showed that the lipid oxidation of lamb meat increased during irradiation and chilled storage. Previous researches found a decrease in heme iron content during heat treatment of meat and meat products,

accompanied by an increase in non-heme iron content (Buchowski *et al.*, 1988; Carpenter and Clark, 1995; Han *et al.*, 1993) and it is believed that iron is liberated from the heme complex due to denaturation of myoglobin, followed by a release and degradation of the heme molecule (Awad and Deranleau, 1968; Kristensen and Andersen, 1997).

6.4.2 Heating temperature

Kristensen and Purslow (2001) reported that heating temperature has a gross effect on heme/non-heme iron with a decrease in heme iron content of 62 % after heating at 80 °C for 2 h. The decrease of heme iron content at 80 °C was observed more than at other lower temperatures (55-75°C). The decrease in heme iron content is followed by an increase in non-heme iron content of the meat in meat processed at 80 °C. The inverse relaship between heme and non-heme iron contents, as a function of heating temperature, is in agreement with previous work where both heme and non-heme iron were measured in meat heat-treated at different temperatures (Buchowski *et al.*, 1988; Carpenter and Clark, 1995; Han *et al.*, 1993). Han *et al.* (1993) measured heme iron content in beef and chicken as a function of heating temperature and showed no significant differences between a raw unheated control and meat heated to 55 °C. However, between 55 and 70 °C a large decrease in heme iron content was observed. The major part of heme iron in meat is located in myoglobin and the thermal stability of myoglobin is highly dependent on an intact heme molecule (Chanthai *et al.*, 1996a, b; Harbrove and Olson, 1996). A destruction of the heme molecule will therefore easily lead to denaturation of myoglobin. Thus, loss of iron from the molecule will not occur in heat treated meat without denaturation of the myoglobin molecule. Geileskey *et al.* (1998) have investigated the kinetics of myoglobin denaturation in meat and found a very low denaturation rate at 55 °C, a large increase in denaturation rate was observed.

6.5 Storage

Alasnier *et al.* (2000b) determined the change in free fatty acid amount and thiobarbituric reactive substabce in chicken breast and thigh muscles at intervals between 1 and 14 days of storage at 4 °C and found the rates of lipid hydrolysis were fast in the first 3 days and then slowed until day 14 and oxidation increased linearly during storage. According to the study of Novelli *et al.* (1997), who studied on oxidative processes of fresh and stored frozen pork and of two pork products (salame Milano and mortadella). sale pork cuts (shoulder, ham trimmings, belly and backfat) were checked fresh and after frozen storage (1, 3 and 6 months). They found raw materials stored frozen up to six months, peroxide and TBARS values are low and oxysterols dangerous for human health are either absent or their concentration is below the levels considered to be necessary for toxic responses in cell culture

(Guardiola *et al.,* 1996). The refrigerated storage largely affected free fatty acid (Alasnier *et al.*, 2000a). The amount of free fatty acid increased significantly in the muscles during the storage, immediately after slaughter, lipids were slightly hydrolysed and free fatty acid represented 2-10 mg/100 g of muscle. Alasnier *et al.* (2000a) reported free fatty acid amount was multiplied by 3-9 and reached 11.5-32.4 mg/100 g of chicken rabbit muscle after 7 days of storage. Lipolysis which releases free fatty acid is also suspected to favor lipid oxidation because free fatty acids are very sensitive to lipid oxidation (Nawar, 1996).

OBJECTIVES

1. To determine the chemical and fatty acid compositions of breast and thigh muscles from broiler, spent hen and Thai indigenous chicken.
2. To investigate the change in chemical and fatty acid compositions, pH value, color and lipid and cholesterol oxidation of breast and thigh muscles from broiler, spent hen and Thai indigenous chicken during refrigerated storage.
3. To study the effect of heating method and heat temperature on the change in chemical and fatty acid compositions, color, and lipid and cholesterol oxidation of chicken muscle.

CHAPTER 2
MATERIALS AND METHODS

1. Chemical compositions and fatty acid profiles of breast and thigh meat from different chicken breeds

1.1 Sample preparation

Eight Thai indigenous chickens (*Gallus domesticus*) aged 16 weeks, 8 commercial broilers (*CP-707* Arbor Acres) aged 40 days and 8 spent hens (*Isa brown*) aged 53 weeks at the market weights of 1.5±0.2 kg were randomly obtained from different local commercial poultry farms in Songkla. The commercial broilers were fed with commercial formula feed in 2 stages, i.e. for grower (0-6 weeks) with 2,800 kcal/kg metabolically energy and 24.0% concentrated protein (CP) and for finisher (4 weeks up) with 3,000 kcal/kg and 22.0% CP. Spent hens were fed with commercial formula feed in 4 stages, i.e. for 0-3 weeks with 3,200 kcal/kg metabolically energy and 19.0% CP, for 6-14 weeks with 3,000 kcal/kg and 16.0% CP, for 14-20 weeks with 2,800 kcal/kg and 13.0% CP and for 20 weeks up with 3,000 kcal/kg and 16.0% CP. Thai indigenous chicken in Southern part reared under extensive production system where chicken were allowed to scavenge on natural food around the homestead during the day and supplement with the concentrated feed in the evening when they came back to roost and sheltered at night.

The animals were transferred to the Food Technology department, Faculty of Agro-Industry, Prince of Songkla University. After resting at a minimum of 6 h without feeding, the animals were slaughtered and dressed according to the method mentioned by Wattanachant *et al.* (2004). The carcasses were cooled in ice cold water till the body temperature dropped down to 8 °C then, packed in plastic bag (polyethylene) and placed in a cold room at 4±1 °C for 30 min weighed and kept in the cold room for 24 h. The carcasses were cut and separated into portions of breasts and thighs. All portions were weighed and deboned. Boneless breasts and thighs were used for chemical analyses. The skin was removed and the meats were trimmed off obvious fat and connective tissue. Breast and thigh meat of 4 chickens for each breed were randomly sampled for chemical analysis, minced using a domestic meat chopper (Moulinex 327, Spain) then vacuum packed and stored at -20 °C until used. While breast and thigh meat of other 4 chickens for each breed were randomly sampled, the middle part from breast (*pectoralis major* muscle) and thigh (*biceps femoris* muscle) were cut to the size 2.0 x 2.0 x 6.0 cm for color measurement. The samples were kept on ice during preparation and analysis.

1.2 pH value and proximate analysis

The pH of meat was determined by homogenizing the meat samples with distilled water at ratio of 1:5 (w/v). The homogenate was subjected to pH measurement using a combined glass electrode pH meter (SevenGO SG2-FK2, METTLER TOLEDO).

The samples were finely ground to a paste consistent and determined chemical composition according to standard techniques. The proximate analyses included moisture by hot air oven technique (AOAC, 2000), crude lipid by the method of Folch et al. (1957) modified by Du *et al.* (1999), protein with Kjeldahl method (AOAC, 2000) and ash with muffle furnace at 600 °C (AOAC, 2000) were determined.

1.3 Fatty acids composition and cholesterol analysis

1.3.1 Lipid extraction

The meat and skin samples were extracted based on the method of Folch *et al.* (1957) modified by Du *et al.* (1999). The samples were finely ground to a paste consistency and a 2 g representative sample was weighed accurately and placed in a 50 ml beaker. Then the sample was added with 20 ml of chloroform-methanol (2:1; v/v) solution and homogenized with Ultra Turrax T25 homogenizer for 5 min, and then added with 25 µl of 10 % BHA in 98 % ethanol with well mixing. The homogenised mixture of the solvent and samples were then filtered through No.1 filter paper into a screw cap tube and the filter paper was rinsed with chloroform-methanol. Five ml of NaCl solution (0.88 %) was added into the tube of sample which was sealed firmly and shaken on a vortex thereafter. The mixture was then allowed to separate into two layers and the top layer (methanol aqueous fraction) was discarded while the bottom layer containing the fatty acids was transferred to a scintillation vial and dried under nitrogen gas. Before extraction, one ml of internal standard solution (C19:0, 2 mg/ml in hexane) was added to the dried samples and left to dry for further methylation or saponofication.

1.3.2 Calculation of relative response factor (RRF)

Each standard fatty acid and nonadecanoic acid (internal standard) solution were mixed in the 1:1 (v/v) combinations together and then subjected to GC analysis. Peak area obtained from above analysis was applied to equation (1) for RRF calculation.

$$RRF = (A_{standard}) / (W_{standard}) \div (A_{IS}) / (W_{IS}) \quad (1)$$

Where $A_{standard}$ is the peak area of each standard obtained from GC analysis, A_{IS} is the peak area of internal standard obtained from GC analysis, $W_{standard}$ is the weight (µg) of standard fatty acid used in analysis and W_{IS} is the weight (µg) of internal standard used in analysis.

1.3.3 Fatty acids methylation

Sample lipid extracted was methylated based on the procedure of Folch *et al.* (1957) with a slight modification. A 50 mg sample extracted was weighed accurately and placed into screw cap tube, and mixed with 2 ml of methanol/hexane (4:1 v/v). Then, 200 µl of acetyl chloride was dropped carefully into the screw cap tube and vortexed together. The reaction was carried out at 100 °C for 1 h in water bath. Then, the mixture solution was centrifuged at 1000 rpm for 15 min with 5 ml of 6 % K_2CO_3 and consequence, the fatty acids methyl esters were extracted in hexane at the top layer. The fatty acids methyl esters was transferred to 1.5 ml vial and covered with parafilm to be stored at -20 °C until analysis.

1.3.4 Cholesterol methylation

Pre-treatment of lipid samples with tetramethylammonium hydroxide (TMAH) methylation was adapted from the method described by Lee *et al.* (1990). Triplicate samples with about 0.1 g lipid were weighed in a 7 ml capped vial, and 1 ml of 10% tetramethylammonium hydroxide methanol solution (TMAH/MeOH) was added to start methylation reaction. The reaction was continued for 10 min under shaking. Then, 0.5 ml of distilled water was added to terminate the reaction and 100 ml of internal standard (5α-cholestane) were added before extraction with 2 ml of ether. Ether extraction was performed twice, and the extracts were combined for GC analysis.

1.3.5 GC analysis

The fatty acid methyl esters were separated using a HP-innowax capillary column, 30 m x 0.25 mm i.d., 0.25 µm film thicknesses, connected to Agilent Technology model 6890N Gas Chromatograph fitted with a flame ionization detector. High purity helium was used as the carrier gas set at 1.0 ml/min and the sample injected into the GC was 1 µl. The initial column temperature of 40 °C which was increased at 25 °C/min to 195 °C, increased at 3 °C/min to 205 °C, increased at 8 °C/min to 230 °C and hold for 1 min. The injector and detector temperatures were set at 250 °C. Split/splitless injection mode (10:1 for each injection) was used. Fatty acid profiles and concentrations of samples were then measured against the internal standard and calculated as by equation (2).

$$\text{Target content } (\mu g/g) = (A_{target} \div A_{IS}) \times (W_{IS} \div \text{RRF}) \times (1 \div W_{sample}) \quad (2)$$

Where W_{sample} is the weight of lipid sample (g), target mean each fatty acid or cholesterol.

The analysis of cholesterol was conducted in a HP-innowax capillary column, 10 m x 0.10 mm i.d., 0.17 µm film thicknesses, connected to Agilent Technology model 6890N Gas Chromatograph fitted with a flame ionization detector. High purity helium was used as the carrier gas set at 1.0 ml/min and the sample injected into the GC was 1 µl. The temperature at injector port was set at 275 °C. Split/splitless injection mode (10:1 for each

injection) was used. Oven temperature was set at 250 °C and hold for 15 min and detector temperature was set at 300 °C. The content of cholesterol for each sample was obtained from triplicate tests and calculated by described equation (2) as mentioned above.

1.4 Myoglobin analysis

The myoglobin content was determined by using method of Geileskey *et al.* (1998). Five gram grounded meat were homogenised at 15000 rpm to fine slurry in 12 ml of 0.1 M phosphate buffer (pH 6.5) for 20 s. The slurry was centrifuged at 5000 g for 15 min at 4 °C, using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). The supernatant was filtered through filter paper No.4 into a 25 ml volumetric flask. The insoluble residue was homogenised in a further 12 ml of phosphate buffer for 20 s, centrifuged and the supernatant was filtered again into the volumetric flask. The extract was made up to volume. The absorbance was read at 525 nm using a UV-1601 spectrophotometer (Shimadzu, Japan). Myoglobin content was calculated from the millimolar extinction coefficient of 0.132 (Eder, 1996) and a molecular weight of 16110 (Gomez-Basauri and Regenstein, 1992). The myoglobin content was expressed as mg/g sample.

1.5 Color measurement

The color of meat samples was determined in sixteen replications (4 chickens x 4 determinations) of each sample using a Hunterlab colorimeter and reported as the complete International Commission on Illumination (CIE) system color profile of Lightness (L*), redness (a*), and yellowness (b*).

1.6 Statistics analysis

A Completely Randomized Design was used to determine the effect of chicken breeds and meat types on chemical compositions and fatty acids compositions. Chemical compositions were done for 8 replicates (4 chickens x 2 determinations), fatty acid compositions were done for 3 replicates (3 determinations) while for physical characteristics were done for 16 replicates (4 chickens x 4 determinations). Data was subjected to analysis of variance (ANOVA) and mean comparison between 3 chicken breeds was carried out using Duncan's Multiple Range Test (DMRT), whereas Paired sample T-test was applied to analyze the significant difference of means between meat types. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows version 11.5: SPSS Inc.).

2. Lipid oxidation of breast and thigh meat from different chicken breeds

2.1 Sample preparation

Sixty-four Thai indigenous chickens (TI) (*Gallus domesticus*) aged 16 weeks, 64 commercial broilers (BC) (*CP-707* Arbor Acres) aged 38 days and 64 spent hens (SP) (*Isa*

brown) aged 53 weeks at the market weights of 1.6±0.2 kg were randomly obtained from different poultry farms in Songkla. Chicken rearing and preparing chicken meat samples same as described in part 1. The breast and thigh meat with bone were individually packed into tightly sealed plastic bags with and without skin at 4 °C for 9 days. During storage, 8 samples of each chicken breed meat were taken at 0, 3, 6 and 9 days, for analyses. All samples were removed skin and bone (for thigh meat) and also trimmed of obvious fat and connective tissue before analysed. Eight breast and thigh meat from 4 chickens (8 meats/chicken breeds) were randomly sampled for chemical analysis, minced using a domestic meat chopper (Moulinex 327, Spain) then vacuum packed and stored at -20 °C until used. While eight breast and thigh meat from 4 chickens (8 meats/chicken breeds) were randomly sampled, the middle part from breast (*pectoralis major* muscle) and thigh (*biceps femoris* muscle) were cut to the size 2.0 x 2.0 x 6.0 cm for color measurement. The samples were kept on ice during preparation and analysis. The highest lipid oxidation sample was selected to further study on part 3.

2.2 Chemical analysis

Samples were finely ground to a paste consistency and determined chemical composition according to standard techniques. The analyses included moisture by hot air oven technique (AOAC, 2000), crude lipid modified method of Folch *et al.* (1957) (Du et al., 1999) and pH determinations.

2.3 Fatty acids composition, cholesterol and cholesterol oxidation products (COPs) analysis

2.3.1 Lipid extraction

The meat samples were extracted as the same method mentioned in item 1.3.1.

2.3.2 Calculation of relative response factor (RRF)

Each standard solution; fatty acids used nonadecanoic acid as internal standard, cholesterol and cholesterol oxidation product (COPs) used 5α-cholestane as internal standard, were subjected to GC analysis. Peak area obtained from the analysis was applied to equation (1) for RRF calculation.

2.3.3 Fatty acid and cholesterol methylation

The extracted lipid samples were methylated based on the method mentioned in item 1.3.3.

2.3.4 Cholesterol oxidation products (COPs) saponification

The extracted lipid samples were saponified by the method of Park and Addis (1986). A sample of 0.2 g of cold extracted lipid was mixed with 5 ml methanol in a screw-capped test tube. The test tube was left for 15 min in a water bath at 40°C and then 15 ml 2 N

KOH in methanol were added. Saponification was conducted at room temperature for 15 hr, followed by mixing with 20 ml of distilled water, and the sample was transferred into a separating funnel and the non saponified part was extracted five times with 30 ml of diethyl ether. The ether fractions were merged and repeatedly washed, first with 20 ml 0.5 N KOH and second with 20 ml of distilled water, until the washing solution was colorless with phenolphtalein. At the end of the washings the ether fraction was made anhydrous by adding Na_2S0_4, filtered on paper and dried with rotary evaporator. The dried extract was redissolved in 1 ml ether and then dried under nitrogen until sample volume was 0.2 ml. The sample was mixed with 200 µl of Sylon HTP and left at 65°C for 55 min.

2.3.5 GC analysis

The fatty acid and cholesterol methyl esters were separated using GC as described previously in item 1.3.5.

For measurement COPs, gas chromatography was performed in a HP-innowax capillary column, 10 m x 0.10 mm i.d., 0.17 µm film thicknesses, connected to Agilent Technology model 6890N Gas Chromatograph fitted with a flame ionization detector. Helium was used as carrier gas and the chromatographic conditions were set as following: oven temperature programmed 1 min at 210 °C, increased at 2 °C/min to 264 °C, at 3.5 °C/min to 290 °C, and kept for 43 min at 290 °C in order to purge the column; injector temperature at 290 °C; detector temperature at 350 °C; split ratio was 1:4; head pressure was 30 psi; injected sample volume was 1.4 µl. The content of COPs for each sample was obtained from triplicate tests and calculated by using equation (2) (as shown in item 1.3.5).

2.4 Lipid fractionation

Lipid fraction of sample was determined by method of Estévez and Helleur (2005). Samples with a total lipid loading between 0.4 and 10.0 mg were applied onto Chromarods SIII (5 mm silica gel-coated quartz rod, Iatron Labs, Tokyo, Japan) below the origin using a 2.0 ml blunt-tipped Hamilton syringe (Hamilton Co., Reno, USA). The rods were developed with two-solvent system followed by total scanning using an Iatroscan MK-5 TLC–FID analyzer (Iatron Laboratories, Inc., Tokyo, Japan). Typical operation conditions for the Iatroscan were: 2 L/min air flow, 200 mL/min hydrogen flow and 35 s/scan speeds. The first development, hexane:diethyl ether:formic acid (80:20:1, v/v) for 40 min to move triglyceride and free fatty acids, the second development, chloroform:methanol (67:37, v/v) for 35 min to move the phospholipids. Each lipid fraction was expressed as the percentage of total lipid.

2.5 Myoglobin analysis

The analysis of myoglobin content was described previously in item 1.5

2.6 Metmyoglobin measurement

The analysis of metmyoglobin content was performed as described by Lee *et al.* (1999). The sample solution was prepared in the same manner as that for myoglobin determination. The supernatant was subjected to absorbance measurement at 700, 572, and 525 nm. The percentage of metmyoglobin was calculated using the following equation (Krzywicki, 1982):

$$\% \text{ Metmyoglobin} = \{1.395 - [(A_{572} - A_{700})/(A_{525} - A_{700})]\} \times 100$$

2.7 Determination of heme iron content

The heme iron content was determined by using the acidified acetone extraction method of Hornsey (1956) with modifications. Grounded sample (5 g) was transferred to a 50 ml poly-propylene centrifuge tube and 10.0 ml acidified acetone (acetone:hydrochloric acid = 95.7:4.3, v/v) was added. The suspension was homogenized for 30 s at 13500 rpm, using homogenizer which afterwards was rinsed 3 times with 3 ml of acidified acetone. The suspension was briefly mixed and stored on ice for 1 h. Insoluble substances were precipitated by centrifugation (1 h, 0 °C, 10000 xg) and 5 ml supernatant was filtered through No.4 filter paper. The absorbance of the filtrate at 640 nm was measured, and the heme iron content was calculated using a molar extraction coefficient of 4800 M^{-1} cm^{-1} and expressed as µg Fe/g sample. All filtered samples were visually examined for turbidity before measurement; turbid samples were filtered once more.

2.8 Determination of non-heme iron content

The non-heme iron content was determined by method of Ahn *et al.* (1993) and Carter (1971) with slight modifications. Grounded meat (5 g) was weighted into a 50 ml poly-propylene centrifuge tube and 15.0 ml of 0.1 M citrate-phosphate buffer at pH 5.5 was added. The suspension was homogenized for 30 s at 13500 rpm using Ultra Turrax T25 homogenizer (before use, rinse the dispersing element twice with 250 ml citrate-phosphate buffer for 3 min at 13500 rpm to minimize release of iron during homogenization of the sample). Homogenate 1.5 g was transferred to a 5 ml sample tube, treated with 0.50 ml of 2 % ascorbic acid in 0.2 M HCl and incubated at room temperature for 15 min. One milliliter of 11.3 % TCA was then added to the sample and mixed thoroughly, then 2 ml was transferred to a 2.5 ml centrifuge tube and centrifuged for 10 min at 20000 xg. One milliliter of the clear supernatant was mixed with 0.40 ml of 10 % ammonium acetate and 0.10 ml of ferrozine color reagent and then the mixture was filtered through No.4 filter paper, transferred to a disposable semi-micro cuvette and absorbance read at 562 nm. The non-heme iron content was calculated using standard curve and expressed as µg Fe/g sample.

2.9 Determination of thiobarbituric acid reactive substance (TBARS) content

The analysis of TBARS was performed essentially according to AOAC (2000). Grounded meat (2 g) was mixed with 10 ml 0.25 N HCl (plus thiobarbituric acid (TBA) 0.375% and trichloroacetic acid (TCA) 15 %). The dispersion was heated at 100 °C for 30 min and then cooled by cooled water for 10 min. The dispersion was centrifuged at 10000 xg for 15 min at 4 °C, supernatant was transferred to a disposable semi-micro cuvette and absorbance was read at 532 nm. The TBARS content was calculated using standard curve and expressed as μg MDA/g sample.

2.10 Color measurement

The color value of breast and thigh meat with and without skin was measured as described previously in item 1.5.

2.11 Statistics analysis

A Completely Randomized Design was used to determine the effect of chicken breeds, meat types and storage times on chemical compositions, fatty acids compositions and lipid oxidation. Chemical compositions were done for 8 replicates (4 chickens x 2 determinations), fatty acid compositions, cholesterol and cholesterol oxidation products were done for 3 replicates (3 determinations) while for physical characteristics were done for 16 replicates (4 chickens x 4 determinations). Data was subjected to analysis of variance (ANOVA) and mean comparison between 3 chicken breeds and 4 storage times was carried out using Duncan's Multiple Range Test (DMRT), whereas Paired sample T-test was applied to analyze the significant difference of means between both meat types and both storage types. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows version 11.5: SPSS Inc.). The correlation between lipid oxidation and independent value present in R^2 model linear and exponential relation using the excel program (Microsoft windows 2003: Microsoft Inc.).

3. Effect of heating methods on lipid oxidation of chicken meat

3.1 Sample preparation

One hundred and sixteen thigh meat from one breed chicken which had the highest lipid oxidation selected from part 2 obtained from a commercial poultry farm in Songkhla Thailand, were slaughtered by methods mentioned by Wattanachant *et al.* (2004). Chicken rearing and preparing chicken meat samples same as described in part 1. Meat sample with skin were dissected from the carcasses after chilling and washed with tap water and drained on a plastic sieve for 10 min. All samples were divided into six portions (8 meat pieces/portion/storage times). One portion was analyzed as a raw sample, the second portion

was cooked in a 180 °C microwave oven (Arcelik MD 581, 2550 W, 230 v, 50 Hz, 2450-50 MHz) with 750 W available cooking power for 18 min one side and 7 min for the other side, the third portion was grilled by using an electrical grill (Arcelik Mini Firin ARMF 4, 1000 W, 220 v, 50 Hz) at 230 °C (the distance between heat source and the samples was 5 cm) for a total of 21 min; 15 min for one side and 6 min for the other side, the fourth portion was steamed in a top sieve on stainless steel pot for 15 min one side and 7 min for the other side, the fifth portion was boiled in a stainless steel pot for 25 min and the sixth portion was fried with deep-fat frying in 4-capacity stainless steel teflon-coated electric fryers. Electric fryers were filled with palm oil about 2 liters and refill every session of frying (4 meat pieces/session). The frying was performed at 160 °C of frying palm oil for 16 min one side and 5 min the other side. All samples were allowed to internal end point temperature at 85 °C and then individually packed into tightly sealed plastic bags and kept with at 4 °C for 15 days. The cooked meat was removed skin and trimmed of obvious fat and connective tissue before determination. The samples were taken for chemical and physical analyses at 0, 5, 10 and 15 days refrigerated storage. Meat sample of 8 chicken pieces from each cooking metohs were randomly sampled for chemical analysis minced using a domestic meat chopper (Moulinex 327, Spain) then vacuum packed and stored at -20 °C until used. While other 8 cooked meat sample from each cooking methods were randomly sampled, the middle part were cut to the size 2.0 x 2.0 x 6.0 cm for color measurement. The samples were kept on ice during preparation and analysis. Two highest lipid oxidation samples from wet and dry heating were selected to further study on part 4.

3.2 Chemical compositions of chicken meat after heating

3.2.1 Moisture content

Moisture content of samples was determined by hot air oven method (AOAC, 2000).

3.2.2 Crude lipid content

Crude lipid content was measured as described by Folch *et al.* (1957) modified by Du *et al.* (1999).

3.2.3 Fatty acid composition

Fatty acid composition was determined as mentioned in Part 1 item 1.3.

3.2.4 Cholesterol content

Cholesterol measurement was determined as mentioned in Part 1 item 1.3.

3.2.5 Lipid fraction

Lipid fraction was determined as mentioned in Part 2 item 2.4.

3.2.6 Myoglobin content

Total myoglobin content was determined as mentioned in Part 1 item 1.4.

3.2.7 Metmyoglobin formation

Metmyoglobin formation was determined as mentioned in Part 2 item 2.6.

3.2.8 Heme iron and non-heme iron content

Heme iron and non-heme iron was determined as mentioned in Part 2 item 2.7 and 2.8).

3.3 Physical characteristics of chicken meat after heating

3.3.1 Color value

Color measurement using a Hunterlab colorimeter and reported as the complete International Commission on Illumination (CIE) system color profile of Lightness (L*), redness (a*), yellowness (b*) and redness index (a*/b*).

3.3.2 Cooking loss (%)

Cooking loss was described by Murphy and Marks (2000). The cooking loss of sample was calculated from differences in the weight of raw and cooked meat strips. The measurements were conducted in eight replications. Cooking losses were calculated by applying the following equation:

$$\text{Cooking loss (\%)} = [(W_b - W_a) / W_b] \times 100$$

Where W_a = weight of thigh meat after heating in gram; W_b = weight of thigh meat before heating in gram.

3.4 Chemical compositions of cooked meat during refrigerated storage

All chemical compositions were analysed as mentioned in item 3.2

3.5 Statistics analysis

A Completely Randomized Design was used to determine the effect of heating methods and storage times on chemical compositions, fatty acids compositions and lipid oxidation. Chemical compositions were done for 8 replicates (4 chickens x 2 determinations), fatty acid compositions, cholesterol and cholesterol oxidation products were done for 3 replicates (3 determinations) while for physical characteristics were done for 16 replicates (4 chickens x 4 determinations). Data was subjected to analysis of variance (ANOVA) and mean comparison between 5 heating methods and 4 storage times was carried out using Duncan's Multiple Range Test (DMRT). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows version 11.5: SPSS Inc.).

4. Effect of heating temperatures on lipid oxidation of chicken meat

4.1 Sample preparation

Two hundred and forty chicken meat for one breed which had the highest lipid oxidation selected from part 3 obtained from a local farm in Songkhla Thailand, were slaughtered by methods mentioned by Wattanachant *et al.* (2004). Chicken rearing and preparing chicken meat samples same as described in part 1. Meat samples were heated with 2 selected heating methods (wet and dry heating, 8 pieces/method/storage time) from part 3 allowed to internal end point temperature at 75, 85 and 95 °C. Cooked meats were individually packed into tightly sealed plastic bags at 4 °C for 15 days. For cooked meat with skin, the skin was removed and all meat samples were trimmed off obvious fat and connective tissue before determination. The samples were taken for chemical and physical analyses at 0, 5, 10 and 15 days refrigerated storage. All samples were prepared and determined for chemical and physical characteristics after heating and during refrigerated storage as the same described in item 3.1-3.4.

4.2 Statistics analysis

A Completely Randomized Design was used to determine the effect of heating temperatures and storage times on chemical compositions, fatty acids compositions and lipid oxidation. Chemical compositions were done for 8 replicates (4 chickens x 2 determinations), fatty acid compositions, cholesterol and cholesterol oxidation products were done for 3 replicates (3 determinations) while for physical characteristics were done for 16 replicates (4 chickens x 4 determinations). Data was subjected to analysis of variance (ANOVA) and mean comparison between 3 heating temperatures and 4 storage times was carried out using Duncan's Multiple Range Test (DMRT). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows version 11.5: SPSS Inc.).

CHAPTER 3
RESULTS AND DISUSSION

1. Chemical and fatty acid compositions of breast and thigh meat from different chicken breeds

1.1 Proximate composition

The proximate composition and pH values of breast and thigh meats from broiler, spent hen and Thai indigenous chicken are presented in Table 7. Thai indigenous chicken meats contained significantly higher percentages of protein, but lower in moisture, fat, ash, and pH values than those of broiler and spent hen meats ($P<0.05$) when compared within meat type. Breast meat was higher in protein and moisture content ($P<0.05$) but lower in pH values, fat and ash content ($P<0.05$) as compared to thigh meat among three chicken breeds. The results were in agreement with those reported by Wattanachant *et al.* (2003) and Intarapichet *et al.* (2008). Wattanachant *et al.* (2003) studied in Southern Thai indigenous chicken while Intarapichet *et al.* (2008) studied in Northern Thai hybrid native chicken. Both researchers reported Thai indigenous chicken breast and thigh meats had higher content in protein and lower in moisture, fat and ash when compared with those of broiler. This could be due to the fact that native hybrid or indigenous chickens normally have a slower growth rate and are older than broilers and spent hens of the same weight (Choprakarn *et al.*, 1998a). Berge *et al.* (1997) reported that protein contents of the bird increased with animal age. Fat contents of leg and thigh of chicken meats were reported (Piironen *et al.*, 2002) having higher amounts compared with those of breasts which similar to the results of this work. The proximate contents found in this study were slightly different from those previously reported, probably due to differences in source of chicken supply rearing system and feeds.

1.2 Myoglobin content and color value

Myoglobin content was observed higher in spent hen breast meat than that of broiler and Thai indigenous chicken ($P<0.05$), respectively (Table 7). However, there was observed higher in myoglobin content of broiler thigh meat than that of spent hen and Thai indigenous chicken ($P<0.05$), respectively. Thigh meat contained significantly higher myoglobin than that of breast meat among three chicken breeds. The result indicated that thigh meat contained more red meat fibres than did breast meat. Myoglobin content of chicken meats found in this study was in ranges (0.1-5.0 mg/g meat) reported by Nishida and Nishida (1985). Myoglobin concentration generally depends on species, breed, sex and age of

animal, training and nature of nutrition, muscular activity, oxygen availability, blood circulation and meat type, as well as the way the meat is treated (Giddings, 1974; Livingston and Brown, 1981).

Table 7 Proximate composition, pH and color values of raw breast and broiler thigh, spent hen and Thai indigenous chicken meat

	Broiler	Spent hen	Indigenous
Breast meat			
Protein (%)	21.45 ± 3.88^{b}	20.50 ± 3.11^{c}	22.82 ± 3.94^{a}
Moisture (%)	75.59 ± 0.23^{a}	74.64 ± 0.78^{b}	73.97 ± 0.30^{b}
Crude fat (%)	0.64 ± 0.13^{b}	0.89 ± 0.19^{a}	0.06 ± 0.01^{c}
Ash (%)	1.25 ± 0.13^{a}	1.19 ± 0.25^{ab}	1.01 ± 0.19^{b}
Myoglobin (mg/g meat)	3.51 ± 0.94^{b}	3.99 ± 0.31^{a}	1.28 ± 0.20^{c}
pH	5.79 ± 0.14^{a}	5.84 ± 0.07^{a}	5.56 ± 0.03^{b}
L*	46.43 ± 3.67^{b}	50.87 ± 3.16^{a}	44.35 ± 2.08^{b}
a*	1.63 ± 0.52^{a}	-1.59 ± 0.41^{c}	1.20 ± 0.48^{b}
b*	13.31 ± 1.64^{a}	8.76 ± 1.70^{b}	8.07 ± 1.33^{c}
Thigh meat			
Protein (%)	19.42 ± 1.60^{ab}	17.58 ± 1.26^{b}	21.01 ± 2.48^{a}
Moisture (%)	75.80 ± 1.58^{a}	70.61 ± 1.25^{b}	70.51 ± 6.13^{b}
Crude fat (%)	1.87 ± 0.55^{b}	2.06 ± 0.43^{a}	0.74 ± 0.19^{c}
Ash (%)	1.18 ± 0.23^{a}	1.10 ± 0.21^{ab}	0.93 ± 0.10^{b}
Myoglobin (mg/g meat)	7.51 ± 1.60^{a}	5.17 ± 1.07^{b}	3.95 ± 0.35^{c}
pH	6.12 ± 0.07^{b}	6.23 ± 0.05^{a}	6.10 ± 0.01^{b}
L*	42.95 ± 2.85^{b}	48.70 ± 3.19^{a}	40.46 ± 4.45^{b}
a*	2.14 ± 1.56^{a}	1.50 ± 0.70^{b}	1.92 ± 1.32^{a}
b*	11.64 ± 2.76^{b}	12.37 ± 3.33^{a}	6.38 ± 1.90^{c}

Data are presented as mean ± standard deviation. N = 8 for chemical compositions, n = 16 for color values.

[a-c]Means with differing superscripts in the same row are significantly different ($P<0.05$).

Heamoglobin and myoglobin are important factors determining meat quality. These factors affect the color of meat and can cause undesirable discoloration when they exudate from muscle tissue or extravasate from the circulatory system. Sams and Jankey (1990) suggested that myoglobin and hemoglobin levels were lowest in the glycolytic meats, *Pectineus* and *P. superficialis*, and highest in the oxidative *Adductor* meat and the heart. The metabolic type of the fibres is a major factor involved in the heterogeneity of muscle quality within a carcess (Alasnier *et al.*, 2000).

The initial lightness value of breast meat had higher ($P<0.05$) than that of thigh meat among three chicken breeds, conversely, redness value of breast meat had lower ($P<0.05$) than that of thigh meat among three chicken breeds while yellowness value of breast broiler and Thai indigenous chicken meat had higher ($P<0.05$) than that of thigh meat but for spent hen, thigh meat had higher ($P<0.05$) yellowness value than that of breast meat. This was probably related to the difference in meat pH and myoglobin content between the meat types. Barbut (1993) stated that there was a significant negative correlation between meat pH and lightness of the meat. The oxidative muscles are more tasty, juicy and redder thaqn the glycolytic ones (Valin *et al.*, 1982). While the mixed type (glycolytic-oxidative) *Sartorius* and the oxidative *Adductor* differed considerably with respect to their myoglobin level, in accordance with the difference in meat type (Crow and Stockdale, 1986). The results clearly indicate that heme protein levels, especially myoglobin, had correlation with meat fiber composition. The pale fillets had significantly greater lightness values, less redness, greater yellowness, less total pigments, less myoglobin, less iron, but higher pH. The dark fillets had significantly greater total pigment, myoglobin, iron, pH, and redness values and significantly lower lightness and yellowness values (Boulianne and King, 1998).

1.3 Fatty acid composition

Fatty acid composition of broiler, spent hen and Thai indigenous chicken meats are shown in Figure 14. Breast meat among three breeds contained higher of saturated fatty acids (SFAs) ($P<0.05$) and lower content of polyunsaturated fatty acids (PUFAs) ($P<0.05$) as compared with thigh meat. There was no significant difference between breast and thigh of Thai indigenous chicken meat for monounsaturated fatty acids (MUFAs) whereas broiler and spent hen thigh meat contained a higher of MUFAs ($P<0.05$) than breast meat. Both breast and thigh Thai indigenous chicken meat had a higher SFAs ($P<0.05$) percentage that those of spent hen and broiler, while both meat of broiler had a higher content of MUFAs ($P<0.05$) than those of spent hen and Thai indigenous chicken. The higher percentage of PUFAs ($P<0.05$) was found in both meat of spent hen than those of broiler and

Thai indigenous chicken. The fatty acid profile of broiler, spent hen and Thai indigenous chicken in this study were different among breeds. In addition, the fatty acid profile of broiler and Thai indigenous chicken was not relatively similar to previously reports (Smith *et al.*, 1993, Alasnier *et al.*, 2000, Wattanachant *et al.*, 2003). However, no report on the fatty acid profiles of spent hen meat was found. As shown in Table 8, fatty acid such as C24:1 was found only in thigh meat for all chicken breeds whereas C12:0 and C17:0 were found only in breast of spent hen and Thai indigenous chicken meat. While fatty acid C22:6*n*-3 was obtained only in broiler meat. Fatty acid C16:1, C20:1 and C18:3*n*-6 were found higher content in broiler thigh meat than those of spent hen and Thai indigenous chicken while C17:0 was found highest in Thai indigenous chieken breast meat and both C18:2*n*-6 *cis* and *trans* were found highest in spent hen thigh meat. These fatty acid compositions were affected by several factors in chicken production, by extrinsic factors such as rearing system, feed diet, quality and quantity of feed consumed and body weight and by intrinsic factors in the animal such as breed, meat type, age and sex of animal which determined by the proportions of the different meat fibres (Enser *et al.*, 1996; Rhee *et al.*, 2000). In addition, the difference of fatty acid compositions between chicken meats was probably due to differences in eating behavior among chicken breeds (Wattanachant *et al.*, 2003).

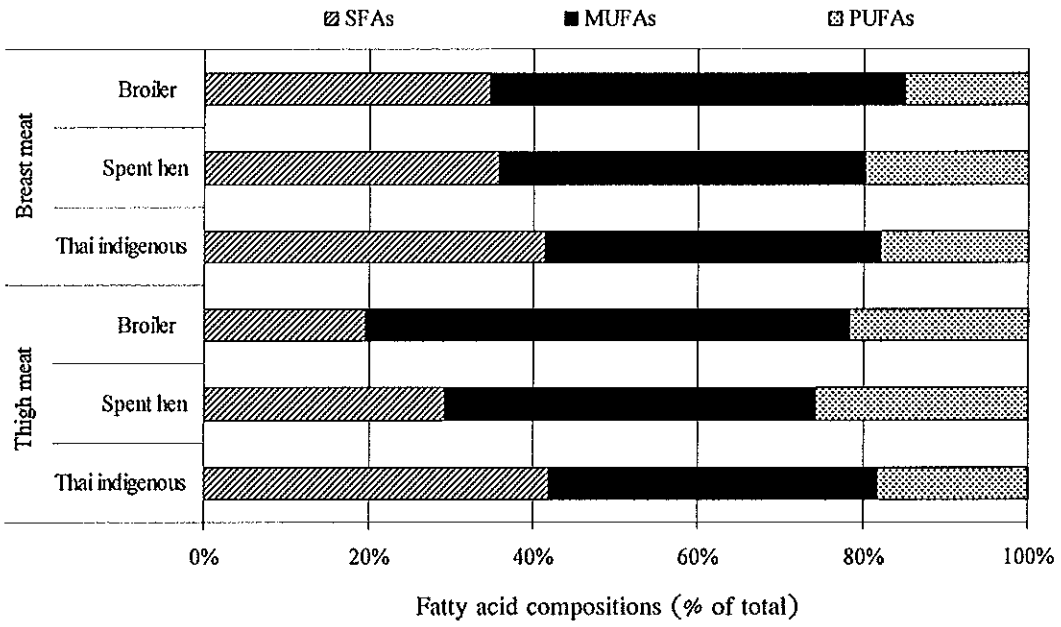


Figure 14 fatty acid compositions (% of total) of broiler, spent hen and Thai indigenous chicken breast and thigh meat.

SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids.

Table 8 Fatty acid compositions (% of total fatty acid) of breast and thigh meat from broiler, spent hen and Thai indigenous chicken

Fatty acid	Broiler		Spent hen		Thai indigenous chicken	
	Breast	Thigh	Breast	Thigh	Breast	Thigh
C12:0	0.00	0.00	9.02 ± 0.01^{b}	0.00	9.31 ± 0.01^{a}	0.00
C14:0	0.85 ± 0.00^{c}	0.49 ± 0.00^{d}	1.02 ± 0.03^{b}c	1.15 ± 0.01^{b}	1.07 ± 0.04^{bc}	2.36 ± 0.02^{a}
C16:0	28.65 ± 0.04^{a}	14.85 ± 0.06^{e}	20.30 ± 0.05^{d}	20.04 ± 0.07^{d}	21.68 ± 0.09^{c}	23.37 ± 0.07^{b}
C17:0	0.00	0.00	9.54 ± 0.01^{b}	1.17 ± 0.01^{d}	12.72 ± 0.03^{a}	2.41 ± 0.02^{c}
C18:0	5.15 ± 0.00^{cd}	4.16 ± 0.02^{d}	5.82 ± 0.01^{c}	6.84 ± 0.01^{b}	5.83 ± 0.01^{c}	13.66 ± 0.03^{a}
SFAs	**34.71 ± 0.01^{c}**	**19.41 ± 0.01^{e}**	**35.67 ± 0.05^{b}**	**29.21 ± 0.07^{d}**	**41.29 ± 0.07^{a}**	**41.80 ± 0.05^{a}**
C16:1	4.35 ± 0.00^{b}	12.21 ± 0.03^{a}	1.81 ± 0.02^{d}	1.86 ± 0.01^{d}	1.57 ± 0.04^{e}	3.77 ± 0.02^{c}
C18:1n-9c,t	45.54 ± 0.07^{a}	34.57 ± 0.13^{d}	41.51 ± 0.15^{b}	38.93 ± 0.16^{c}	38.00 ± 0.20^{c}	27.85 ± 0.09^{e}
C20:1	0.39 ± 0.00^{e}	6.34 ± 0.01^{a}	1.23 ± 0.02^{d}	2.10 ± 0.01^{c}	1.18 ± 0.04^{d}	4.25 ± 0.02^{b}
C24:1	0.00	5.78 ± 0.01^{a}	0.00	2.03 ± 0.01^{c}	0.00	4.10 ± 0.02^{b}
MUFAs	**50.27 ± 0.06^{b}**	**58.89 ± 0.17^{a}**	**44.57 ± 0.11^{e}**	**44.93 ± 0.13^{e}**	**40.75 ± 0.11^{d}**	**39.98 ± 0.04^{e}**
C18:2n-6c,t	11.05 ± 0.00^{c}	11.55 ± 0.01^{c}	13.47 ± 0.01^{b}	19.59 ± 0.03^{a}	11.66 ± 0.03^{c}	5.85 ± 0.01^{d}
C18:3n-6	0.87 ± 0.02^{d}	3.63 ± 0.03^{a}	1.26 ± 0.04^{c}	1.99 ± 0.05^{b}	0.93 ± 0.04^{d}	3.93 ± 0.02^{a}
C18:3n-3	0.91 ± 0.02^{a}c	1.94 ± 0.04^{b}	1.42 ± 0.03^{bc}	1.56 ± 0.06^{bc}	1.73 ± 0.04^{b}	3.08 ± 0.02^{a}
C20:3n-6	0.42 ± 0.02^{d}	1.20 ± 0.04^{b}	1.08 ± 0.04^{c}	0.00	1.47 ± 0.04^{a}	0.00
C20:3n-3	0.87 ± 0.02^{d}	1.16 ± 0.04^{bc}	1.29 ± 0.04^{b}	1.34 ± 0.06^{b}	1.00 ± 0.04^{c}	2.63 ± 0.02^{a}
C22:2	0.44 ± 0.02^{c}	1.17 ± 0.04^{b}	1.27 ± 0.04^{b}	1.39 ± 0.06^{b}	1.18 ± 0.04^{b}	2.73 ± 0.02^{a}
C22:6n-3	0.52 ± 0.02^{b}	0.97 ± 0.04^{b}	0.00	0.00	0.00	0.00
PUFAs	**15.08 ± 0.11^{e}**	**21.61 ± 0.25^{b}**	**19.79 ± 0.16^{b}**	**25.87 ± 0.19^{a}**	**17.96 ± 0.18^{d}**	**18.22 ± 0.09^{c}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

[a-e]Means with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

1.4 Cholesterol

Cholesterol content of breast and broiler thigh, spent hen and Thai indigenous chicken meats are shown in Table 9. There was significant difference in cholesterol content between breast and thigh meat from three breeds. The result showed that thigh meat contained a higher (P<0.05) cholesterol as compared with breast meat. Breast and broiler thigh meat had higher cholesterol content than those of spent hen and Thai indigenous chicken meat. In meat, the cholesterol contents in meat ranged from 45 to 84 mg/100 g; which for pork, beef and chicken meats were in range of 45-54, 52-55 and 56-84 mg/100 g, respectively (Piirone *et al.*, 2002). Intarapichet *et al.* (2008) reported the breast of native chicken breeds contained similar amounts of cholesterol and lower than those of broiler. In contrast to red meat, Piirone *et al.* (2002) reported that two chicken meat types differed markedly in their cholesterol levels (56 and 84 mg/100g meat; breast and thigh met). Different methods for cholesterol determination, sampling and trimming of adipose tissue may be responsible for differences between the various published values (Chizzolini *et al.*, 1999). As reviewed by Chizzolini *et al.* (1999) and Piirone *et al.* (2002), the magnitude of variation of the cholesterol contents of different species and muscles appears to be low. Instead, significant differences have been reported between muscle types due to differences in their fiber types. This might be the most likely reason for some of the variations observed in the cholesterol contents between different muscles of the same species and between the same muscles in different species (Chizzolini *et al.*, 1999).

Table 9 Cholesterol content (mg/g lipid) of breast and thigh meat from broiler, spent hen and Thai indigenous chicken

Chicken breed	Meat type	Cholesterol (mg/g lipid)
Broiler	Breast	75.08 ± 0.51^{b}
	Thigh	105.02 ± 1.10^{d}
Spent hen	Breast	52.98 ± 0.92^{b}
	Thigh	83.34 ± 0.49^{c}
Thai indigenous chicken	Breast	17.57 ± 0.47^{a}
	Thigh	66.54 ± 0.43^{b}

Data are presented as mean ± standard deviation. N = 3.

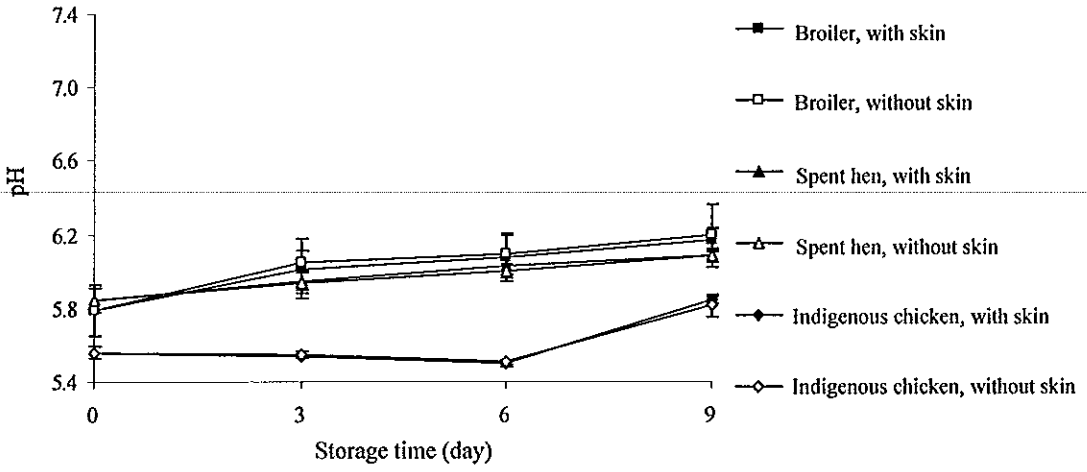
[a-d]Means with differing superscripts in the same column are significantly different (P<0.05).

2. Changes in chemical and fatty acid compositions and heme pigment of breast and thigh meat from different chicken breeds during refrigerated storage

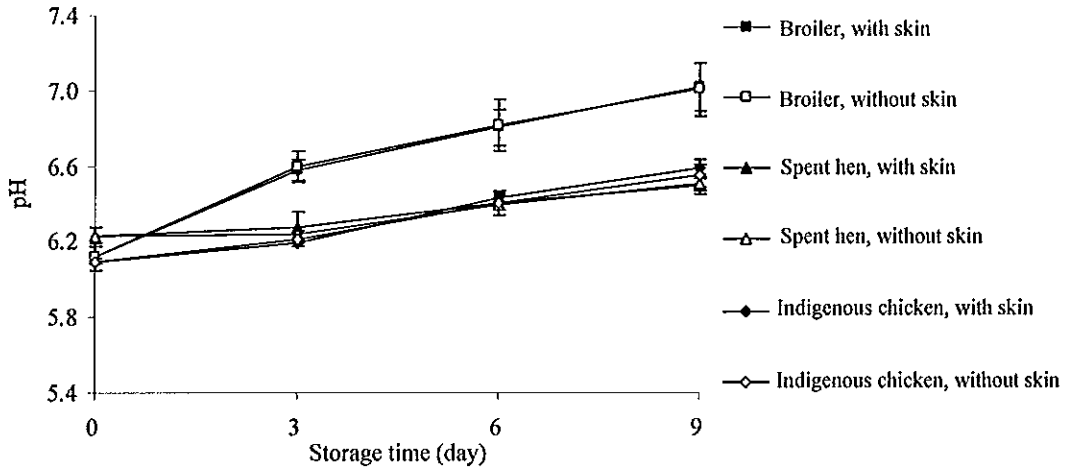
2.1 Changes in muscle pH

As shown in Figure 15 and 16, the initial pH between breast and thigh meat was different. Thigh meat had higher pH than breast meat ($P<0.05$). Muscle glycogen at the time of slaughter is the main metabolic fuel for the anaerobic glycolysis that takes place postmortem when muscles are no longer supplied with oxygen. Accumulation of lactate (from conversion of glycogen to lactic acid) and H^+ ions (from hydrolysis of adenosine triphosphate, ATP) in the tissue cause a decline in pH when muscle is converted to meat. Thus, in mammals and poultry, the pH of meat chiefly depends on the muscle glycogen content at time of slaughter (Bendall, 1973). However, the pH decline can stop, even in the presence of high residual glycogen content (Lawrie, 1955; Bendall, 1973). According to Sahlin (1978) and Young *et al.* (2004), glycolytic enzymes are probably inactivated when the pH reaches a low value (acidic conditions). This would suggest that the biochemical determinism of pH in poultry species differs from that in mammals. This could be because broilers of different genetic types had different metabolic activities and different perimortem glycogen content and ATP stores. It is generally assumed that the pH of meat is dependent on glycogen concentration at the time of slaughter. In fasting broiler chickens, high pH in broiler pectoralis muscle was caused by low glycogen concentration immediately postmortem (Bendall, 1973).

A gradual increase in the pH was observed in thigh and breast meat of broiler and spent hen during refrigerated storage with and without skin for 9 days ($P<0.05$) (Figure 15 (a) and (b)). While those of Thai indigenous chicken meat was rather unchange for 6 days storage and then increased ($P<0.05$) sharply at 9 days. It was presumable that Thai indigenous chicken meat had higher buffering capacity than the other two chicken breeds. The increase in muscle pH during storage depended also on the liberation of inorganic phosphate and ammonia due to the enzymatic degradation of ATP (Sikerski *et al.*, 1990). Moreover, it was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes (Chaijan *et al.*, 2005). Also, the decomposition of nitrogenous compounds caused an increase in pH in muscle (Greaser, 1986). Puolanne and Kivikari (2000) mentioned that the change in muscle pH depended on a variety of factor such as species, handling, feeding, storage temperature and buffering capacity of meat. The change of pH in breast and thigh meat of both storages was different among three chicken breeds but no significant difference of pH value between meat storage with and without skin.



(a)



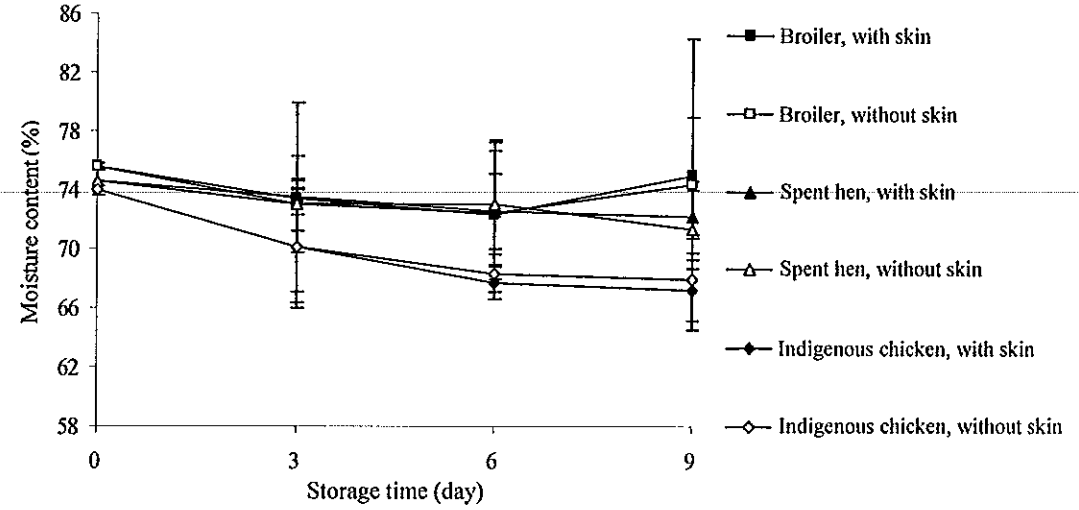
(b)

Figure 15 Change in pH values of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.

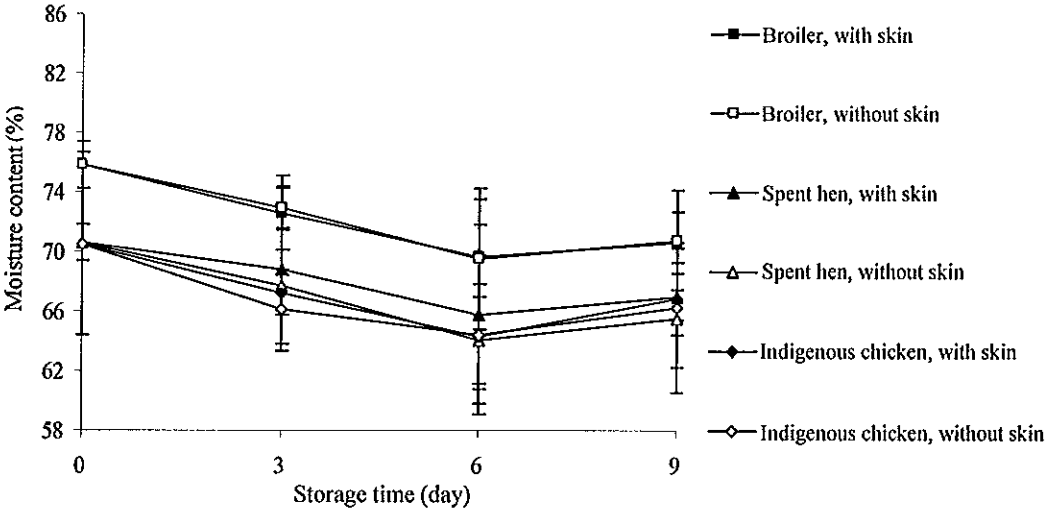
Moreover, the pH value of meat also is influenced not only by lactic acid but also by phosphoric acid, high-powered ions (Šimek *et al.*, 2003), H^+ ions from hydrolysis of adenosine triphosphate (ATP) in the tissue (Rammouz *et al.*, 2004) and a different buffering capacity of meat (Šimek *et al.*, 2003). During storage, breast meat of three chicken breeds tended to slightly change in muscle pH when compared with those of thigh meat (Figure 15 (a) and (b)). This is consistent, because they are comprised primarily of white muscle fibers, which have a high content of glycolytic enzymes. The end product of glycolytic metabolism is lactic acid, which tends to lower the pH. Thus, white fibers need a more effective buffering mechanism than red ones (Puolanne and Kivikari, 2000). Besides, the pH value is also influenced by other acids, especially free amino acids (Šimek *et al.*, 2003).

2.2 Changes in moisture and lipid content

The initial moisture content of breast meat was not significant difference among three chicken breeds similarly to that of spent hen meat and Thai indigenous chicken thigh meat while the highest moisture content was observed in broiler thigh meat. As shown in Figure 16 (a) and (b), the moisture content of all meat samples sharply decreased ($P<0.05$) during first 6 days storage ($P<0.05$) excepted for those of breast spent hen and Thai indigenous chicken meat that gradually decreased until 9 days. No significant difference in moisture change of breast and thigh chicken meats between storage with and without skin was observed. The moisture content of breast meat was higher ($P<0.05$) than that of thigh meat for spent hen and Thai indigenous chicken whereas for broiler meat that was no significantly difference between both meats. The lipid contents of three chicken breeds thigh meat were higher than those of breast meat as presented in Figure 17 (a) and (b). The lowest lipid content was found in Thai indigenous chicken and the highest content was found in spent hen both breast and thigh meats. When extended storage time, lipid content of broiler and spent hen breast and thigh increased with storage time during 6 days and then decreased at 9 days while those of Thai indigenous chicken breast and thigh meats were rather unchange during first 3 days of storage but increased at the day after until 6 days and then stable until 9 days of storage (Figure 17 (a) and (b)). No significant difference in lipid content between meat storage with and without skin was obtained. The change of lipid content of chicken meat was observed conversely to the change of moisture content during refrigerated storage for 9 days. The decrease of moisture content due to drip loss occurred in meat during refrigerated storage and resulted in the increase in lipid content when determined based on wet basis.

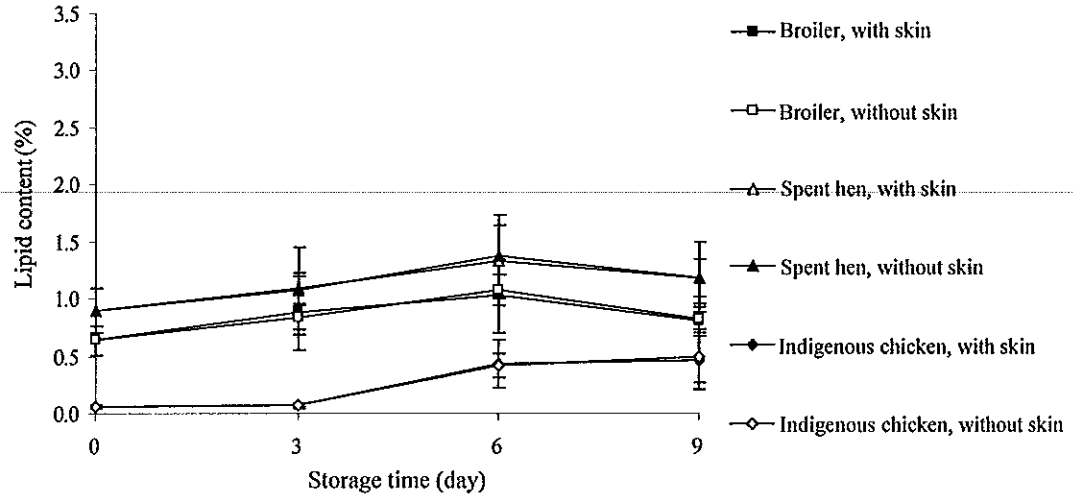


(a)

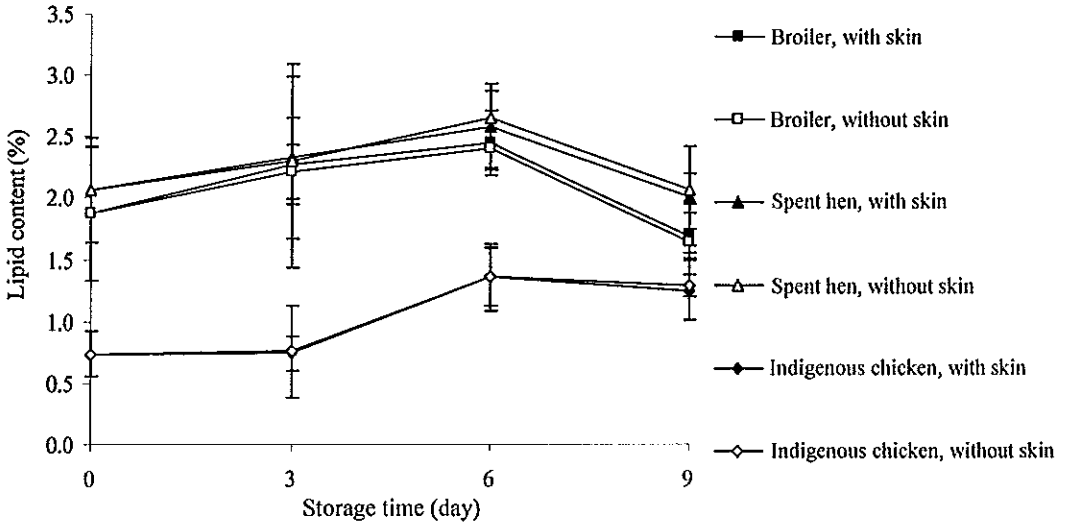


(b)

Figure 16 Change in moisture content (%) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.



(a)



(b)

Figure 17 Change in lipid content (%) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.

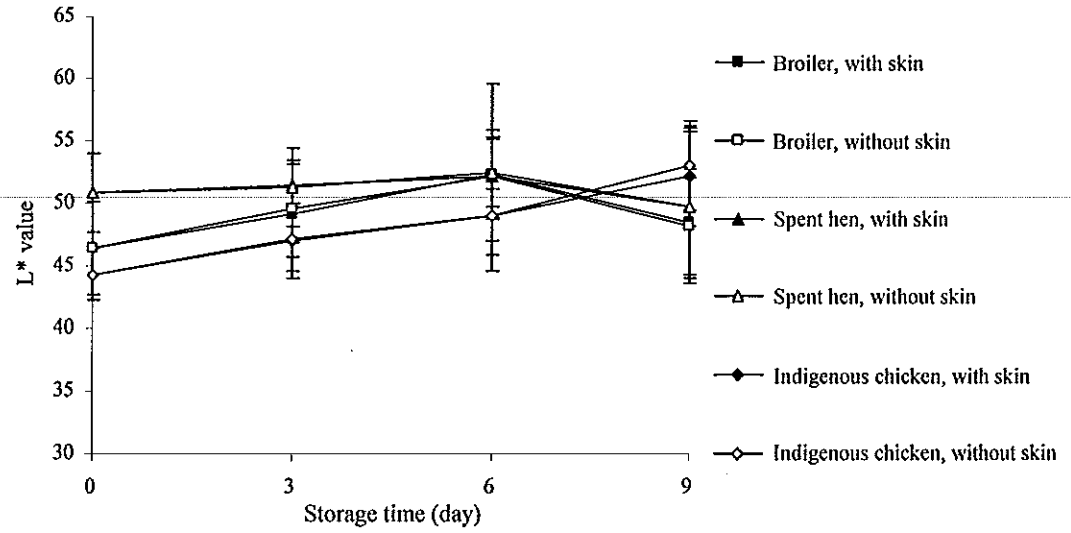
2.3 Change in color and redness index (a*/b*)

The change in color L*, a* and b* values of broiler, spent hen and Thai indigenous chicken breast and thigh meat are showed in Figure 18-21, respectively. The lightness (L*) value and yellowness value (b*) of all chicken meats tended to increase when extended storage time, while the redness (a*) tended to decreased. The oxidation of myoglobin to form metmyoglobin resulted to discolor of chicken meat during storage. Haard (1992) reported that discoloration of tuna during frozen storage is caused by the formation of metmyoglobin.

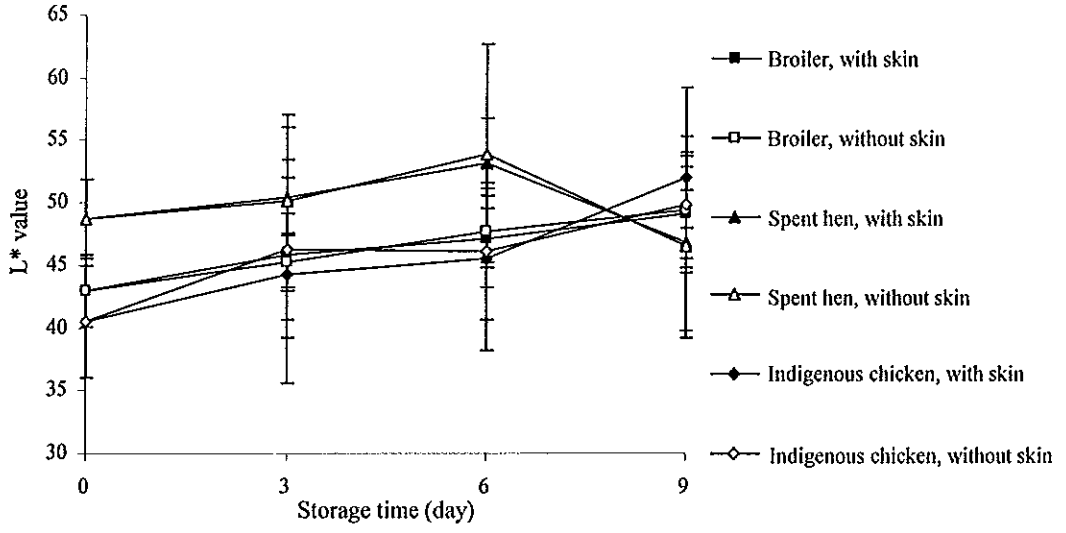
The redness index (a*/b* ratio) of breast and broiler thigh, spent hen and Thai indigenous chicken meat decreased when the storage time increased (Figure 27 and 28). The redness index of breast meat had lower ($P<0.05$) than that of thigh meat for the three chicken breeds. Notably, the lowest redness index was found in spent hen meat and the highest was found in Thai indigenous chicken meat. The ratio was used as an index of apparent change in redness (Chaijan *et al.*, 2005) and used to more efficiency approach to evaluate the discoloration in meat during storage than consider only L*, a* or b* value.

2.4 Changes in myoglobin content

As shown in Figure 22 (a) and (b), thigh meat of three chicken breeds contained much greater myoglobin content than that of breast meat ($P<0.05$). The result indicated that thigh muscle contained more red muscle fibres than did breast muscle. For broiler thigh meat of both storage with and without skin contained higher myoglobin than ($P<0.05$) those of thigh spent hen and Thai indigenous chicken, respectively. There was no significant difference in myoglobin content between broiler and spent hen breast meat while the lowest myoglobin content ($P<0.05$) was observed in Thai indigenous chickens meat. The myoglobin content markedly decreased when storage time extended in concomitance with the reduction of redness index especially for broiler thigh and breast meat (Figure 21). However, for those of spent hen and Thai indigenous chicken breast meat, slight change was observed during storage.

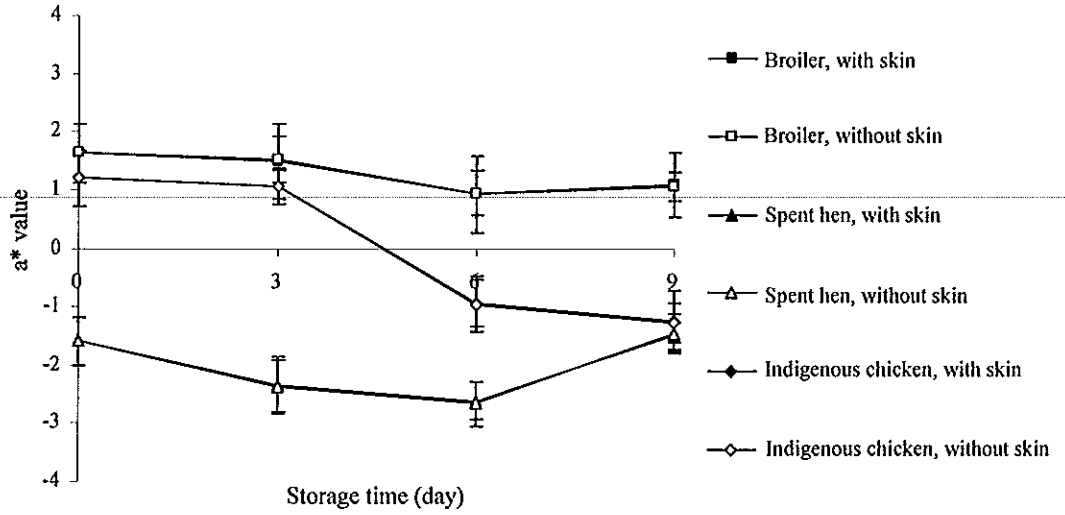


(a)

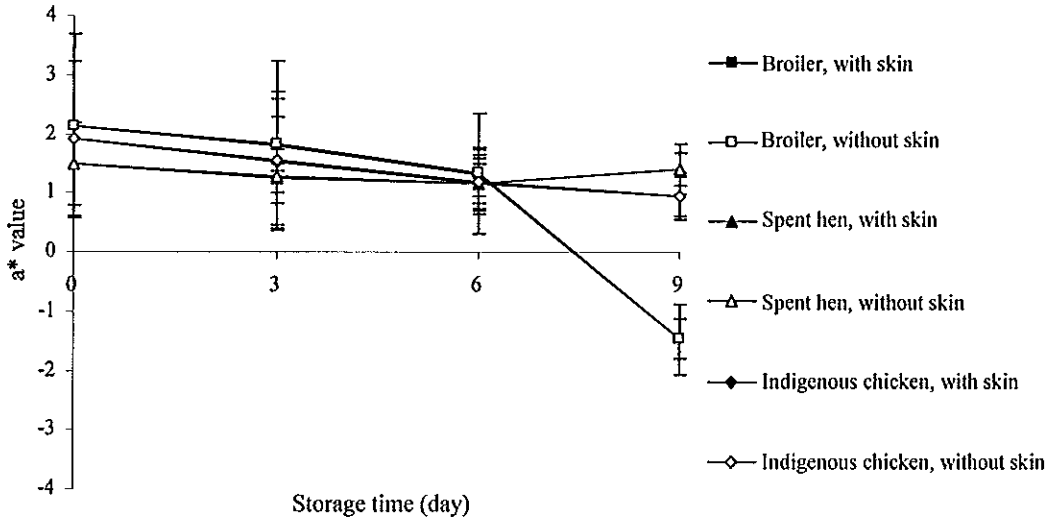


(b)

Figure 18 Change in L* value of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from sixteen replicate determinations.

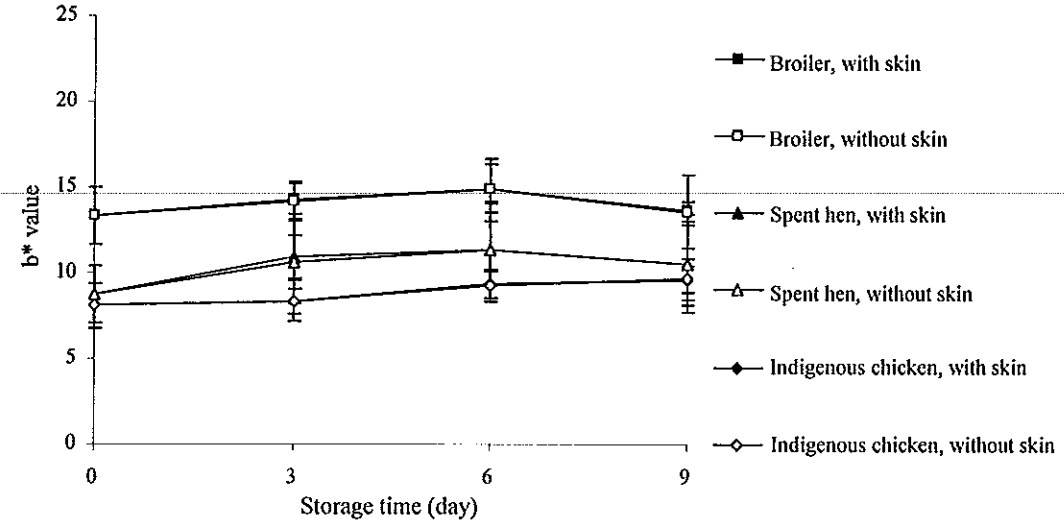


(a)

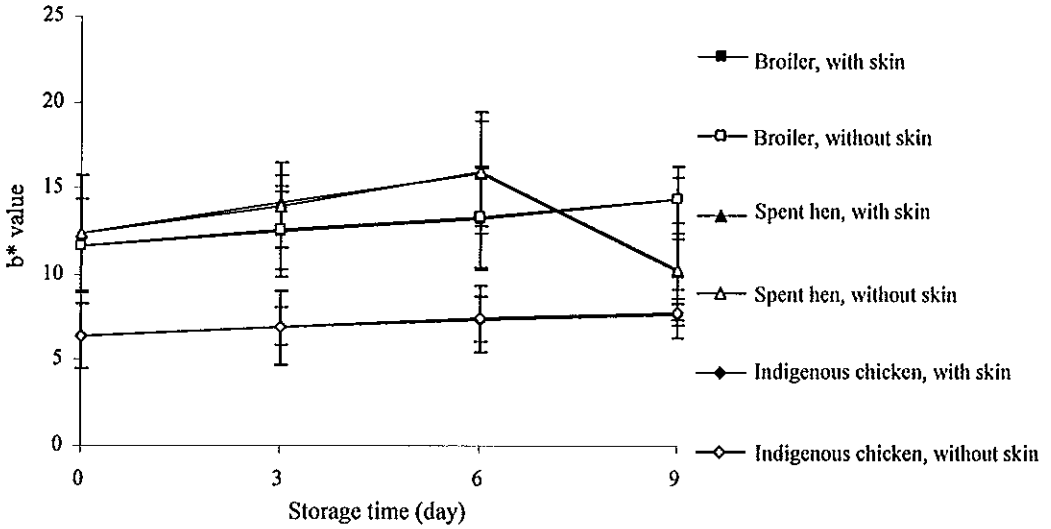


(b)

Figure 19 Change in a* value of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from sixteen replicate determinations.

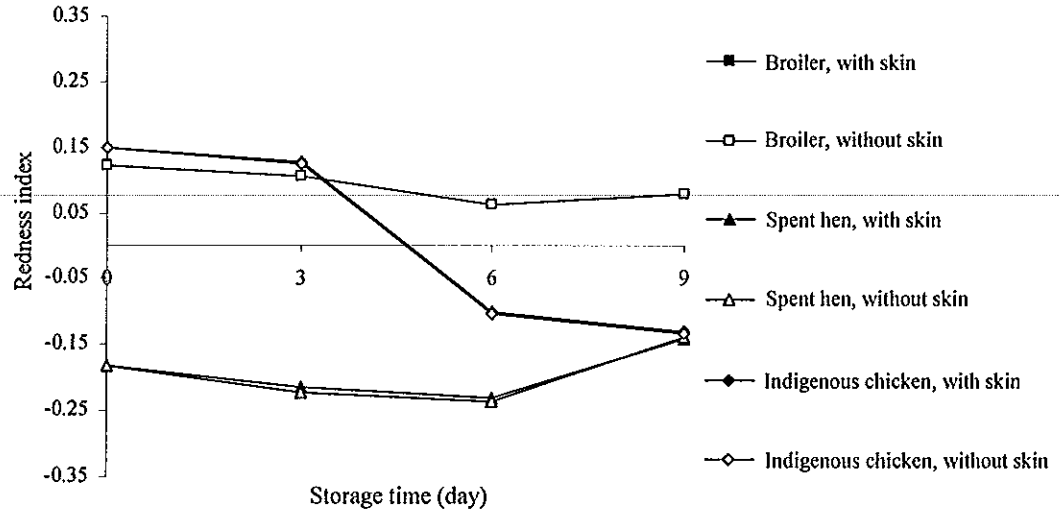


(a)

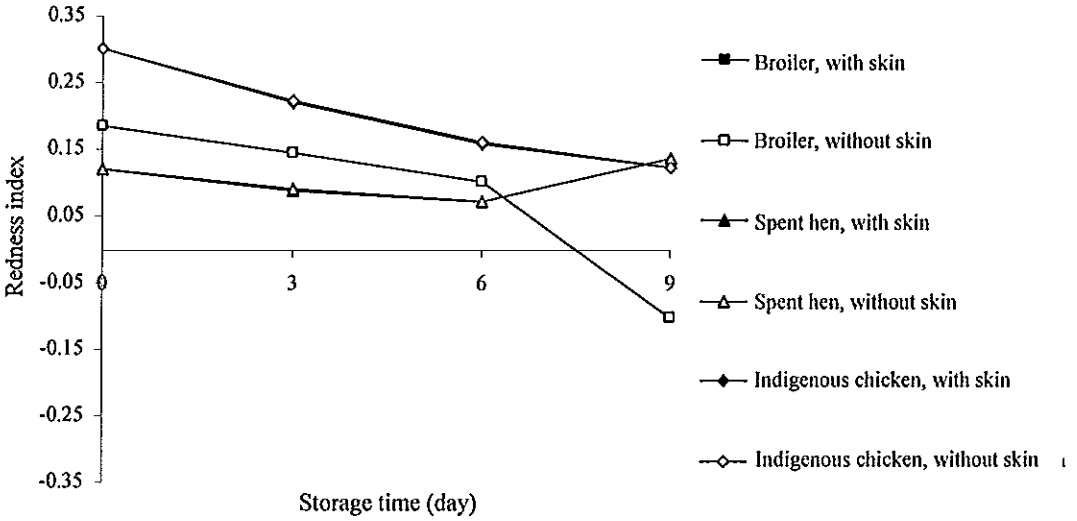


(b)

Figure 20 Change in b* value of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from sixteen replicate determinations.



(a)



(b)

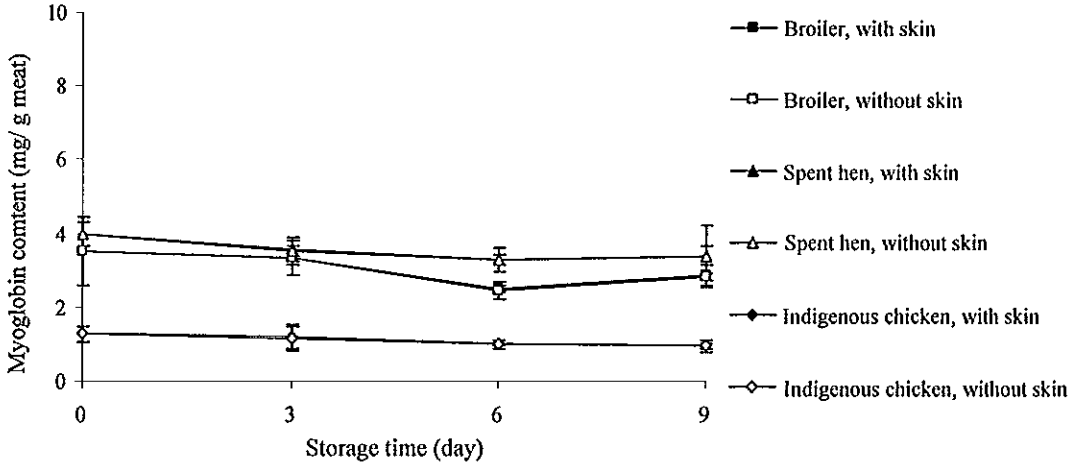
Figure 21 Change in redness index (a*/b*) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from sixteen replicate determinations.

The decrease in myoglobin content with extended storage time was presumably that the pigment possibly underwent oxidation or denaturation during storage, leading to the higher pigment content remaining or bound in the muscle (Chaijan *et al.*, 2005). Insolubility and binding of oxidized myoglobin to the muscle resulted in less removal of myoglobin when drip loss occurred (Chen, 2003). This phenomenon can be influenced by many factors such as pH, temperature, ionic strength and oxygen consumption reaction (Renerre and Labas, 1987). Metmyoglobin formation is positively correlated with the lipid oxidation (Chan *et al.*, 1997; Lee *et al.*, 2003), which associated with the higher lipid oxidation in dark muscle, which had a high fat content (Chaijan *et al.*, 2005). Myoglobin and other heme compounds at high concentration in dark muscles function as pro-oxidants in muscle tissue (Love, 1983). Furthermore, metmyoglobin forms cross-linkages with myosin in the presence of hydrogen peroxide (Hanan and Shaklai, 1995).

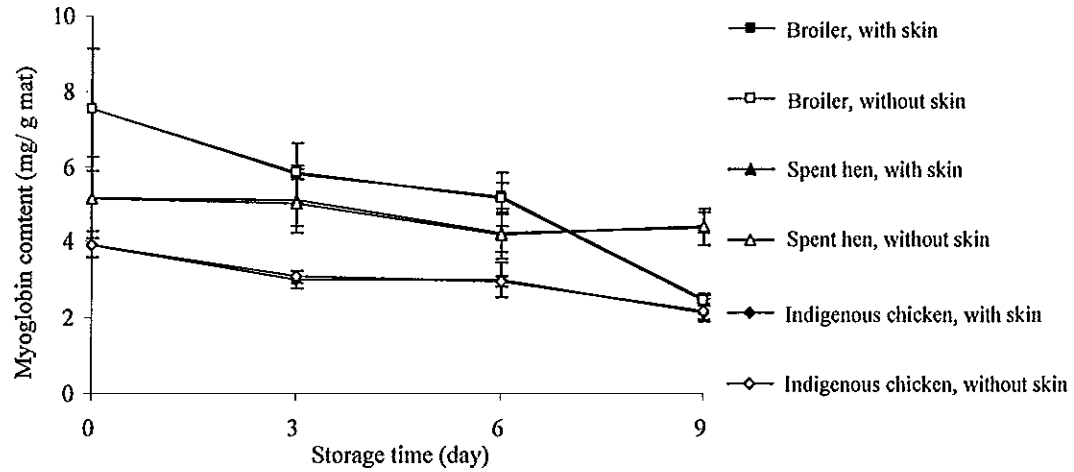
2.5 Changes in metmyoglobin formation

The formation of metmyoglobin in breast and thigh meat of broiler, spent hen and Thai indigenous chicken are shown in Figure 23 (a) and (b). No significantly difference in metmyoglobin formation was found in meat stored with and without skin during 9 days. For broiler breast and thigh meat, a marked higher metmyoglobin at the initial storage as compared with those of spent hen and Thai indigenous chicken meat. All samples were observed increase in the metmyoglobin formation when extended storage time. The sharp increase in metmyoglobin formation with extended storage time suggested that myoglobin underwent more oxidation (Benjakul and Bauer, 2001). In fresh meat, reducing substances such as NAD^+ or FAD^+ are endogenously produced, and they are responsible for the constant reduction of the brown-gray metmyoglobin or the purple myoglobin (Eder, 1996). Metmyoglobin reductase remaining in the muscle might reduce metmyoglobin to other forms. From the result, the metmyoglobin formation in thigh meat of three chicken breeds tended to increase more rapidly than those in breast meat. This might be due to the lower metmyoglobin reductase in ordinary or white muscle. It has been known that metmyoglobin reductase is a component of red blood cells (Al-Shaibani *et al.*, 1977). Since some blood was retained in the muscle, especially dark muscle, residual activity of this enzyme could be present and could result in the retardation of color deterioration. Distribution and localisation of myoglobin in both muscles might also be different, leading to the different susceptibility of myoglobin to oxidation. Many factors have been known to increase myoglobin oxidation, including pH, salt concentration and species (Trout, 1990). Surprisingly, the metmyoglobin formation in broilers and spent hens breast meat was reduced at the end of storage time (days 9). This might be due

to the rapid deterioration of subcellular organelles, including the mitochondria, of this muscle, resulting in the release of pyridine nucleotides such as NAD(P)H that might be involved in metmyoglobin reduction (Chan *et al.*, 1997).

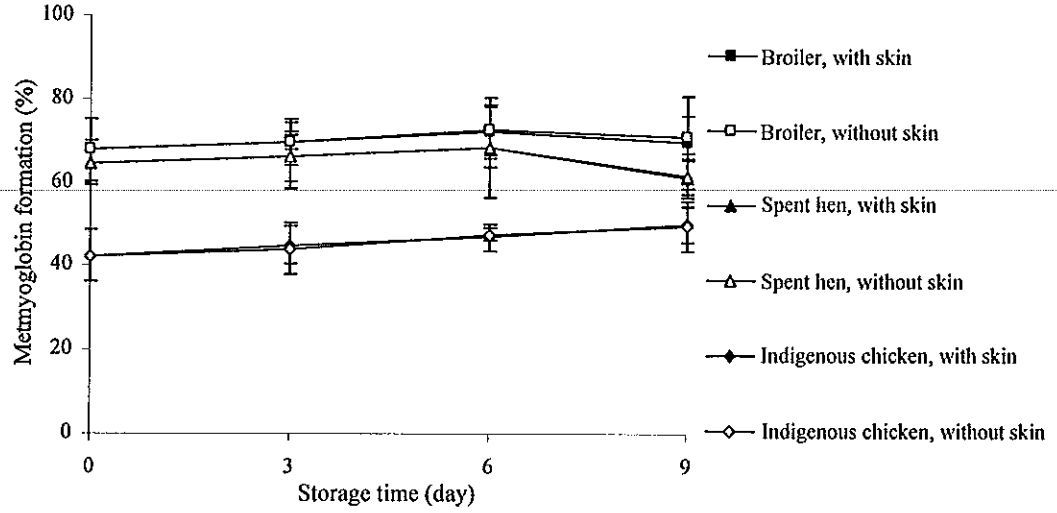


(a)

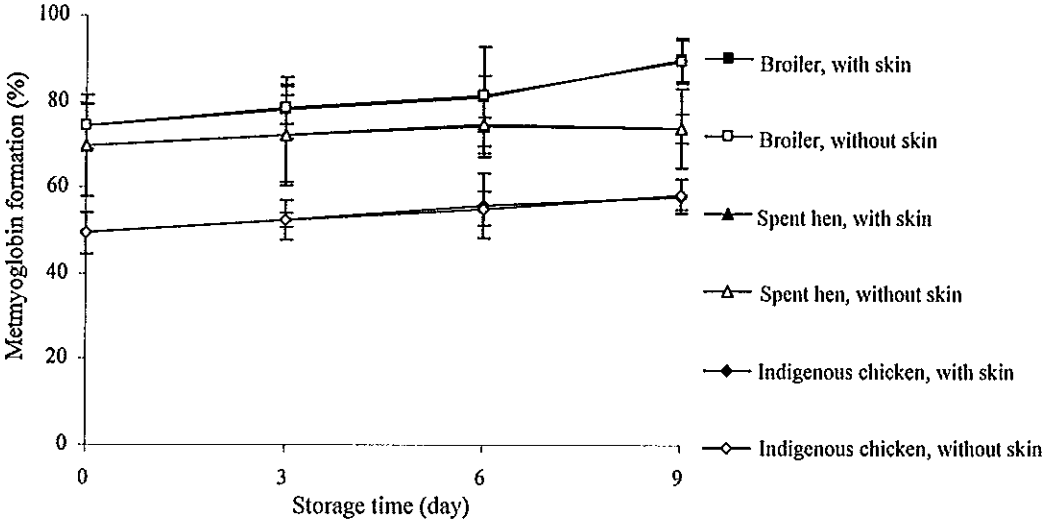


(b)

Figure 22 Change in myoglobin content (mg/g meat) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.



(a)



(b)

Figure 23 Change in metmyoglobin formation (%) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.

2.6 Changes in heme and non-heme iron content

For breast meat, initial heme iron content of 5.61, 3.09 and 3.64 μg/g meat were found for broiler, spent hen and Thai indigenous chicken, respectively and 13.20, 19.79 and 16.89 μg/g meat were obtained for those of thigh meat, respectively. As shown in Figure 24, thigh meat from three chicken breeds had a higher heme iron content than did breast meat. Broiler breast meat had greater heme iron content than those of spent hen and Thai indigenous chicken meat during storage with and without skin. Conversely, lower heme iron content was observed in broiler thigh meat, compared with those of spent hen and Thai indigenous chicken meat. The presence of larger amounts of iron in the dark muscle reflected higher contents of haemoglobin and myoglobin, as well as mitochondrial, iron-containing enzymes (Dulavik *et al.*, 1998). Kongkachuichai *et al.* (2002) reported that chicken breast and drumsticks contained small amounts of heme iron is 1 and 3 μg/ g edible portion, respectively.

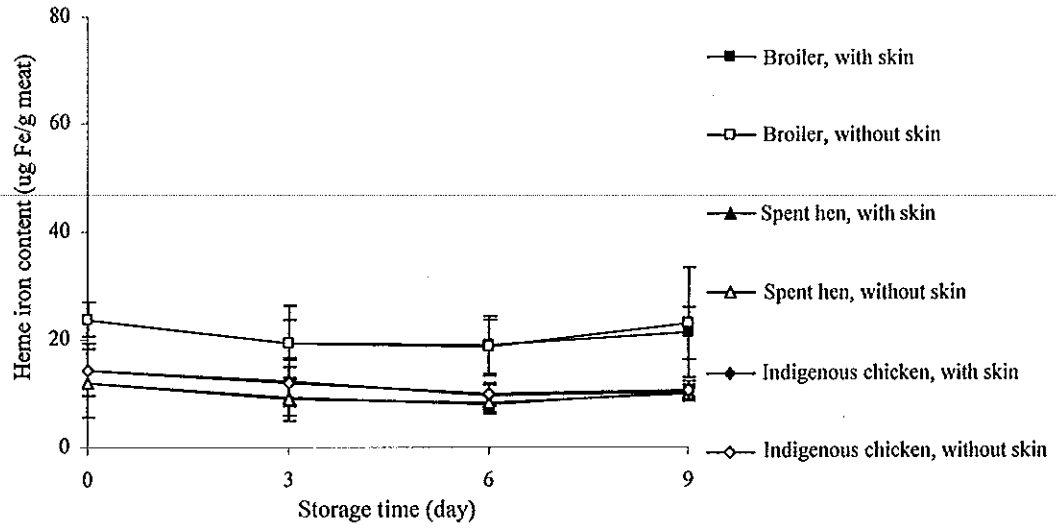
During storage from 0 to 9 days, heme iron content of three chicken breeds breast meat had a slightly decreasing when compared to those of thigh meat (Figure 24). The first 6 days of storage, the heme iron content sharply decreased ($P<0.05$) in thigh meat of broiler and spent hen while those of Thai indigenous chicken was observed gradually decreased. No significantly difference of heme iron content between meat storage with and without skin. The decrease in heme iron content on first storage time was presumably due to the release of free iron from heme (Chaijan *et al.*, 2005) and may be some heme iron was soluted with water when drip loss occurred. The increase in heme iron content observed with extended storage time was presumably that the pigment possibly underwent oxidation or denaturation during storage.

The changes in non-heme iron content in broiler, spent hen and Thai indigenous chicken breast and thigh meat are shown in Figure 25. Initial content of non-heme iron found in fresh broiler, spent hen and Thai indigenous chicken breast meat were 0.32, 0.26 and 0.33 μg Fe/g meat, respectively, while in thigh meats were 0.42, 0.52 and 0.43 μg Fe/g meat, respectively. Generally, red muscle contained higher amounts of non-heme iron than white muscle. Total, heme and nonheme iron contents in meat differ among species and vary with the type of tissue (Kongkachuichai *et al.*, 2002).

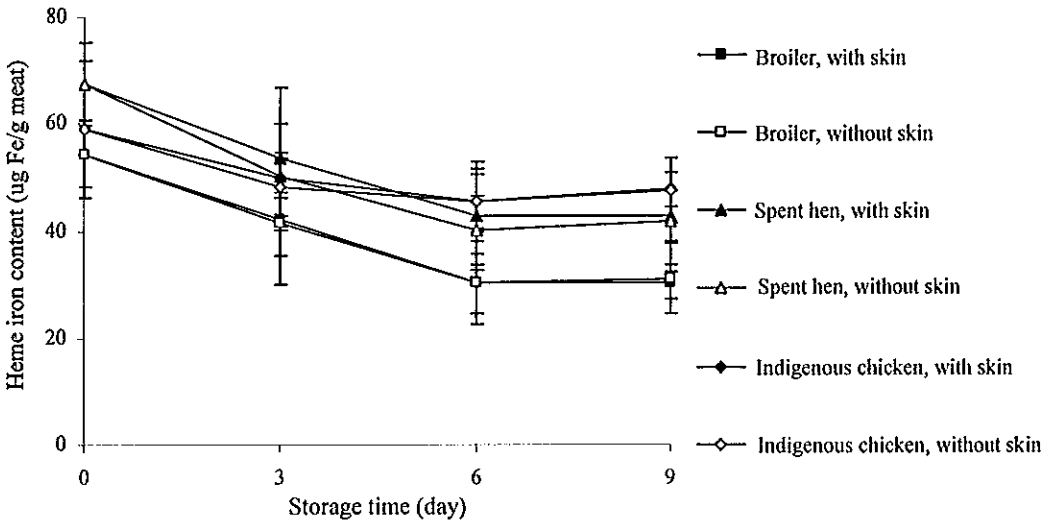
Kongkachuichai *et al.* (2002) reported that chicken breast and drumsticks contained non-heme iron for 3 and 6 μg/g edible portion, respectively. Broiler breast meat had a greater ($P<0.05$) non-heme iron content than those of spent hen and Thai indigenous chicken meat, respectively while thigh meat of spent hen had a greater ($P<0.05$) non-heme iron content than those of broiler and Thai indigenous chicken meat. The results indicated that

the non-heme iron content of chicken meat corresponding to heme iron content. Spent hen breast meat had the marked increase in non-heme iron content while those of the other chicken breeds had a slight changing.

Hazell (1982) reported that iron was distributed between five components, including insoluble fraction, ferritin, haemoglobin, myoglobin and a low-molecular-weight fraction. Schricker *et al.* (1982) reported that total iron, heme iron and nonheme iron concentrations were significantly different between species and muscle types. The differences may relate to inherent differences in residual blood between white and red muscles in normal post mortem muscle (Schricker *et al.*, 1982). The increment of non-heme iron content might be due to the much greater release of free iron from the muscle which was extensively degraded. Muscle proteins undergo degradation with increasing storage times in refrigerate. The results suggested that the heme pigments, or other iron-containing proteins, are possibly denatured with increasing storage time, resulting in the release of iron. The denaturation of those components possibly contributes to the increase in non-heme iron content (Decker and Hultin, 1990a, 1990b). Decker and Hultin (1990a, 1990b) reported that the deterioration of subcellular organelles, e.g. mitochondria, and the release of cytochrome c, could be responsible for the increase in soluble hemin. But the released iron might bind tightly with the muscle components, which were denatured and easily interacted with those free irons, caused to non-heme iron content decreased on extended storage times in some samples (Schricker *et al.*, 1982).

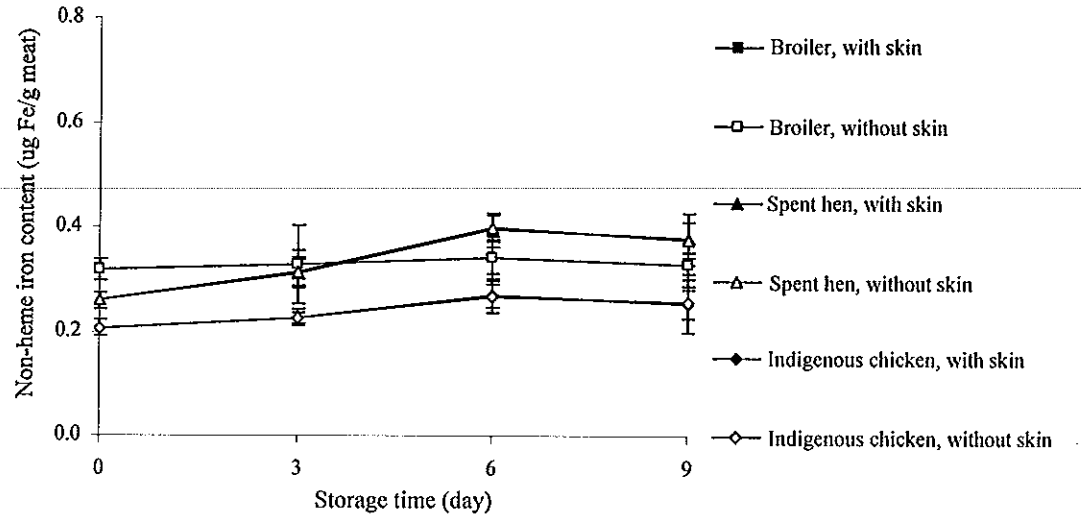


(a)

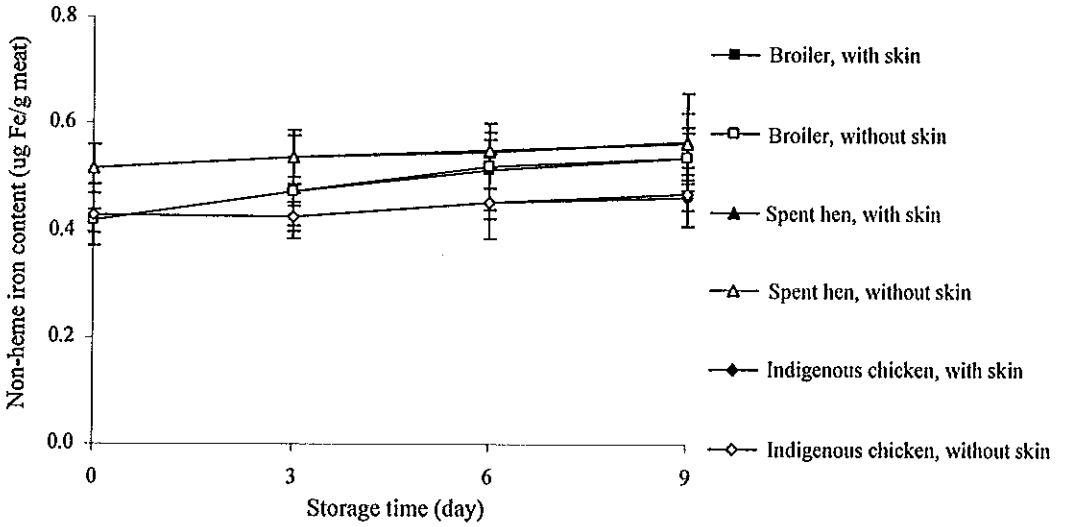


(b)

Figure 24 Change in heme iron content (μg Fe/g meat) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.



(a)



(b)

Figure 25 Change in non-heme iron content (μg Fe/g meat) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.

2.7 Change in lipid fractions and cholesterol content

The lipid fractions of broiler, spent hen and Thai indigenous chicken breast and thigh meat are shown in Table 10. Triglyceride, phospholipids and free fatty acid content in Thai indigenous chicken was not significantly different between breast and thigh meat. While triglyceride and free fatty acid content of broiler thigh meat had higher ($P<0.05$) than those of breast meat, conversely, phospholipids content of breast meat was higher ($P<0.05$) than that of thigh meat. Phospholipids and free fatty acid of spent hen breast meat had higher ($P<0.05$) content than those of thigh meat while triglyceride content of thigh meat was higher ($P<0.05$) than that of breast meat.

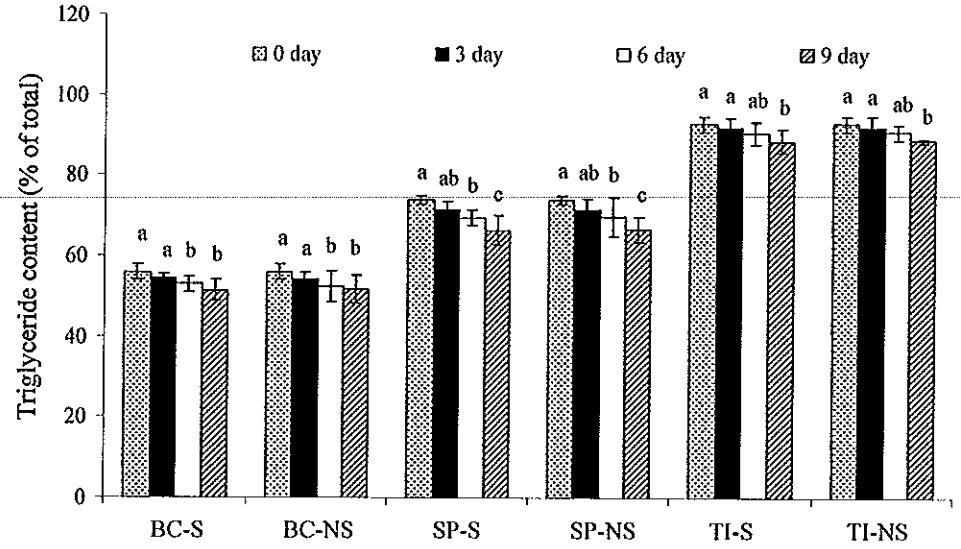
Table 10 Lipid fraction (% of total) of raw breast and thigh meats from broiler, spent hen and Thai indigenous chickens

	Broiler	Spent hen	Indigenous
Breast meat			
Triglyceride	55.92 ± 1.93^{c}	73.83 ± 1.02^{b}	92.78 ± 1.95^{a}
Phospholipids	36.61 ± 2.16^{a}	21.03 ± 1.71^{b}	5.71 ± 2.13^{c}
Free fatty acid	7.47 ± 1.68^{a}	5.14 ± 1.53^{b}	1.51 ± 0.31^{c}
Thigh meat			
Triglyceride	60.86 ± 5.46^{b}	90.34 ± 2.62^{a}	92.59 ± 1.52^{a}
Phospholipids	27.12 ± 5.41^{a}	7.72 ± 2.67^{b}	6.06 ± 1.34^{b}
Free fatty acid	12.02 ± 3.96^{a}	1.94 ± 0.20^{b}	1.35 ± 0.32^{b}

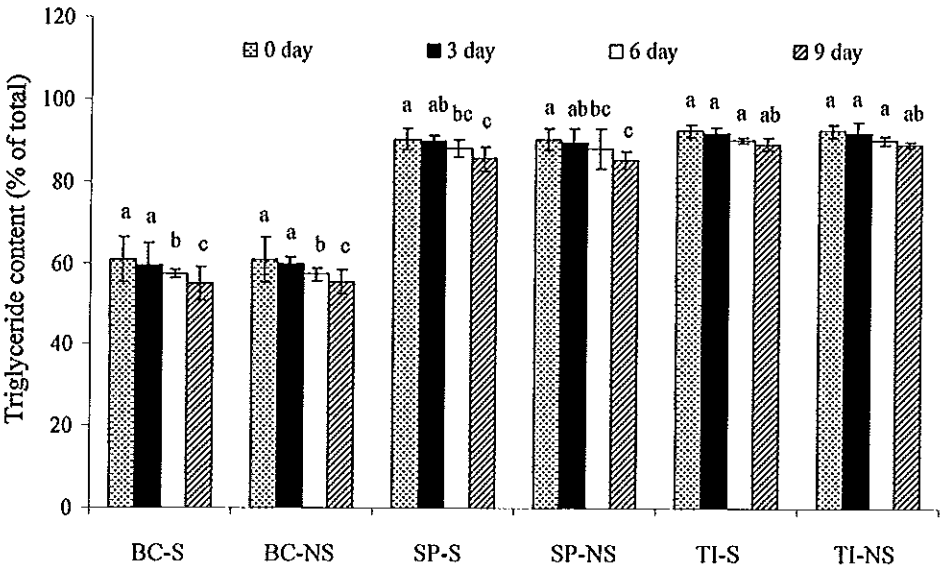
Data are presented as mean ± standard deviation.

N = 8 for lipid fraction, n = 3 for cholesterol.

$^{a-c}$Means with differing superscripts in the same row are significantly different ($P<0.05$).

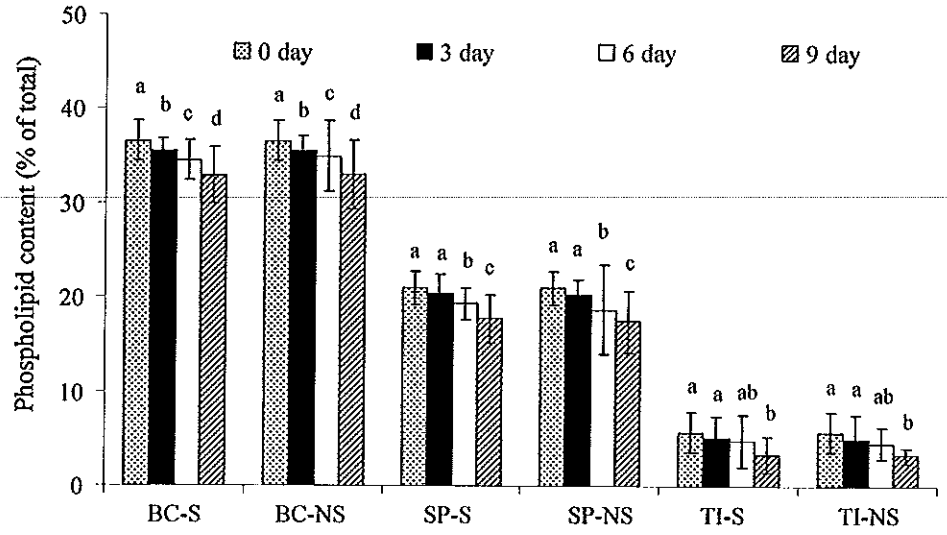


(a) Breast meat

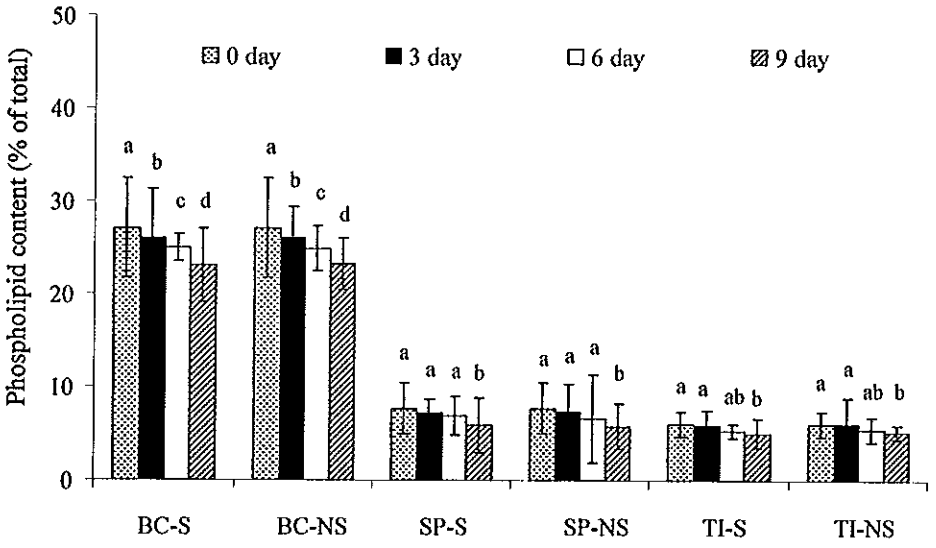


(b) Thigh meat

Figure 26 Change in triglyceride content (% of total) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations. Different letters under the same breeds and meat types indicate significant differences ($P<0.05$). BC-S: Broiler-with skin storage; BC-NS: Broiler-without skin storage; SP-S: Spent hen-with skin storage; SP-NS: Spent hen-without skin storage; TI-S: Thai indigenous-with skin storage; TI-NS: Thai indigenous-without skin storage.

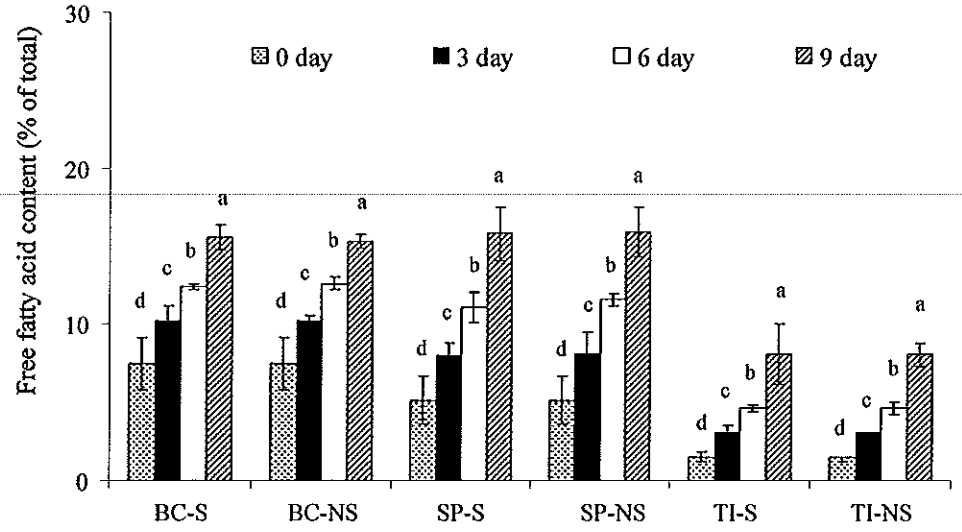


(a) Breast meat

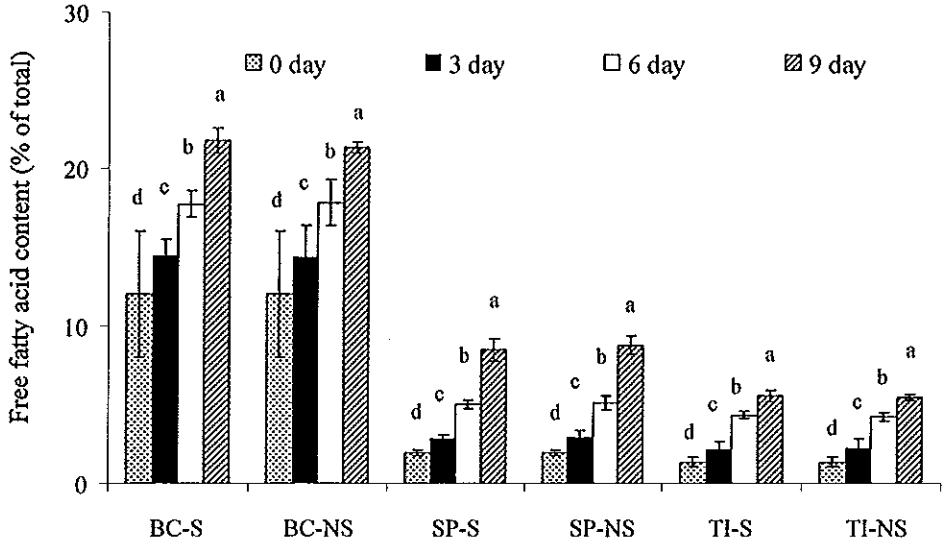


(b) Thigh meat

Figure 27 Change in phospholipids content (% of total) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations. Different letters under the same breeds and meat types indicate significant differences ($P<0.05$). BC-S: Broiler-with skin storage; BC-NS: Broiler-without skin storage; SP-S: Spent hen-with skin storage; SP-NS: Spent hen-without skin storage; TI-S: Thai indigenous-with skin storage; TI-NS: Thai indigenous-without skin storage.

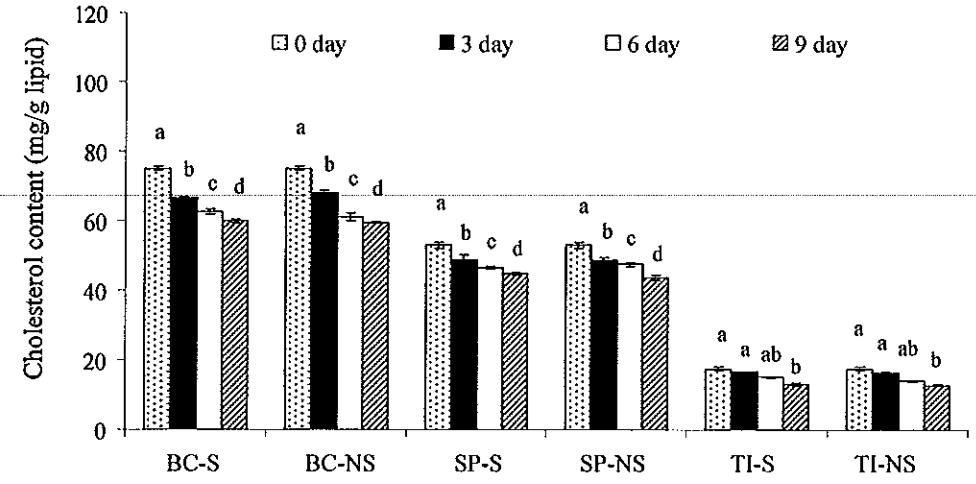


(a) Breast meat

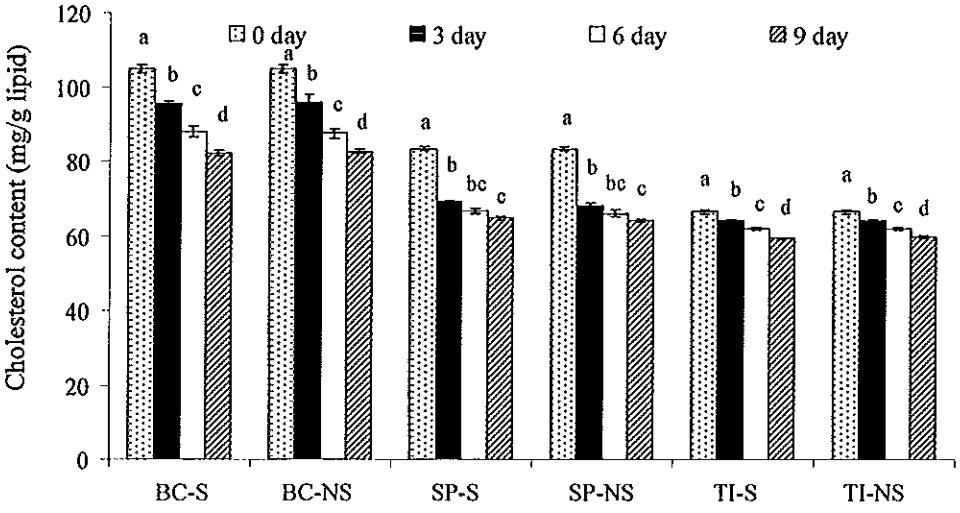


(b) Thigh meat

Figure 28 Change in free fatty acid content (% of total) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations. Different letters under the same breeds and meat types indicate significant differences ($P<0.05$). BC-S: Broiler-with skin storage; BC-NS: Broiler-without skin storage; SP-S: Spent hen-with skin storage; SP-NS: Spent hen-without skin storage; TI-S: Thai indigenous-with skin storage; TI-NS: Thai indigenous-without skin storage.



(a) Breast meat



(b) Thigh meat

Figure 29 Change in cholesterol content (mg/g of lipid) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations. Different letters under the same breeds and meat types indicate significant differences ($P<0.05$). BC-S: Broiler-with skin storage; BC-NS: Broiler-without skin storage; SP-S: Spent hen-with skin storage; SP-NS: Spent hen-without skin storage; TI-S: Thai indigenous-with skin storage; TI-NS: Thai indigenous-without skin storage.

The cholesterol content of thigh meat was higher ($P<0.05$) than those of breast meat among three chicken breeds. The highest triglyceride content was observed in Thai indigenous chicken meat compared to those of spent hen and broiler meat, respectively. While the highest phospholipids, free fatty acid and cholesterol content were observed in broiler meat than those found in spent hen and Thai indigenous chicken meat, respectively. From previous report, the phospholipids and triglyceride content depended on the metabolic type of the muscles (Alasnier *et al.*, 2000). The results were in disagreement with Sklan *et al.* (1983) who reported that phospholipids, triglyceride and free fatty acids were higher concentration in thigh muscle than breast muscle. The lipid fraction contents found in this study were different from those previous reports, probably due to difference in chicken breeds, ages and feeds.

2.8 Change is fatty acid compositions

Table 11-16 present the fatty acid compositions (% of total) in broiler, spent hen and Thai indigenous chicken breast and thigh meat during storage, respectively. When the fatty acid contents were calculated in relation to the total fatty acid compositions of the samples, it shown that there were significant difference ($P<0.05$) within chicken breeds, meat types and storage times but was no significant difference between storage with and without skin. When storage times extended, most of meat samples were found the decrease in PUFAs while MUFAs and SFAs increased ($P<0.05$). However, SFAs content of spent hen thigh meat and Thai indigenous breast meat rather unchange during storage ($P>0.05$). The decrease in PUFAs might be related to the fatty acid hydrolysis or lipolysis resulted in breaking down of long chain fatty acids to short chain fatty acids that also resulted in some short chain of SFAs and MUFAs increased. Conversly, Alasnier *et al.* (2000) reported the decrease of proportion of SFAs and MUFAs but the proportion of PUFAs increased in raw rabbit meat. The fatty acid composition depened on muscles but only the proportions of MUFAs and long chain PUFAs were related to the metabolic type of the muscle. Alasnier *et al.* (2000) found that after 7 days of storage, the proportions of C16:1, C18:1, C20:4*n*-6 and long chain *n*-6 PUFAs were higher in the oxidative muscles than in the glycolytic one. While the proportion of other fatty acids such as C16:0 and *n*-3 PUFAs varied according to the muscles but they were nit related to the metabolic type. But in this study, the changes in the each fatty acid compositions of all samples trended to similarity.

Table 11 Fatty acid compositions (% of total fatty acid) of broiler breast meat during refrigerated storage for 9 days

Fatty acid	0 day	3 day		6 day		9 day	
		With skin	Without skin	With skin	Without skin	With skin	Without skin
C12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14:0	0.85 ± 0.00^{a}	0.78 ± 0.01^{b}	0.77 ± 0.01^{b}	0.76 ± 0.01^{b}	0.76 ± 0.01^{b}	0.63 ± 0.06^{c}	0.64 ± 0.04^{c}
C16:0	28.65 ± 0.04^{b}	29.20 ± 0.04^{a}	29.01 ± 0.05^{a}	29.45 ± 0.04^{a}	29.23 ± 0.01^{a}	29.76 ± 0.03^{a}	29.51 ± 0.04^{a}
C17:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:0	5.15 ± 0.00^{a}	5.18 ± 0.01^{a}	5.15 ± 0.01^{a}	5.20 ± 0.01^{a}	5.17 ± 0.01^{a}	5.14 ± 0.05^{a}	5.11 ± 0.04^{a}
SFAs	**34.71 ± 0.01^{a}**	**34.45 ± 0.03^{a}**	**34.45 ± 0.03^{a}**	**34.38 ± 0.03^{a}**	**34.41 ± 0.04^{a}**	**33.96 ± 0.17^{a}**	**34.03 ± 0.11^{a}**
C16:1	4.35 ± 0.00^{a}	4.29 ± 0.01^{a}	4.27 ± 0.01^{a}	4.23 ± 0.01^{a}	4.26 ± 0.02^{a}	4.12 ± 0.02^{a}	4.15 ± 0.01^{a}
C 18:1n-9c,t	45.54 ± 0.07^{c}	46.39 ± 0.05^{b}	46.10 ± 0.07^{b}	46.74 ± 0.06^{b}	46.42 ± 0.05^{b}	47.27 ± 0.03^{a}	46.90 ± 0.03^{ab}
C20:1	0.39 ± 0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MUFAs	**50.27 ± 0.06^{b}**	**50.67 ± 0.05^{b}**	**50.36 ± 0.07^{b}**	**50.97 ± 0.05^{b}**	**50.68 ± 0.08^{b}**	**51.39 ± 0.01^{a}**	**51.05 ± 0.03^{a}**
C18:2n-6c,t	11.05 ± 0.00^{a}	10.55 ± 0.04^{a}	10.57 ± 0.03^{a}	9.95 ± 0.02^{b}	9.97 ± 0.02^{b}	9.33 ± 0.03^{c}	9.35 ± 0.03^{c}
C18:3n-6	0.87 ± 0.02^{a}	0.81 ± 0.02^{a}	0.89 ± 0.02^{a}	0.81 ± 0.02^{a}	0.90 ± 0.01^{a}	0.83 ± 0.02^{a}	0.93 ± 0.02^{a}
C18:3n-3	0.91 ± 0.02^{a}	0.85 ± 0.02^{a}	0.94 ± 0.02^{a}	0.86 ± 0.02^{a}	0.95 ± 0.01^{a}	0.88 ± 0.02^{a}	0.97 ± 0.02^{a}
C20:3n-6	0.42 ± 0.02^{a}	0.34 ± 0.02^{a}	0.43 ± 0.02^{a}	0.35 ± 0.02^{a}	0.44 ± 0.01^{a}	0.36 ± 0.02^{a}	0.46 ± 0.02^{a}
C20:3n-3	0.87 ± 0.02^{a}	0.80 ± 0.02^{b}	0.89 ± 0.02^{a}	0.81 ± 0.02^{b}	0.90 ± 0.01^{a}	0.83 ± 0.02^{b}	0.92 ± 0.02^{a}
C22:2	0.44 ± 0.02^{a}	0.37 ± 0.02^{a}	0.46 ± 0.02^{a}	0.37 ± 0.02^{a}	0.46 ± 0.01^{a}	0.39 ± 0.02^{a}	0.48 ± 0.02^{a}
C22:6n-3	0.52 ± 0.02^{a}	0.45 ± 0.02^{a}	0.53 ± 0.02^{a}	0.45 ± 0.02^{a}	0.54 ± 0.01^{a}	0.46 ± 0.02^{a}	0.56 ± 0.02^{a}
PUFAs	**15.08 ± 0.11^{a}**	**14.17 ± 0.10^{b}**	**14.71 ± 0.13^{b}**	**13.61 ± 0.11^{c}**	**14.16 ± 0.07^{b}**	**13.09 ± 0.12^{c}**	**13.68 ± 0.15^{c}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-c}$Means with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 12 Fatty acid compositions (% of total fatty acid) of broiler thigh meat during refrigerated storage for 9 days

Fatty acid	0 day	3 day		6 day		9 day	
		With skin	Without skin	With skin	Without skin	With skin	Without skin
C12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14:0	0.49 ± 0.00^{a}	0.42 ± 0.01^{a}	0.42 ± 0.01^{a}	0.34 ± 0.01^{b}	0.35 ± 0.01^{b}	0.25 ± 0.01^{c}	0.26 ± 0.00^{c}
C16:0	14.85 ± 0.06^{d}	15.24 ± 0.08^{c}	15.17 ± 0.08^{c}	15.75 ± 0.12^{b}	15.67 ± 0.12^{b}	16.10 ± 0.14^{a}	15.98 ± 0.11^{a}
C17:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:0	4.16 ± 0.02^{a}	4.21 ± 0.02^{a}	4.19 ± 0.02^{a}	4.28 ± 0.0^{2a}	4.27 ± 0.02^{a}	4.30 ± 0.04^{a}	4.28 ± 0.03^{a}
SFAs	**19.41 ± 0.01^{a}**	**19.16 ± 0.03^{a}**	**19.16 ± 0.03^{a}**	**18.89 ± 0.04^{a,b}**	**18.92 ± 0.04^{a,b}**	**18.62 ± 0.01^{b}**	**18.65 ± 0.01^{b}**
C16:1	12.21 ± 0.03^{a}	12.37 ± 0.03^{a}	12.38 ± 0.04^{a}	12.56 ± 0.07^{a}	12.57 ± 0.08^{a}	12.71 ± 0.07^{a}	12.67 ± 0.07^{a}
C 18:1n-9c,t	34.57 ± 0.13^{d}	35.44 ± 0.16^{c}	35.34 ± 0.18^{c}	36.54 ± 0.26^{b}	36.42 ± 0.28^{b}	37.38 ± 0.27^{a}	37.13 ± 0.23^{a}
C20:1	6.34 ± 0.01^{a}	6.31 ± 0.02^{a}	6.35 ± 0.02^{a}	6.26 ± 0.03^{a}	6.31 ± 0.04^{a}	6.23 ± 0.03^{a}	6.24 ± 0.02^{a}
C24:1	5.78 ± 0.01^{a}	5.74 ± 0.02^{a}	5.78 ± 0.02^{a}	5.67 ± 0.03^{a}	5.72 ± 0.04^{a}	5.62 ± 0.03^{a}	5.63 ± 0.02^{a}
MUFAs	**58.89 ± 0.17^{b}**	**59.87 ± 0.18^{b}**	**59.85 ± 0.22^{b}**	**61.04 ± 0.34^{a}**	**61.02 ± 0.39^{a}**	**61.95 ± 0.34^{a}**	**61.67 ± 0.33^{a}**
C18:2n-6c,t	11.55 ± 0.01^{a}	11.63 ± 0.02^{a}	11.60 ± 0.01^{a}	11.73 ± 0.02^{a}	11.70 ± 0.02^{a}	11.78 ± 0.01^{a}	11.76 ± 0.01^{a}
C18:3n-6	3.63 ± 0.03^{a}	3.45 ± 0.04^{b}	3.47 ± 0.04^{b}	3.24 ± 0.06^{c}	3.25 ± 0.07^{c}	3.04 ± 0.07^{d}	3.10 ± 0.07^{d}
C18:3n-3	1.94 ± 0.04^{a}	1.71 ± 0.05^{b}	1.73 ± 0.05^{b}	1.43 ± 0.08^{c}	1.45 ± 0.08^{c}	1.18 ± 0.09^{d}	1.25 ± 0.08^{d}
C20:3n-6	1.20 ± 0.04^{a}	0.94 ± 0.05^{b}	0.97 ± 0.06^{b}	0.63 ± 0.08^{c}	0.66 ± 0.09^{c}	0.36 ± 0.10^{d}	0.44 ± 0.08^{d}
C20:3n-3	1.16 ± 0.04^{a,d}	0.91 ± 0.05^{b}	0.94 ± 0.06^{b}	0.60 ± 0.08^{c}	0.62 ± 0.09^{c}	0.33 ± 0.10^{d}	0.40 ± 0.08^{d}
C22:2	1.17 ± 0.04^{a}	0.92 ± 0.05^{b}	0.94 ± 0.06^{b}	0.60 ± 0.08^{c}	0.63 ± 0.09^{c}	0.33 ± 0.10^{d}	0.41 ± 0.08^{d}
C22:6n-3	0.97 ± 0.04^{a}	0.71 ± 0.05^{b}	0.73 ± 0.06^{b}	0.38 ± 0.08^{c}	0.41 ± 0.09^{c}	0.38 ± 0.08^{c}	0.46 ± 0.07^{c}
PUFAs	**21.61 ± 0.25^{a}**	**20.27 ± 0.27^{b}**	**20.37 ± 0.31^{b}**	**18.60 ± 0.46^{c}**	**18.70 ± 0.52^{c}**	**17.40 ± 0.51^{d}**	**17.81 ± 0.46^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 13 Fatty acid compositions (% of total fatty acid) of spent hen breast meat during refrigerated storage for 9 days

Fatty acid	0 day	3 day		6 day		9 day	
		With skin	Without skin	With skin	Without skin	With skin	Without skin
C12:0	9.02 ± 0.01^{a}	9.06 ± 0.02^{a}	9.06 ± 0.02^{a}	9.04 ± 0.02^{a}	9.04 ± 0.01^{a}	9.09 ± 0.02^{a}	9.05 ± 0.02^{a}
C14:0	1.02 ± 0.03^{a}	0.86 ± 0.03^{b}	0.87 ± 0.02^{b}	0.63 ± 0.04^{c}	0.64 ± 0.04^{c}	0.45 ± 0.04^{d}	0.47 ± 0.04^{d}
C16:0	20.30 ± 0.05^{b}	20.62 ± 0.07^{b}	20.60 ± 0.07^{b}	20.91 ± 0.09^{ab}	20.89 ± 0.07^{ab}	21.26 ± 0.11^{a}	21.15 ± 0.09^{a}
C17:0	9.54 ± 0.01^{a}	9.59 ± 0.02^{a}	9.59 ± 0.02^{a}	9.59 ± 0.02^{a}	9.59 ± 0.01^{a}	9.65 ± 0.03^{a}	9.61 ± 0.02^{a}
C18:0	5.82 ± 0.01^{a}	5.78 ± 0.01^{a}	5.79 ± 0.01^{a}	5.68 ± 0.01^{a}	5.69 ± 0.01^{a}	5.64 ± 0.01^{a}	5.62 ± 0.01^{a}
SFAs	**35.67 ± 0.05^{a}**	**35.99 ± 0.09^{a}**	**35.99 ± 0.09^{a}**	**36.19 ± 0.10^{a}**	**36.16 ± 0.06^{a}**	**36.55 ± 0.14^{a}**	**36.37 ± 0.11^{a}**
C16:1	1.81 ± 0.02^{a}	1.74 ± 0.03^{a}	1.75 ± 0.02^{a}	1.67 ± 0.02^{a}	1.68 ± 0.03^{a}	1.54 ± 0.04^{a}	1.54 ± 0.04^{a}
C 18:1n-9c,t	41.51 ± 0.15^{d}	42.42 ± 0.17^{c}	42.36 ± 0.15^{c}	43.43 ± 0.23^{b}	43.36 ± 0.19^{b}	44.38 ± 0.26^{a}	44.12 ± 0.22^{a}
C20:1	1.23 ± 0.02^{a}	1.15 ± 0.03^{a}	1.16 ± 0.02^{a}	1.07 ± 0.03^{ab}	1.08 ± 0.03^{ab}	0.92 ± 0.05^{b}	0.92 ± 0.04^{b}
C24:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MUFAs	**44.57 ± 0.11^{d}**	**45.32 ± 0.11^{c}**	**45.26 ± 0.12^{c}**	**46.17 ± 0.18^{b}**	**46.12 ± 0.13^{b}**	**46.85 ± 0.18^{a}**	**46.58 ± 0.16^{ab}**
C18:2n-6c,t	13.47 ± 0.01^{a}	13.54 ± 0.02^{a}	13.53 ± 0.01^{a}	13.64 ± 0.02^{a}	13.63 ± 0.02^{a}	13.74 ± 0.03^{a}	13.75 ± 0.02^{a}
C18:3n-6	1.26 ± 0.04^{a}	1.03 ± 0.04^{b}	1.04 ± 0.04^{b}	0.80 ± 0.06^{c}	0.81 ± 0.04^{c}	0.57 ± 0.07^{d}	0.66 ± 0.06^{d}
C18:3n-3	1.42 ± 0.03^{a}	1.19 ± 0.04^{b}	1.21 ± 0.04^{b}	0.97 ± 0.06^{bc}	0.98 ± 0.04^{bc}	0.74 ± 0.07^{c}	0.83 ± 0.06^{c}
C20:3n-6	1.08 ± 0.00^{a}	0.84 ± 0.04^{b}	0.85 ± 0.04^{b}	0.60 ± 0.06^{c}	0.62 ± 0.04^{c}	0.37 ± 0.07^{d}	0.46 ± 0.06^{d}
C20:3n-3	1.29 ± 0.04^{a}	1.06 ± 0.04^{b}	1.08 ± 0.04^{b}	0.83 ± 0.06^{c}	0.85 ± 0.04^{c}	0.60 ± 0.07^{d}	0.69 ± 0.06^{d}
C22:2	1.27 ± 0.04^{a}	1.03 ± 0.04^{b}	1.05 ± 0.04^{b}	0.80 ± 0.06^{c}	0.82 ± 0.04^{c}	0.57 ± 0.07^{d}	0.66 ± 0.06^{d}
C22:6n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PUFAs	**19.79 ± 0.16^{a}**	**18.69 ± 0.19^{b}**	**18.75 ± 0.21^{b}**	**17.64 ± 0.28^{c}**	**17.72 ± 0.19^{c}**	**16.60 ± 0.32^{d}**	**17.05 ± 0.26^{cd}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 14 Fatty acid compositions (% of total fatty acid) of spent hen thigh meat during refrigerated storage for 9 days

Fatty acid	0 day	3 day		6 day		9 day	
		With skin	Without skin	With skin	Without skin	With skin	Without skin
C12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14:0	1.15 ± 0.01^{a}	0.99 ± 0.03^{b}	1.03 ± 0.04^{b}	0.86 ± 0.05^{bc}	0.88 ± 0.06^{bc}	0. 68 ± 0.04^{c}	0.68 ± 0.02^{c}
C16:0	20.04 ± 0.07^{a}	20.42 ± 0.10^{a}	20.30 ± 0.09^{a}	20.73 ± 0.13^{a}	20.69 ± 0.15^{a}	21.07 ± 0.10^{b}	21.06 ± 0.12^{b}
C17:0	1.17 ± 0.01^{a}	1.01 ± 0.03^{b}	1.05 ± 0.04^{b}	0.89 ± 0.05^{bc}	0.91 ± 0.06^{bc}	0.71 ± 0.04^{c}	0.71 ± 0.02^{c}
C18:0	6.84 ± 0.01^{a}	6.84 ± 0.02^{a}	6.83 ± 0.03^{a}	6.84 ± 0.03^{a}	6.85 ± 0.03^{a}	6.82 ± 0.03^{a}	6.82 ± 0.03^{a}
SFAs	**29.21 ± 0.07^{a}**	**29.25 ± 0.08^{a}**	**29.21 ± 0.10^{a}**	**29.31 ± 0.13^{a}**	**29.32 ± 0.12^{a}**	**29.27 ± 0.13^{a}**	**29.28 ± 0.13^{a}**
C16:1	1.86 ± 0.01^{a}	1.73 ± 0.02^{a}	1.75 ± 0.02^{a}	1.60 ± 0.04^{ab}	1.62 ± 0.05^{ab}	1.44 ± 0.02^{b}	1.44 ± 0.04^{b}
C 18:1n-9c,t	38.93 ± 0.16^{d}	39.85 ± 0.23^{c}	39.56 ± 0.20^{c}	40.58 ± 0.30^{b}	40.47 ± 0.35^{b}	41.44 ± 0.22^{a}	41.41 ± 0.24^{a}
C20:1	2.10 ± 0.01^{a}	1.97 ± 0.02^{a}	2.00 ± 0.02^{a}	1.85 ± 0.04^{ab}	1.87 ± 0.05^{ab}	1.69 ± 0.02^{b}	1.70 ± 0.04^{b}
C24:1	2.03 ± 0.01^{a}	1.90 ± 0.02^{a}	1.92 ± 0.02^{a}	1.77 ± 0.04^{ab}	1.79 ± 0.05^{ab}	1.61 ± 0.02^{b}	1.62 ± 0.04^{b}
MUFAs	**44.93 ± 0.13^{c}**	**45.45 ± 0.17^{b}**	**45.23 ± 0.15^{b}**	**45.79 ± 0.19^{b}**	**45.74 ± 0.21^{b}**	**46.18 ± 0.15^{a}**	**46.17 ± 0.15^{a}**
C18:2n-6c,t	19.59 ± 0.03^{a}	19.88 ± 0.05^{a}	19.82 ± 0.05^{a}	20.14 ± 0.08^{b}	20.10 ± 0.10^{b}	20.47 ± 0.06^{b}	20.46 ± 0.06^{b}
C18:3n-6	1.99 ± 0.05^{a}	1.79 ± 0.07^{ab}	1.87 ± 0.06^{ab}	1.63 ± 0.08^{ab}	1.65 ± 0.09^{ab}	1.47 ± 0.06^{b}	1.48 ± 0.07^{b}
C18:3n-3	1.56 ± 0.06^{a}	1.34 ± 0.07^{a}	1.43 ± 0.06^{a}	1.18 ± 0.08^{ab}	1.20 ± 0.09^{ab}	1.01 ± 0.07^{b}	1.02 ± 0.07^{b}
C20:3n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3n-3	1.34 ± 0.06^{a}	1.12 ± 0.07^{a}	1.20 ± 0.06^{a}	0.95 ± 0.08^{ab}	0.97 ± 0.09^{ab}	0.77 ± 0.07^{b}	0.78 ± 0.08^{b}
C22:2	1.39 ± 0.06^{a}	1.16 ± 0.07^{ab}	1.25 ± 0.06^{a}	1.00 ± 0.08^{b}	1.02 ± 0.09^{b}	0.82 ± 0.07^{c}	0.83 ± 0.08^{c}
C22:6n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PUFAs	**25.87 ± 0.19^{a}**	**25.30 ± 0.23^{a}**	**25.56 ± 0.20^{a}**	**24.90 ± 0.25^{ab}**	**24.94 ± 0.27^{ab}**	**24.54 ± 0.22^{b}**	**24.55 ± 0.24^{b}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

[a-c]Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 15 Fatty acid compositions (% of total fatty acid) of Thai indigenous chicken breast meat during refrigerated storage for 9 days

Fatty acid	0 day	3 day		6 day		9 day	
		With skin	Without skin	With skin	Without skin	With skin	Without skin
C12:0	9.31 ± 0.01^{a}	9.29 ± 0.01^{a}	9.28 ± 0.01^{a}	9.31 ± 0.01^{a}	9.30 ± 0.02^{a}	9.34 ± 0.02^{a}	9.35 ± 0.02^{a}
C14:0	1.07 ± 0.04^{a}	0.92 ± 0.06^{b}	0.95 ± 0.05^{b}	0.85 ± 0.06^{c}	0.88 ± 0.07^{c}	0.70 ± 0.08^{d}	0.68 ± 0.06^{d}
C16:0	21.68 ± 0.09^{b}	21.85 ± 0.09^{b}	21.80 ± 0.08^{b}	22.00 ± 0.11^{ab}	21.94 ± 0.12^{b}	22.32 ± 0.15^{a}	22.35 ± 0.11^{a}
C17:0	12.72 ± 0.03^{a}	12.75 ± 0.03^{a}	12.73 ± 0.03^{a}	12.80 ± 0.04^{a}	12.78 ± 0.04^{a}	12.92 ± 0.05^{a}	12.93 ± 0.04^{a}
C18:0	5.83 ± 0.01^{a}	5.75 ± 0.03^{a}	5.76 ± 0.02^{a}	5.73 ± 0.03^{a}	5.74 ± 0.03^{a}	5.69 ± 0.03^{a}	5.69 ± 0.03^{a}
SFAs	**41.29 ± 0.07^{a}**	**41.28 ± 0.06^{a}**	**41.24 ± 0.06^{a}**	**41.39 ± 0.07^{a}**	**41.34 ± 0.08^{a}**	**41.62 ± 0.10^{a}**	**41.65 ± 0.08^{a}**
C16:1	1.57 ± 0.04^{a}	1.49 ± 0.04^{a}	1.51 ± 0.04^{a}	1.43 ± 0.05^{ab}	1.45 ± 0.05^{ab}	1.29 ± 0.07^{b}	1.27 ± 0.05^{b}
C 18:1n-9c,t	38.00 ± 0.20^{a}	38.50 ± 0.22^{a}	38.38 ± 0.20^{a}	38.81 ± 0.25^{a}	38.69 ± 0.27^{a}	39.51 ± 0.35^{a}	39.58 ± 0.24^{a}
C20:1	1.18 ± 0.04^{a}	1.10 ± 0.04^{a}	1.13 ± 0.04^{a}	1.03 ± 0.05^{a}	1.06 ± 0.05^{a}	0.88 ± 0.07^{ab}	0.87 ± 0.05^{ab}
C24:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MUFAs	**40.75 ± 0.11^{b}**	**41.09 ± 0.14^{b}**	**41.02 ± 0.13^{b}**	**41.27 ± 0.15^{ab}**	**41.20 ± 0.16^{ab}**	**41.68 ± 0.21^{a}**	**41.72 ± 0.15^{a}**
C18:2n-6c,t	11.66 ± 0.03^{a}	11.74 ± 0.03^{a}	11.72 ± 0.03^{a}	11.78 ± 0.04^{a}	11.76 ± 0.04^{a}	11.87 ± 0.05^{a}	11.88 ± 0.03^{a}
C18:3n-6	0.93 ± 0.04^{a}	0.84 ± 0.04^{ab}	0.87 ± 0.04^{ab}	0.77 ± 0.05^{b}	0.80 ± 0.06^{b}	0.62 ± 0.07^{c}	0.60 ± 0.05^{c}
C18:3n-3	1.73 ± 0.04^{c}	1.65 ± 0.04^{b}	1.68 ± 0.04^{b}	1.59 ± 0.04^{b}	1.62 ± 0.05^{b}	1.46 ± 0.06^{a}	1.44 ± 0.05^{a}
C20:3n-6	1.47 ± 0.04^{a}	1.40 ± 0.04^{a}	1.42 ± 0.04^{a}	1.33 ± 0.05^{ab}	1.36 ± 0.05^{ab}	1.19 ± 0.07^{b}	1.17 ± 0.05^{b}
C20:3n-3	1.00 ± 0.04^{a}	0.91 ± 0.04^{ab}	0.94 ± 0.04^{ab}	0.84 ± 0.05^{ab}	0.87 ± 0.06^{ab}	0.69 ± 0.07^{b}	0.67 ± 0.05^{b}
C22:2	1.18 ± 0.04^{a}	1.09 ± 0.04^{a}	1.12 ± 0.04^{a}	1.02 ± 0.05^{a}	1.05 ± 0.05^{a}	0.88 ± 0.07^{b}	0.86 ± 0.05^{b}
C22:6n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PUFAs	**17.96 ± 0.18^{a}**	**17.63 ± 0.18^{a}**	**17.74 ± 0.16^{a}**	**17.34 ± 0.21^{a}**	**17.46 ± 0.23^{a}**	**16.69 ± 0.30^{b}**	**16.63 ± 0.21^{b}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

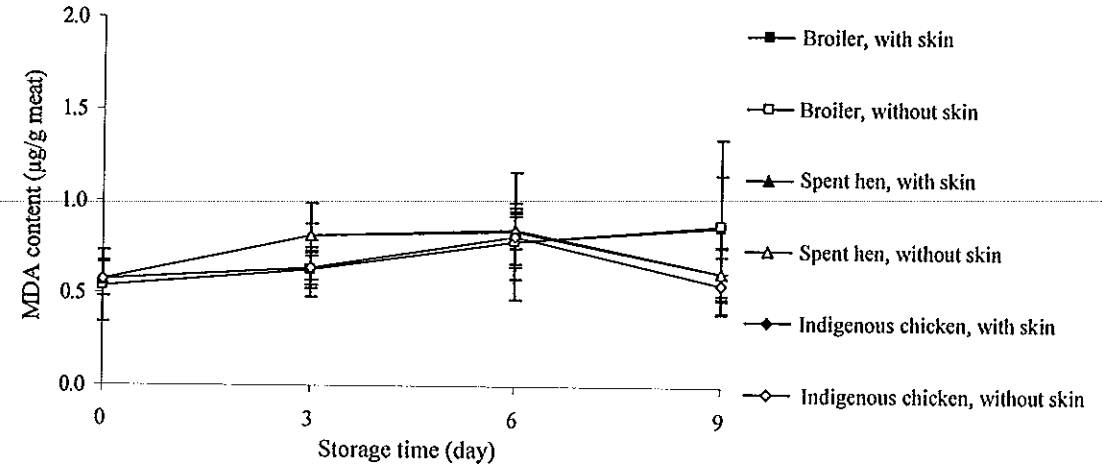
Table 16 Fatty acid compositions (% of total fatty acid) of Thai indigenous chicken thigh meat during refrigerated storage for 9 days

Fatty acid	0 day	3 day		6 day		9 day	
		With skin	Without skin	With skin	Without skin	With skin	Without skin
C12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14:0	2.36 ± 0.02^{a}	2.09 ± 0.03^{a}	2.13 ± 0.02^{a}	1.87 ± 0.05^{ab}	1.91 ± 0.04^{ab}	1.57 ± 0.02^{b}	1.60 ± 0.03^{b}
C16:0	23.37 ± 0.07^{c}	24.14 ± 0.09^{b}	24.05 ± 0.07^{b}	24.80 ± 0.14^{b}	24.69 ± 0.11^{b}	25.70 ± 0.05^{a}	25.59 ± 0.08^{a}
C17:0	2.41 ± 0.02^{a}	2.15 ± 0.03^{b}	2.18 ± 0.02^{b}	1.93 ± 0.05^{bc}	1.96 ± 0.04^{bc}	1.62 ± 0.02^{c}	1.66 ± 0.03^{c}
C18:0	13.66 ± 0.03^{a}	13.96 ± 0.03^{a}	13.92 ± 0.02^{a}	14.21 ± 0.05^{a}	14.17 ± 0.04^{a}	14.55 ± 0.02^{a}	14.51 ± 0.03^{a}
SFAs	**41.80 ± 0.05^{c}**	**42.34 ± 0.06^{b}**	**42.27 ± 0.05^{b}**	**42.81 ± 0.10^{b}**	**42.73 ± 0.08^{b}**	**43.44 ± 0.03^{a}**	**43.36 ± 0.06^{a}**
C16:1	3.77 ± 0.02^{a}	3.58 ± 0.02b^{a}	3.60 ± 0.02^{a}	3.42 ± 0.03^{ab}	3.44 ± 0.03^{ab}	3.19 ± 0.01^{b}	3.22 ± 0.02^{b}
C 18:1n-9c,t	27.85 ± 0.09^{c}	28.85 ± 0.12^{b}	28.72 ± 0.08^{b}	29.70 ± 0.18^{ab}	29.56 ± 0.14^{ab}	30.84 ± 0.06^{a}	30.70 ± 0.11^{a}
C20:1	4.25 ± 0.02^{a}	4.08 ± 0.02^{a}	4.10 ± 0.01^{a}	3.93 ± 0.03^{a}	3.96 ± 0.02^{a}	3.74 ± 0.01^{b}	3.76 ± 0.02^{a}
C24:1	4.10 ± 0.02^{a}	3.92 ± 0.02^{a}	3.95 ± 0.01^{a}	3.77 ± 0.03^{ab}	3.80 ± 0.03^{ab}	3.57 ± 0.01^{b}	3.59 ± 0.02^{b}
MUFAs	**39.98 ± 0.04^{c}**	**40.43 ± 0.05^{b}**	**40.37 ± 0.04^{b}**	**40.82 ± 0.08^{b}**	**40.75 ± 0.07^{b}**	**41.34 ± 0.03^{a}**	**41.28 ± 0.05^{a}**
C18:2n-6c,t	5.85 ± 0.01^{a}	5.76 ± 0.01^{a}	5.78 ± 0.01^{a}	5.69 ± 0.02^{a}	5.70 ± 0.01^{a}	5.58 ± 0.01^{a}	5.60 ± 0.01^{a}
C18:3n-6	3.93 ± 0.02^{a}	3.75 ± 0.02^{ab}	3.77 ± 0.02^{ab}	3.59 ± 0.03^{b}	3.61 ± 0.03^{b}	3.37 ± 0.01^{c}	3.40 ± 0.02^{c}
C18:3n-3	3.08 ± 0.02^{a}	2.85 ± 0.03^{b}	2.88 ± 0.02^{b}	2.65 ± 0.04^{bc}	2.69 ± 0.03^{bc}	2.39 ± 0.01^{c}	2.42 ± 0.02^{c}
C20:3n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3n-3	2.63 ± 0.02^{a}	2.39 ± 0.03b^{b}	2.40 ± 0.02^{b}	2.17 ± 0.04^{c}	2.21 ± 0.04^{c}	1.89 ± 0.02^{d}	1.92 ± 0.03^{d}
C22:2	2.73 ± 0.02^{a}	2.49 ± 0.03^{b}	2.52 ± 0.02^{b}	2.28 ± 0.04^{bc}	2.31 ± 0.04^{bc}	1.99 ± 0.02^{c}	2.03 ± 0.03^{c}
C22:6n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PUFAs	**18.22 ± 0.09^{a}**	**17.23 ± 0.12^{b}**	**17.35 ± 0.08^{b}**	**16.37 ± 0.18^{c}**	**16.52 ± 0.14^{c}**	**15.22 ± 0.06^{d}**	**15.36 ± 0.11^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

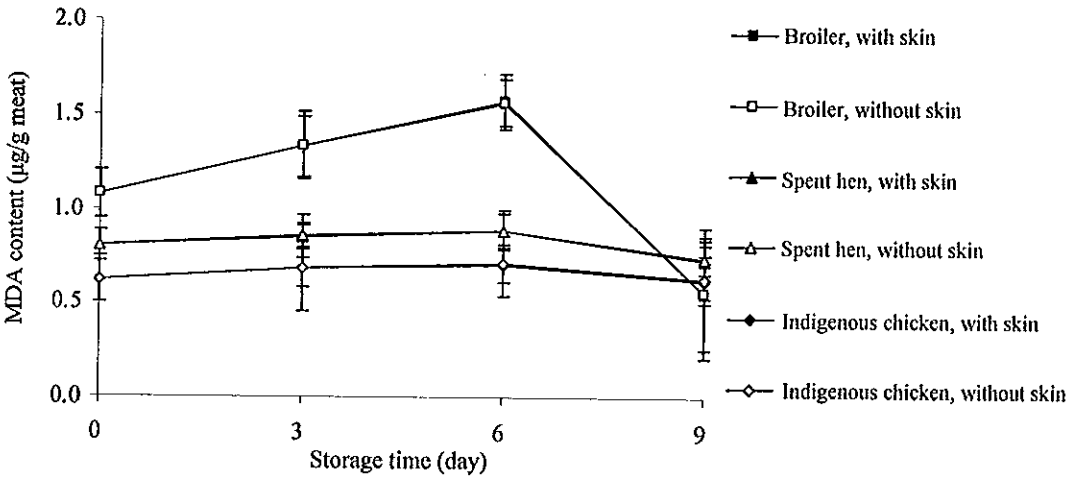
Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.



(a)



(b)

Figure 30 Change in TBARS (µg MDA/g meat) of broiler, spent hen and Thai indigenous chicken breast (a) and thigh (b) meat during refrigerated storage with and without skin (4 °C) for 9 days. Bars indicate SD from eight replicate determinations.

Table 17 Cholesterol oxidation products (μg/g lipid) of breast meat during refrigerated storage for 9 days

Breed	Storage time (day)	Storage type	7β -HC	β-CE	α-CE	CT	25-HC
Broiler	0	-	0.00	0.00	0.00	0.00	0.00
	3	S	0.00	0.00	0.00	0.00	0.00
		NS	0.00	0.00	0.00	0.00	0.00
	6	S	0.18 ± 0.01^{bc}	0.11 ± 0.00^{b}	0.07 ± 0.00^{b}	0.07 ± 0.01^{c}	0.02 ± 0.00^{c}
		NS	0.16 ± 0.00^{bc}	0.13 ± 0.00^{b}	0.09 ± 0.00^{b}	0.19 ± 0.01^{c}	0.04 ± 0.00^{c}
	9	S	0.24 ± 0.00^{b}	0.21 ± 0.00^{a}	0.11 ± 0.00^{b}	0.21 ± 0.01^{a}	0.06 ± 0.00^{b}
		NS	0.25 ± 0.00^{b}	0.22 ± 0.00^{a}	0.12 ± 0.00^{b}	0.23 ± 0.00^{a}	0.09 ± 0.01^{b}
Spent hen	0	-	0.00	0.00	0.00	0.00	0.00
	3	S	0.00	0.00	0.00	0.00	0.00
		NS	0.00	0.00	0.00	0.00	0.00
	6	S	0.22 ± 0.01^{b}	0.17 ± 0.00^{b}	0.11 ± 0.01^{b}	0.16 ± 0.01^{b}	0.07 ± 0.01^{b}
		NS	0.24 ± 0.00^{b}	0.15 ± 0.00^{b}	0.10 ± 0.00^{b}	0.14 ± 0.01^{b}	0.09 ± 0.00^{b}
	9	S	0.31 ± 0.01^{a}	0.22 ± 0.01^{a}	0.19 ± 0.00^{a}	0.24 ± 0.00^{a}	0.12 ± 0.01^{a}
		NS	0.33 ± 0.01^{a}	0.21 ± 0.01^{a}	0.17 ± 0.01^{a}	0.22 ± 0.01^{a}	0.14 ± 0.00^{a}
Thai indigenous chicken	0	-	0.00	0.00	0.00	0.00	0.00
	3	S	0.00	0.00	0.00	0.00	0.00
		NS	0.00	0.00	0.00	0.00	0.00
	6	S	0.06 ± 0.00^{c}	0.03 ± 0.00^{c}	0.00	0.00	0.00
		NS	0.05 ± 0.00^{c}	0.04 ± 0.01^{c}	0.00	0.00	0.00
	9	S	0.17 ± 0.01^{bc}	0.09 ± 0.00^{b}	0.07 ± 0.01^{b}	0.07 ± 0.01^{c}	0.00
		NS	0.15 ± 0.01^{bc}	0.11 ± 0.00^{b}	0.08 ± 0.00^{b}	0.09 ± 0.01^{c}	0.00

Data are presented as mean ± standard deviation. N = 3.

[a-c]Means with differing superscripts in the same column are significantly different ($P<0.05$).

S = storage with skin, NS = storage without skin.

7β-HC = 7β-hydroxy cholesterol, β-CE = cholesterol 5β,6β-epoxide, α-CE = cholesterol 5α,6α-epoxide, CT = 5-cholestane-3β-ol-7-one, 25-HC = 25-hydroxy cholesterol.

Table 18 Cholesterol oxidation products (μg/g lipid) of thigh meat during refrigerated storage for 9 days

Breed	Storage time (day)	Storage type	7β -HC	β-CE	α-CE	CT	25-HC
Broiler	0	-	0.00	0.00	0.00	0.00	0.00
	3	S	0.00	0.00	0.00	0.00	0.00
		NS	0.00	0.00	0.00	0.00	0.00
	6	S	0.27 ± 0.01^{b}	0.17 ± 0.01^{b}	0.15 ± 0.00^{b}	0.27 ± 0.01^{b}	0.11 ± 0.00^{b}
		NS	0.29 ± 0.02^{b}	0.19 ± 0.01^{b}	0.17 ± 0.00^{b}	0.26 ± 0.00^{b}	0.13 ± 0.01^{b}
	9	S	0.36 ± 0.01^{ab}	0.35 ± 0.00^{a}	0.22 ± 0.01^{a}	0.37 ± 0.00^{a}	0.16 ± 0.00^{b}
		NS	0.39 ± 0.01^{a}	0.33 ± 0.01^{a}	0.24 ± 0.01^{a}	0.39 ± 0.02^{a}	0.17 ± 0.00^{b}
Spent hen	0	-	0.00	0.00	0.00	0.00	0.00
	3	S	0.00	0.00	0.00	0.00	0.00
		NS	0.00	0.00	0.00	0.00	0.00
	6	S	0.33 ± 0.01^{ab}	0.23 ± 0.01^{b}	0.19 ± 0.01^{ab}	0.22 ± 0.01^{bc}	0.15 ± 0.00^{b}
		NS	0.34 ± 0.01^{ab}	0.21 ± 0.01^{b}	0.21 ± 0.01^{a}	0.23 ± 0.00^{bc}	0.17 ± 0.01^{b}
	9	S	0.41 ± 0.01^{a}	0.31 ± 0.02^{a}	0.24 ± 0.02^{a}	0.32 ± 0.01^{ab}	0.21 ± 0.01^{a}
		NS	0.43 ± 0.01^{a}	0.29 ± 0.01^{a}	0.23 ± 0.02^{a}	0.33 ± 0.00^{ab}	0.23 ± 0.01^{a}
Thai indigenous chicken	0	-	0.00	0.00	0.00	0.00	0.00
	3	S	0.00	0.00	0.00	0.00	0.00
		NS	0.00	0.00	0.00	0.00	0.00
	6	S	0.12 ± 0.00^{d}	0.07 ± 0.00^{c}	0.04 ± 0.01^{b}	0.00	0.00
		NS	0.13 ± 0.01^{d}	0.09 ± 0.00^{c}	0.04 ± 0.00^{b}	0.00	0.00
	9	S	0.21 ± 0.01^{c}	0.19 ± 0.00^{b}	0.17 ± 0.01^{b}	0.11 ± 0.01^{c}	0.14 ± 0.01^{b}
		NS	0.23 ± 0.01^{c}	0.21 ± 0.01^{b}	0.15 ± 0.01^{b}	0.15 ± 0.00^{c}	0.15 ± 0.02^{b}

Data are presented as mean ± standard deviation. N = 3.

[a-c]Means with differing superscripts in the same column are significantly different ($P<0.05$).

S = storage with skin, NS = storage without skin.

7β-HC = 7β-hydroxy cholesterol, β-CE = cholesterol 5β,6β-epoxide, α-CE = cholesterol 5α,6α-epoxide, CT = 5-cholestane-3β-ol-7-one, 25-HC = 25-hydroxy cholesterol.

2.10 Relationship evaluation

The correlations between some chemical compositions with other chemical compositions and with lipid oxidation are exhibited in Table 19. It was elucidated that in different breeds chicken meat had difference in relation between several chemical compositions and lipid oxidation. From these relations indicated that the lipid oxidation was highly correlated with some chemical composition such as heme pigment, metmyoglobin formation, lipid fraction and fatty acid compositions. Whole thigh meat contained higher lipid, myoglobin, heme and non-heme iron relating to more lipid oxidation compared to whole breast meat were observed.The heme iron content decreased in a coincidence with an increase in non-heme iron content, suggesting to the destruction of the heme protein. The increase of MDA content with extended storage time from 0 to 6 days in each meat sample associated with the increase in non-heme iron, lipid content, metmyoglobin formation, free fatty acid and relating to the decrease in moisture, heme iron, myoglobin, triglyceride, phospholipids content, SFAs, MUFAs and PUFAs proportion of chicken meat. No significant difference in chemical compositions and lipids oxidation of chicken meat between storage with and without skin was observed. In first 6 days storage, lipid oxidation of broiler thigh meat had highest than meat from the other breeds ($P<0.05$) relating to the highest in myoglobin, phospholipids, free fatty acid content and unsaturated fatty acid proportion. Moreover, during refrigerated storage for 6 days, myoglobin and heme iron content decreased in concomitant with the decrease in redness value (a*), suggested to myoglobin might be converted to metmyogobin form which from radicals products from autoxidation of myoglobin could be stimulate lipid oxidation.

The highest lipid oxidation in broiler thigh meat had high relation to myoglobin content than heme and non-heme iron content. So, broiler thigh meat was collected to sample in the next part for investigated effect of cooking methods on lipid oxidation. Inspite of broiler thigh meat was not a highest content of non-heme iron that could be stimulated lipid oxidation. Grunwald and Richards (2006) reported that the lower amount of iron may explain why released iron from myoglobin did not appear to be pro-oxidative in fresh meat. Tappel (1953) suggested that the release of iron from heme proteins due to heme destruction will decrease rates of lipid oxidation due to the destruction of hemin reactant. Moreover, the radicals from autoxidation of myoglobin could be stimulated lipid oxidation. Brantley *et al.* (1993) suggested that the process by which ferrous Mb is converted to ferric metmyoglobin is called autoxidation. Superoxide anion radical (O_2•-) or •OOH is liberated in this process depending on whether deoxy or oxy heme protein undergoes autoxidation. O_2•- and •OOH can readily be converted to hydrogen peroxide (H_2O_2), which enhances the ability

of heme proteins to promote lipid oxidation. Additionally, lipolysis which releases free fatty acids from both triglyceride and phospholipids is also suspected to favour lipid oxidation because free fatty acids are very sensitive to lipid oxidation (Alasnier *et al.*, 2000). Several studies indicated that free fatty acid content was lower in breast meat than in thigh or leg meat, suggesting that lipolysis was less intense in breast meat than in thigh meat (Currie and Wolfe, 1997; Sklan *et al.*, 1983, Alasnier *et al.*, 2000). Thigh meat had more sensitive to oxidation than breast meat. This is partly related to their phospholipids content (Igene *et al.*, 1979) and to their higher long chain polyunsaturated fatty acid content (Alasnier and Gandemer, 1998) because phospholipids rich in long chain polyunsaturated fatty acid such as phosphotidyl ethanolamine are very sensitive to oxidation (Gandemer, 1990) which corresponding to the result in this part of study.

Table 19 The correlation (R^2) in linear correlation between MDA content and chemical compositions of chicken meat from three chicken breeds

Independent value	Broiler				Spent hen				Thai indigenous chicken			
	Breast		Thigh		Breast		Thigh		Breast		Thigh	
	With skin	Without skin	With skin	Without skin	With skin	Without skin	With skin	Without skin	With skin	Without skin	With skin	Without skin
Moisture	0.90	0.76	1.00	0.99	0.88	0.98	0.96	0.98	0.82	0.78	0.91	1.00
Lipid	0.93	0.99	0.97	0.98	0.78	0.71	1.00	0.97	0.96	0.96	0.43	0.51
Myoglobin	0.94	0.95	0.95	0.96	0.91	0.95	0.75	0.77	0.99	0.99	0.96	0.98
Metmyoglobin	0.99	1.00	1.00	0.98	0.79	0.76	1.00	0.98	0.87	1.00	0.86	0.94
Heme iron	0.83	0.89	0.99	1.00	1.00	0.99	0.99	0.99	1.00	0.98	1.00	1.00
Non-heme iron	1.00	0.99	1.00	1.00	0.71	0.73	0.96	1.00	0.99	0.99	0.30	0.37
Triglyceride	0.97	0.99	0.96	0.95	0.86	0.87	0.82	0.89	0.97	0.97	0.69	0.78
Phospholipids	0.96	0.91	1.00	1.00	0.67	0.67	0.99	0.93	0.74	0.82	0.64	0.62
Free fatty acid	0.96	0.97	0.98	0.98	0.81	0.80	0.91	0.90	0.92	0.92	0.68	0.75
SFAs	0.74	0.69	1.00	1.00	0.76	0.75	1.00	0.98	0.78	0.82	0.93	0.94
MUFAs	0.80	0.71	0.99	0.97	0.82	0.83	1.00	0.97	0.88	0.93	0.93	0.94
PUFAs	0.90	0.99	0.99	0.99	0.84	0.84	1.00	0.95	0.88	0.93	0.93	0.94

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

3. Effect of cooking methods on changes in chemical, physical compositions and lipid oxidation of broiler thigh meat

The heating time of broiler thigh meat was recorded during heating as shown in Table 20. All samples were allowed to reach the average end point temperature 85±1 °C of internal thigh meat.

Table 20 Heating condition of broiler thigh meat to end point temperature at 85±1 °C

Cooking method	Convector/Conductor	Convector/Conductor temperature (°C)	Overall Time (min)
Boiling	Water	92 ± 2	25
Steaming	Water	95 ± 2	32
Fring	Frying oil	160 ± 2	21
Grilling	Air	230 ± 2	36
Microwave cooking	Air	180 ± 2	25

Note: Individually meat sample size 146-160 grams with skin.

3.1 Changes in chemical and physical properties of cooked thigh meat

Loss of water in thigh meat was shown to be dependent on the methods of heating process (Table 21). The loss of water in boiled, steamed, fried, grilled and microwaved meat were 22.61, 10.30, 27.78, 6.46 and 15.62 % of their initial water content of raw meat, respectively (Table 21). While the cooking losses were obtained ordinary higher in boiled, steamed, fried, grilled and microwaved meat, respectively (Table 20). According to these results, frying methods caused higher cooking loss and water loss than did boiling, microwave cooking, steaming and grilling, respectively. The difference in cooking temperature and heat penetration period among cooking methods resulted in various level of denaturation of the meat might be contributed to the difference in cooked meat properties. In additionally, fat frying can reach much higher temperatures with the shorter time than water at normal atmospheric pressure. In this study, deep frying was classified as a dry cooking method because no water is used. Due to the high temperature involved and the high heat penetration rate of oil made it cooks food extremely quickly. In deep frying with high temperature of medium oil and short cooking time, much of the water would be lost and oil penetration would be confined only to the outer surface, resulting in high cooking loss and water loss but lower fat content in sample as shown in Table 21.

The initial crude fat content in thigh meat was markedly lost during cooking by frying and grilling while crude fat content was increased in boiling, microwave cooking and steaming (Table 21). The highest crude fat in cooked meat was observed in boiled and steamed meat while the lowest fat content was observed in fried and grilled meat. The lost of lipid after cooking due to some of meat lipid might be dissolved into frying oil or some of lipid might be drip out from meat surface during cooking by other methods.

Table 21 Crude fat and moisture content, cooking loss and water loss of raw and cooked broiler thigh meat

	Raw	Boiled	Steamed	Fried	Grilled	Microwaved
Crude fat (%)	1.87 ± 0.55^{b}	2.29 ± 0.17^{a}	2.25 ± 0.81^{a}	1.65 ± 0.24^{b}	1.79 ± 0.25^{b}	2.06 ± 0.32^{ab}
Water content (%)	75.80 ± 1.58^{a}	58.66 ± 3.61^{d}	67.99 ± 2.71^{bc}	54.74 ± 3.13^{e}	70.90 ± 4.88^{b}	63.96 ± 5.00^{c}
Cooking loss (%)	-	31.07 ± 2.31^{b}	28.47 ± 2.28^{b}	36.14 ± 3.26^{a}	24.45 ± 4.64^{c}	29.51 ± 3.25^{b}
Water loss (%)	-	22.61	10.30	27.78	6.46	15.62

Data are presented as mean ± standard deviation. N =8.

[a-e]Means with differing superscripts in the same row are significantly different ($P<0.05$).

Changes in color of cooked broiler thigh meat after heat treatment are shown in Table 22. The color values of lightness (L*), redness (a*) and redness index (a*/b*) of all cooked samples decreased significantly when compared with raw sample while yellowness (b*) increased in all samples except fried meat. This results were similar not to previous studies which found the increase in L* and b* value and decrease in a* value of muscle after cooked at 95 and 98 °C by deep fat frying (Qiao *et al.*, 2002; Ramírez and Cava, 2004). The lightening is due to an increased reflection of light arising from light scattering by denatured protein, consequence, the L* value increase after heating. While in this study, the L* value decreased might be due to the loss of water from muscle protein and not to be confined within meat surface by this reason, light scattering in meat decreased leading to decrease in L* value of the sample. The decrease in a* value and redness index due to the loss of chroma resulted from the changes in heme pigment especially myoglobin. Myoglobin is almost completely denatured between 80-85 °C (Lawrie, 1991).

Table 22 Color values of raw and cooked broiler thigh meat

Sample	L* value	a* value	b* value	Redness index
Raw meat	42.95 ± 2.85^{a}	2.14 ± 1.56^{a}	11.64 ± 2.76^{cd}	0.18^{a}
Boiled meat	35.36 ± 2.57^{b}	-4.31 ± 0.50^{d}	17.94 ± 1.64^{a}	-0.24^{b}
Steamed meat	37.04 ± 2.79^{b}	-4.57 ± 0.94^{d}	18.15 ± 2.62^{a}	-0.25^{b}
Fried meat	32.09 ± 3.87^{c}	-2.17 ± 0.56^{b}	11.06 ± 2.91^{d}	-0.20^{b}
Grilled meat	41.62 ± 3.68^{a}	-3.18 ± 0.60^{c}	13.25 ± 1.94^{c}	-0.24^{b}
Microwaved meat	37.16 ± 2.87^{b}	-3.65 ± 0.41^{c}	15.97 ± 2.93^{b}	-0.23^{b}

Data are presented as mean ± standard deviation. N =16.

$^{a-d}$Means with differing superscripts in the same column are significantly different (P<0.05).

3.2 Changes in heme pigment

Myoglobin, heme iron, non-heme iron contents and metmyoglobin formation for raw and cooked broiler thigh meat are given in Table 23. The effect of cooking methods on the myoglobin, heme and non-heme iron content and metmyoglobin formation of broiler thigh meat were significantly different when compared with raw meat and within five cooking methods (P<0.05). Similarly, three forms of heme pigment were observed difference between broiler thigh meats baked with electrical oven and microwave oven (Table 23). The lowest content in four form of heme pigments were observed in fried meat because of this method provided high temperature with short time during cooking processes. No significant difference (P>0.05) in myoglobin, heme iron content and metmyoglobin formation between boiled and steamed meat and steamed meat had higher in non-heme iron content than did in boiled meat. Results obtained from this study were different from the results of Gall *et al.* (1983) who found that cooking did not significantly affect the concentration of iron. However, the results from this study were in agreement with the study of Turhan *et al.* (2004) who found that cooking methods (electric oven, grill, microwave and boil) had effected on total and heme iron contents of anchovy (*Engraulis encrasicholus*). The highest total and heme iron losses were found in grilled samples and the lowest were found in boiled samples. According to these results, frying methods caused higher cooking loss than did boiling, microwave cooking, steaming and grilling, respectively.

Table 23 Myoglobin, heme iron, non-heme iron content and metmyoglobin formation of raw and cooked broiler thigh meat

	Raw	Boiled	Steamed	Fried	Grilled	Microwaved
Myoglobin (mg/g meat)	7.51 ± 1.60^{c}	10.28 ± 1.05^{a}	10.96 ± 2.47^{a}	6.93 ± 0.73^{c}	7.09 ± 1.01^{c}	8.58 ± 1.04^{b}
Heme iron (ug/g meat)	13.20 ± 1.96^{d}	43.08 ± 4.47^{a}	45.93 ± 5.10^{a}	28.84 ± 3.07^{c}	32.99 ± 4.29^{bc}	35.82 ± 4.41^{b}
Non-heme iron (ug/g meat)	0.42 ± 0.02^{e}	2.09 ± 0.38^{b}	2.43 ± 0.37^{a}	1.86 ± 0.28^{c}	1.44 ± 0.14^{d}	2.08 ± 0.20^{b}
Metmyoglobin formation (%)	74.18 ± 5.31^{b}	86.38 ± 2.85^{c}	87.61 ± 4.11^{c}	68.27 ± 6.73^{a}	74.77 ± 8.19^{b}	78.51± 4.77^{b}

Data are presented as mean ± standard deviation. N =8.

$^{a-d}$Means with different letters in the same row denote a statistical difference ($P< 0.05$).

The content of all forms of heme pigment in meat was increased after heating presumably due to losing of water in meat as elucidated by the cooking loss and moisture content shown in Table 21. The variety methods of cooking resulted in increase in heme iron, non-heme iron, crude fat content and metmyoglobin formation whereas decrease in myoglobin and moisture content. Notability, the lower of crude fat in fried meat were observed due to lipid losses during heating process. While microwaved meat had lower moisture content but higher lipid content after cooking due to microwave heating is the most effect on liquid water and much less so on fats which have less molecular dipole moment resulted in microwaved meat had lowest crude fat loss after heating. Kongkachuichai *et al.* (2002) reported that cooking processes are important because of their effect on nutrient loss. Results of their study showed that heme iron content in foods decreased during the cooking process, while non-heme iron increased. The increase of non-heme iron was derived from the alteration of hemoglobin and myoglobin structures. These findings are consistent with several observations (Ahn *et al.*, 1993; Wang and Lin, 1994). Cooking processes reduce the amount of heme iron, which is known to be the better absorbed form; however, heating processes are necessary for food safety and human digestibility. Conversely, in this study, heme iron content in cooked meats were increased when compared with that of raw meat because of the calculation based on dry weight basis while previous reports calculated based on edible meat portion.

3.3 Changes in lipid fraction

The lipid fractions; triglyceride, phospholipids and free fatty acid, of cooked broiler thigh meat are shown in Table 24. Triglyceride proportion of all cooked meat decreased ($P<0.05$) while phospholipids and free fatty acid increased ($P<0.05$) when compared with those of raw meat. A significant increase in the phospholipids content of cooked broiler thigh meat after heat processing (Table 24) was in conformity with the findings of earlier researchers (Wilson *et al.*, 1976; Igene *et al.*, 1979; Rao *et al.*, 1996). The significant increase in FFAs content on heat processing was in agreement with the findings of Petro *et al.* (1983), which could be due to thermal hydrolysis (Addis and Warner, 1991) or glycerolipid hydrolysis (Petro *et al.*, 1983). Lipolysis is one of the main processes of the degradation of lipids in fresh meat during processing (Alasnier *et al.*, 2000). Lipolysis is governed by a set of specific enzymes, namely lipases and phospholipases and leads to the formation of free fatty acids (FFAs). The decrease of triglyceride might be due to in fat cells consist more proportion of triglyceride than phospholipids so triglyceride could be loss during heating with loss of total lipid more than losing of phospholipids (Allen and Foegeding, 1981). In this study, the phospholipids and free fatty acid content of boiled, steamed and microwaved meat had a higher content than those of fried and grilled meat. On the other hand, some previous study showed that the free fatty acid content of the grilled samples were significantly lower than those of the raw meat (Rodriguez-Estrada *et al.*, 1997). This indicates that the FFAs were lost in the cooking medium and/or combined with other molecules during the treatments.

Table 24 Triglyceride, phospholipids and free fatty acid content (% of total) of raw and cooked broiler thigh meat

Sample	Triglyceride (%)	Phospholipids (%)	Free fatty acid (%)
Raw meat	60.86 ± 5.46^{a}	27.12 ± 5.41^{d}	12.02 ± 3.96^{c}
Boiled meat	32.64 ± 2.11^{d}	44.01 ± 2.81^{a}	23.35 ± 1.85^{a}
Steamed meat	31.06 ± 0.97^{d}	44.39 ± 1.01^{a}	24.54 ± 0.91^{a}
Fried meat	50.37 ± 1.32^{b}	31.09 ± 1.76^{c}	18.53 ± 1.25^{b}
Grilled meat	40.79 ± 1.39^{c}	38.63 ± 1.60^{b}	20.58 ± 1.18^{b}
Microwaved meat	32.97 ± 1.66^{d}	42.89 ± 1.99^{a}	24.15 ± 1.48^{a}

Data are presented as mean ± standard deviation. N = 8.

[a-d]Means with differing superscripts in the same column are significantly different ($P<0.05$).

3.4 Changes in fatty acid compositions

Figure 31 shows the differences in fatty acids compositions of lipid from raw, boiled, steamed, fried, grilled and microwaved meat. Steamed and boiled meat had the highest proportion of unsaturated fatty acids both mono- and polyunsaturated fatty acid. While fried meat was the highest proportion of saturated fatty acids. Notably, steaming and boiling methods were wet heating that involved with water in process resulted in the higher content of unsaturated fatty acid while dry heating: frying, grilling and microwave cooking obtained cooked meat with the higher in saturated fatty acid. This study found significant differences in the percentages of saturated, monounsaturated and polyunsaturated fatty acids between raw and cooked meat excepted between raw and grilled meat which were not significant difference (Figure 31). On the other hand, Rodriguez-Estrada *et al.* (1997) reported that no significant differences were observed among the percentage of saturated and monounsaturated fatty acids of the raw and the cooked hamburgers and the level of polyunsaturated fatty acids in the raw meat, however, was significantly lower than that of the cooked hamburgers. Notably, fried meat had a higher content of saturated fatty acids ($P<0.05$) when compared with other cooked meat. Because of during frying, the culinary fat or oil acts as a heat transfer medium and becomes an important ingredient of the fried food because water loss as well as penetration of oil into the food takes place (Sánchez-Muniz *et al.*, 1994; Castrillón *et al.*, 1997; Varela and Ruiz-Roso, 2000). As shown in Table 24, C12:0 proportion increased in all cooked meat when compared with those in raw meat especially in fried meat due to the lipolysis resulted in releasing of short chain fatty acid.

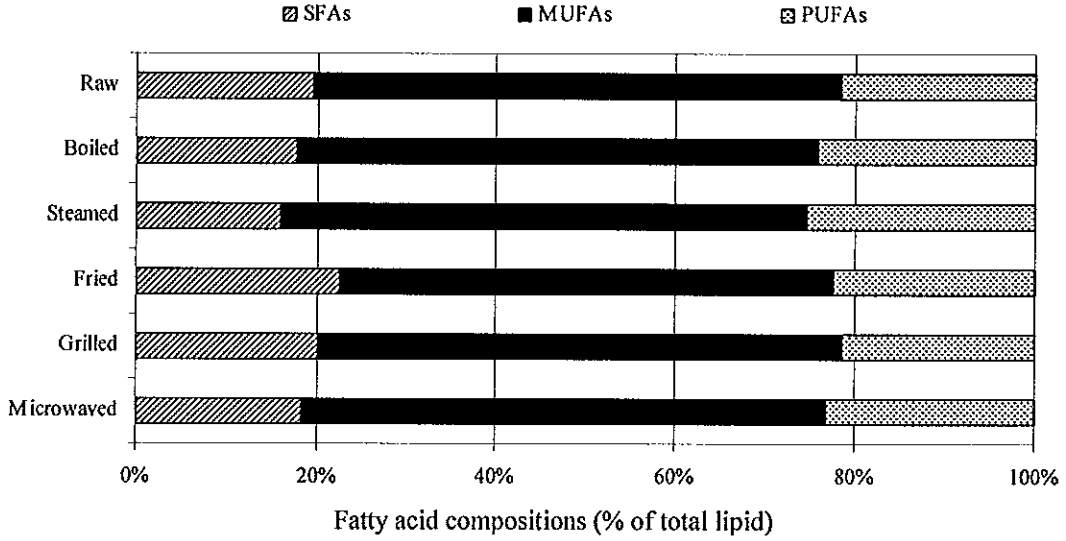


Figure 31 Fatty acid compositions (% of total) of raw and cooked broiler thigh meat.

SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids.

Table 24 Fatty acid compositions (% of total) of raw and cooked broiler thigh meat

Fatty acid	Raw	Boiled	Steamed	Fied	Grilled	Microwaved
C12:0	0.00	0.87 ± 0.01^{bc}	0.73 ± 0.01^{c}	2.01 ± 0.01^{a}	$1.04 \pm 0\ 01^{b}$	0.97 ± 0.01^{b}
C14:0	0.49 ± 0.00^{d}	0.74 ± 0.01^{c}	0.53 ± 0.01^{cd}	1.04 ± 0.01^{a}	0.91 ± 0.01^{b}	0.88 ± 0.01^{b}
C16:0	14.85 ± 0.06^{a}	12.53 ± 0.01^{c}	11.47 ± 0.01^{d}	14.18 ± 0.02^{a}	14.20 ± 0.01^{a}	13.29 ± 0.01^{b}
C17:0	0.00	0.00	0.00	0.00	0.00	0.00
C18:0	4.16 ± 0.02^{b}	3.57 ± 0.01^{c}	3.24 ± 0.01^{d}	5.32 ± 0.01^{a}	3.85 ± 0.01^{c}	3.18 ± 0.01^{d}
SFAs	**19.41 ± 0.01^{b}**	**17.72 ± 0.04^{d}**	**15.96 ± 0.04^{e}**	**22.54 ± 0.06^{a}**	**20.01 ± 0.05^{ab}**	**18.32 ± 0.04^{c}**
C16:1	12.21 ± 0.03^{bc}	12.44 ± 0.01^{b}	13.26 ± 0.01^{a}	10.16 ± 0.02^{d}	11.84 ± 0.02^{c}	12.59 ± 0.01^{b}
C 18:1n-9c,t	34.57 ± 0.13^{a}	33.13 ± 0.01^{ab}	31.80 ± 0.01^{b}	29.07 ± 0.01^{c}	34.24 ± 0.01^{a}	33.92 ± 0.01^{ab}
C20:1	6.34 ± 0.01^{c}	7.12 ± 0.02^{b}	7.59 ± 0.02^{ab}	8.11 ± 0.02^{a}	7.01 ± 0.02^{b}	6.65 ± 0.02^{c}
C24:1	5.78 ± 0.01^{b}	5.25 ± 0.02^{a}	5.95 ± 0.02^{b}	7.55 ± 0.02^{c}	5.36 ± 0.02^{a}	5.27 ± 0.02^{a}
MUFAs	**58.89 ± 0.17^{a}**	**57.94 ± 0.05^{b}**	**58.60 ± 0.05^{a}**	**54.90 ± 0.07^{c}**	**58.45 ± 0.05^{a}**	**58.43 ± 0.05^{a}**
C18:2n-6c,t	11.55 ± 0.01^{a}	11.06 ± 0.01^{b}	11.76 ± 0.01^{a}	9.80 ± 0.02^{e}	9.72 ± 0.01^{e}	11.02 ± 0.01^{b}
C18:3n-6	3.63 ± 0.03^{c}	4.14 ± 0.01^{a}	4.41 ± 0.01^{a}	3.24 ± 0.01^{d}	4.30 ± 0.01^{a}	3.94 ± 0.01^{b}
C18:3n-3	1.94 ± 0.04^{bc}	2.40 ± 0.01^{b}	2.48 ± 0.01^{b}	2.69 ± 0.01^{a}	1.69 ± 0.01^{c}	2.04 ± 0.01^{b}
C20:3n-6	1.20 ± 0.04^{d}	1.81 ± 0.01^{a}	1.83 ± 0.01^{c}	1.76 ± 0.01^{b}	1.43 ± 0.01^{c}	1.76 ± 0.01^{b}
C20:3n-3	1.16 ± 0.04^{c}	1.28 ± 0.01^{b}	1.33 ± 0.01^{b}	1.61 ± 0.01^{a}	1.26 ± 0.01^{b}	1.20 ± 0.01^{ab}
C22:2	1.17 ± 0.04^{a}	1.84 ± 0.01^{c}	1.86 ± 0.01^{c}	1.88 ± 0.02^{c}	1.36 ± 0.01^{b}	1.74 ± 0.01^{b}
C22:6n-3	0.97 ± 0.04^{c}	1.80 ± 0.01^{a}	1.77 ± 0.01^{a}	1.58 ± 0.01^{b}	1.78 ± 0.01^{a}	1.54 ± 0.01^{b}
PUFAs	**21.61 ± 0.25^{e}**	**24.34 ± 0.08^{b}**	**25.43 ± 0.01^{a}**	**22.56 ± 0.10^{d}**	**21.54 ± 0.08^{e}**	**23.24 ± 0.08^{c}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

a-eMeans with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

3.5 Changes in cholesterol and COPs

In this study, cholesterol levels were also analyzed in raw and cooked samples with various methods. All samples, except fried and grilled meat, were observed increase in cholesterol content, the values being the highest increase for the steamed samples (Table 26). A significant increase in the total cholesterol content during heat processing was due to loss of moisture during cooking and was in agreement with the reports of Rathore (1991) in mutton and Rao *et al.* (1996) in buffalo meat.

The COPs of all samples either raw or cooked could not be detected. In the cooked meat without storage, secondary COPs, which can be derived from primary COPs such as 5-cholestane-3β-ol-7-one (cholestanetriol), 25-hydroxycholesterol α-epoxides, β-epoxides and 7β-hydroxy cholesterol were also not detected.

Regarding meat, it is well known that, even during short storage periods after heating, lipid oxidation does occur (De Vore, 1988; Mielche and Bertelsen, 1994; Mielche and Bertelsen, 1995; Kerler and Grosch, 1997). The oxidation of cholesterol proceeds as a part of the lipid oxidation process. Rodriguez-Estrada *et al.* (1997) found no significant differences in the 7-ketocholesterol content of a homogenous meat system (hamburger) among the different cooking treatments (oven cooking, microwave oven cooking, boiling, broiling, barbecue cooking, a combination of traditional oven-microwave oven), although the raw meat already presented a considerably high initial 7-ketocholesterol level (3.5 ppm). On the other hand, oxidation in meat products depends on the quality of the raw materials, the amount of antioxidants added, the processing conditions and the length of ripening and storage.

Table 26 Cholesterol content (mg/g lipid) and COPs (μg/g lipid) of raw and cooked broiler thigh meat

Sample	Cholesterol (mg/g lipid)	Total COPs (μg/g lipid)
Raw meat	105.02 ± 1.10^{c}	ND
Boiled meat	120.56 ± 1.02^{b}	ND
Steamed meat	137.72 ± 0.77^{a}	ND
Fried meat	80.98 ± 0.39^{d}	ND
Grilled meat	102.46 ± 1.33^{e}	ND
Microwaved meat	122.78 ± 0.46^{b}	ND

Data are presented as mean ± standard deviation. N =3. ND = not detected.

[a-d]Means with different letters in the same column are significantly different ($P<0.05$).

Total COPs: 7β-hydroxy cholesterol (7β-HC), cholesterol 5β,6β-epoxide (β-CE), cholesterol 5α,6α-epoxide (α-CE), 5-cholestane-3β-ol-7-one (CT) and 25-hydroxy cholesterol (25-HC).

Detection limit of 7β-HC = 0.0011, β-CE = 0.0009, α-CE = 0.0015, CT = 0.0036 and 25-HC = 0.0022 μg/ml.

3.6 Lipid oxidation

Cooking led to significantly increased oxidation, as reflected by COPs content and, especially, thiobarbituric acid reactive substances (TBARS) values that always expressed as malondialdehyde (MDA) content. As shown in Table 27, the lowest lipid oxidation were observed in fried meat (10.76 μg MDA/g meat) while the highest were observed in steamed meat (18.47 μg MDA/g meat). This cooking related to oxidation has been reported in the literature for different cooking methods (Rao *et al.*, 1996; Kowale *et al*, 1996; Love and Pearson, 1974; Paniangvait *et al.*, 1995; Pikul *et al.*, 1984) and has been attributed to various factors. Among them, protein denaturation, which can lead to the loss of antioxidant enzyme activity (e.g. inactivation of catalase and glutathione peroxidase) or the release of catalytically-active iron from metallo-proteins (mainly myoglobin); disruption of cell membranes, which brings PUFAs into contact with prooxidants; transformation of myoglobin from an antioxidant to a prooxidant species; and thermal decomposition of hydroperoxides to prooxidant species, such as alkoxyl and hydroxyl radicals (Labuza *et al.*, 1969; Frankel, 1998).

Table 27 TBARS value (μg MDA/g meat) of raw and cooked broiler thigh meat

Sample	TBARS (μg MDA /g meat)
Raw meat	1.08 ± 0.13^{a}
Boiled meat	17.59 ± 2.39^{cd}
Steamed meat	18.47 ± 2.41^{d}
Fried meat	10.76 ± 1.96^{b}
Grilled meat	15.41 ± 0.74^{c}
Microwaved meat	17.55 ± 3.12^{cd}

Data are presented as mean ± standard deviation. N =8.

[a-e]Means with different letters in the same column are significantly different ($P< 0.05$).

The lower of crude fat in fried meat were observed due to lipid losses during heating that related to lower lipid oxidation. Whereas the higher lipid oxidation was found in steamed and boiled meat due to there was moisture involved in those methods. Water is known as the important factor could be stimulating lipid oxidation (Lawrie, 1991). Moreover, no significant differences ($P>0.05$) in myoglobin, heme iron content and metmyoglobin

formation were obtained between boiled and steamed meat but steamed meat had higher in non-heme iron content which related to the higher lipid oxidation than boiled meat. The same phenomenon was observed between grilled and microwaved meat. This results indicating to non-heme iron content had higher correlation with the lipid oxidation in cooked meat than another heme pigment form.

4. Effect of cooking methods on changes in chemical and physical compositions and lipid and cholesterol oxidation of cooked broiler thigh meat during refrigerated storage

4.1 Changes in chemical compositions and color values

When storage times extended to 15 days, moisture content of all samples gradually decreased ($P<0.05$) concomitant to the increase ($P<0.05$) in lipid content (Figure 32 and 33). The decrease in moisture content due to drip loss occurred in meat during refrigerated storage and resulted in the decrease in lipid content when concerned based on wet basis.

The a* value and redness index in cooked meat decreased when extended storage time to 15 days coincided to the increase in L* and b* value (Figure 34). The redness index (a*/b* ratio) of all cooked meat decreased when the storage time increased (Figure 35). This ratio was used as an index of apparent change in redness (Chen *et al.*, 1997) and used to evaluate the discoloration in meat during storage (Lee *et al.*, 2003). At day 0, the redness index of fried meat was higher than those of other cooked meats. Boulianne and King (1998) showed a strong positive correlation between total pigment concentration and a* value. The decrease in the redness index was associated with the darkening of meats, resulting from the formation of metmyoglobin (Love, 1983).

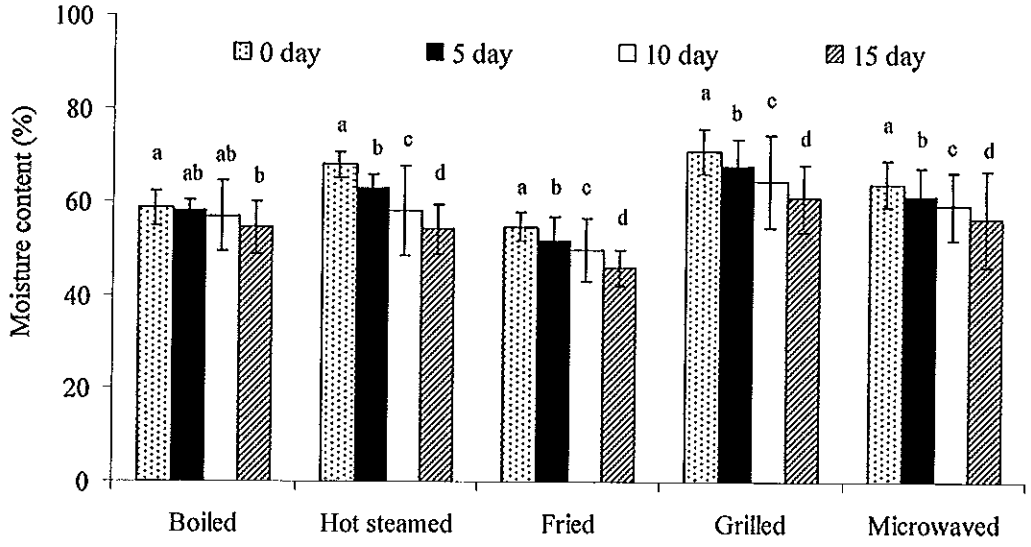


Figure 32 Change in moisture (%) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences ($P<0.05$).

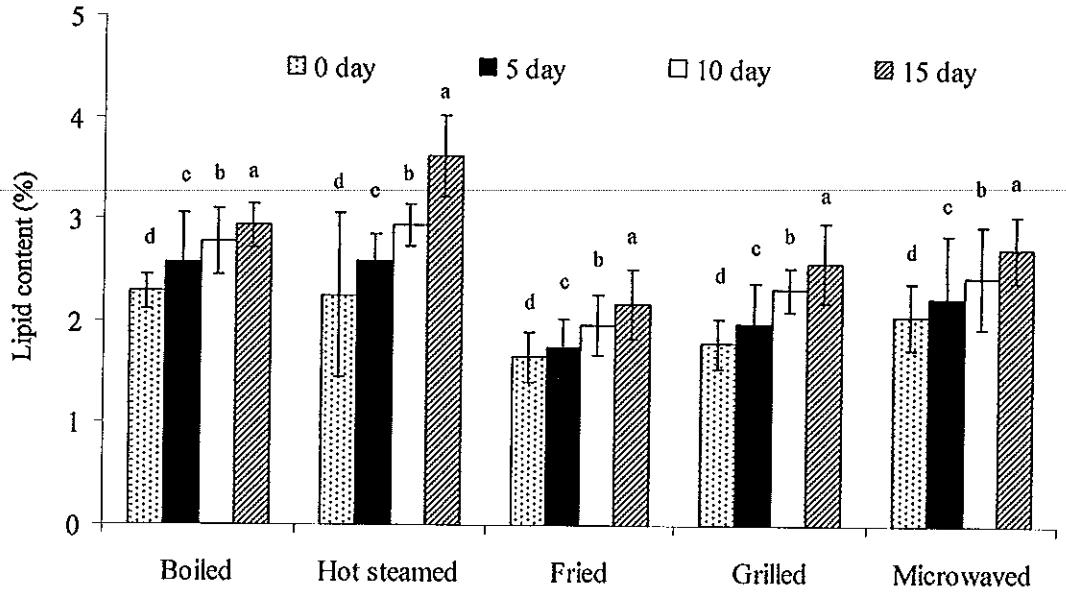
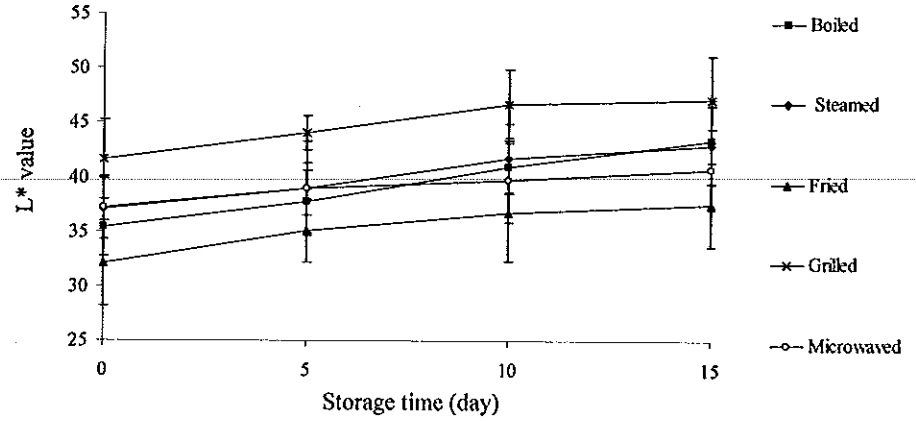


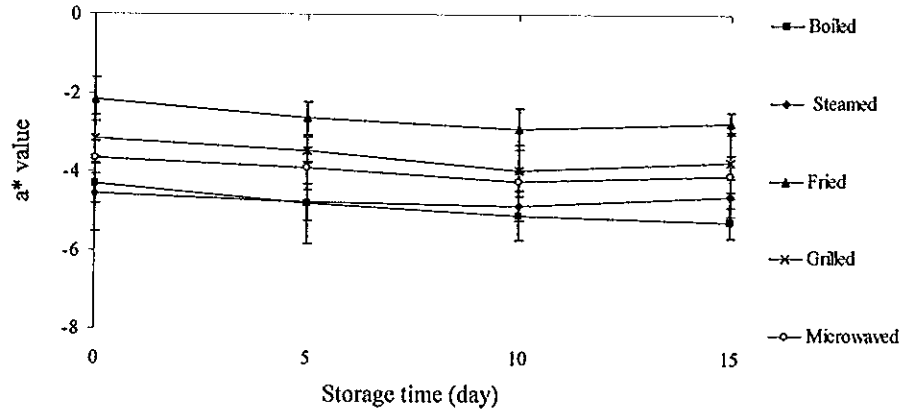
Figure 33 Change in lipid content (% DW) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences ($P<0.05$).

4.2 Changes in heme pigment

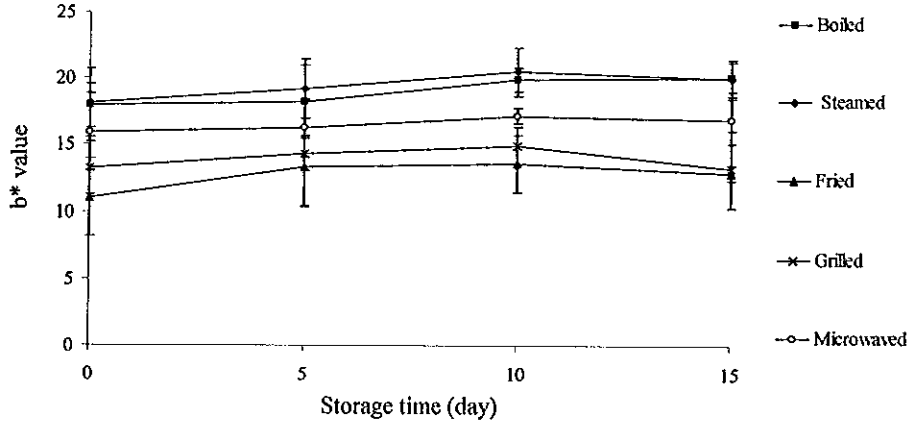
Figure 36-38 and Table 28, show the change of myoglobin, heme iron, and non-heme iron content metmyoglobin formation, respectively of cooked thigh meat by different methods during refrigerated storage. The decrease in myoglobin and heme iron content was observed while non-heme iron content and metmyoglobin formation increased with storage times. The non-heme iron content increased in concomitance with the decrease in heme iron content and metmyoglobin formation increased related to the decrease in myoglobin content. The highest decreasing in myoglobin and heme iron content coincided with the increasing in non-heme iron content and metmyoglobin formation was found in steamed meat during first 6 days of storage while the lowest change was found in fried meat and both case tended to stable until storage at 10 days. Steamed, boiled and grilled meat showed the sharply decrease in myoglobin and heme iron content concomitant with the increase in non-heme iron content when increased storage times when compared with those of fried and microwaved meat.



(a)



(b)



(c)

Figure 34 Change in color values; lightness value (L*) (a), redness (a*) (b) and yellowness (b*) (c), of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from sixteen replicate determinations.

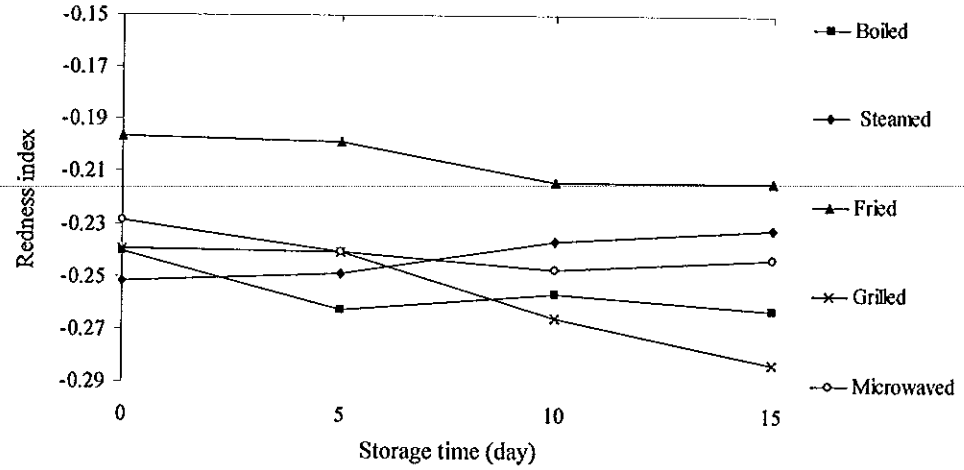


Figure 35 Change in redness index (a*/b*) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 day.

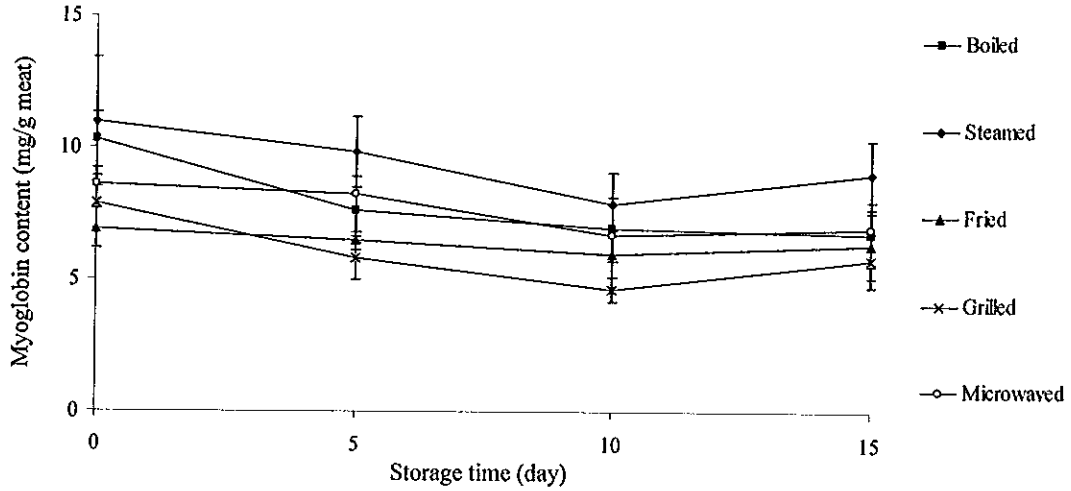


Figure 36 Change in myoglobin content (mg/g meat) of broiler cooked thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

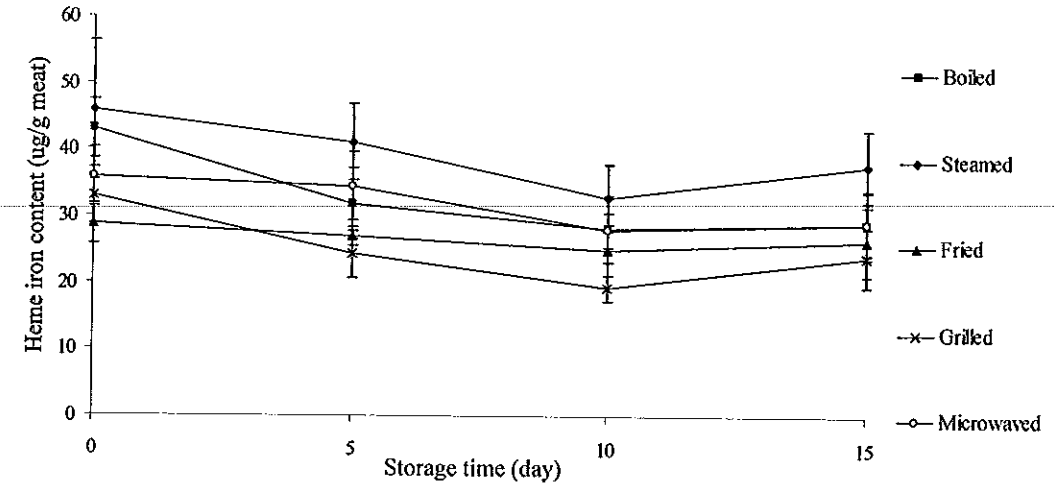


Figure 37 Change in heme iron content (μg/g meat) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

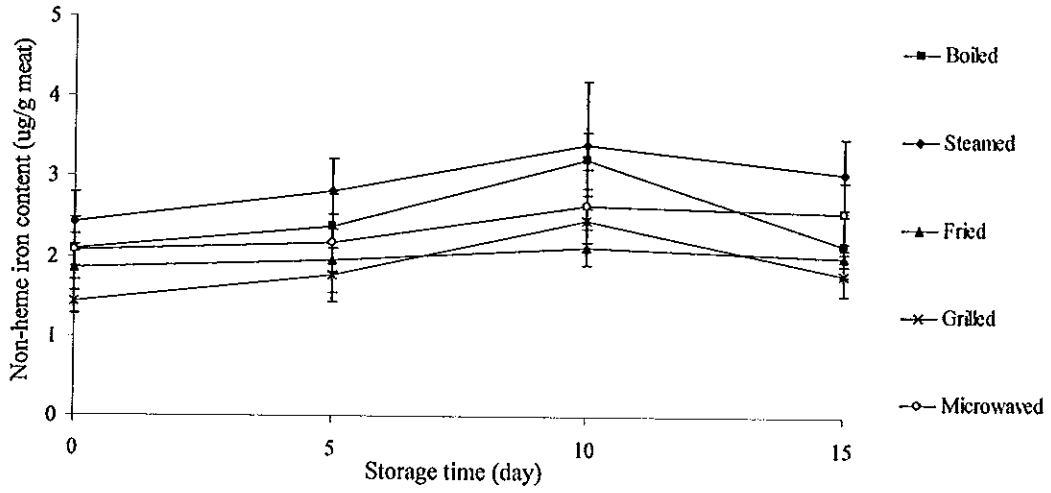


Figure 38 Change in non-heme iron content (μg/g meat) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

Table 28 Metmyoglobin formation of cooked broiler thigh meat during refrigerated storage at 4 °C for 15 days

Storage time (days)	Boiled	Steamed	Fried	Grilled	Microwaved
0	86.38 ± 2.85^{c}	87.61 ± 4.11^{d}	68.27 ± 6.73^{c}	74.77 ± 8.19^{c}	78.51 ± 4.77^{d}
5	93.28 ± 2.59^{b}	90.72 ± 5.50^{b}	71.53 ± 5.90^{b}	80.92 ±11.30^{b}	83.73 ± 8.65^{b}
10	95.12 ± 4.50^{a}	95.80 ± 1.83^{a}	74.10 ± 4.26^{a}	83.36 ± 6.80^{a}	85.79 ± 5.39^{a}
15	95.81 ± 1.51^{a}	89.12 ± 5.96^{c}	73.07 ± 6.42^{a}	80.12 ± 8.60^{b}	81.79± 11.07^{c}

Data are presented as mean ± standard deviation. N =8.

$^{a-d}$Means with different letters in the same column denote a statistical difference (P<0.05).

4.3 Changes in lipid fraction and cholesterol

The changes in triglyceride, phospholipids and free fatty acid content of cooked broiler thigh meat are shown in Figure 39-41, respectively. These results showed the significant decrease in triglyceride and phospholipids content when extended storage time to 15 days, coincided with the increase in free fatty acids content (P<0.05). The alteration of lipid fractions content in cooked meat during refrigerated storage caused by the same reason as described previously in stored raw meat. The increase in free fatty acids content might be due to lipolysis from triglyceride and phospholipids and release free fatty acid during refrigerated storage, moreover, the lipolysis also resulted in the decrease of triglyceride and phospholipids content.

Cholesterol levels were analyzed in cooked meat with different cooking methods during refrigerated storage. The change of cholesterol content among all sample tended to similarly to the change of cholesterol content in raw meat as described in previous section. When extended storage time to 15 days, the decrease of cholesterol content of boiled, steamed, fried, grilled and microwaved meat were observed. At the end of storage, the highest cholesterol content was observed in steamed meat that also had a highest content at the start of storage (Figure 42). The decrease of cholesterol content might be due to cholesterol loss with the oxidation of cholesterol during refrigerated storage.

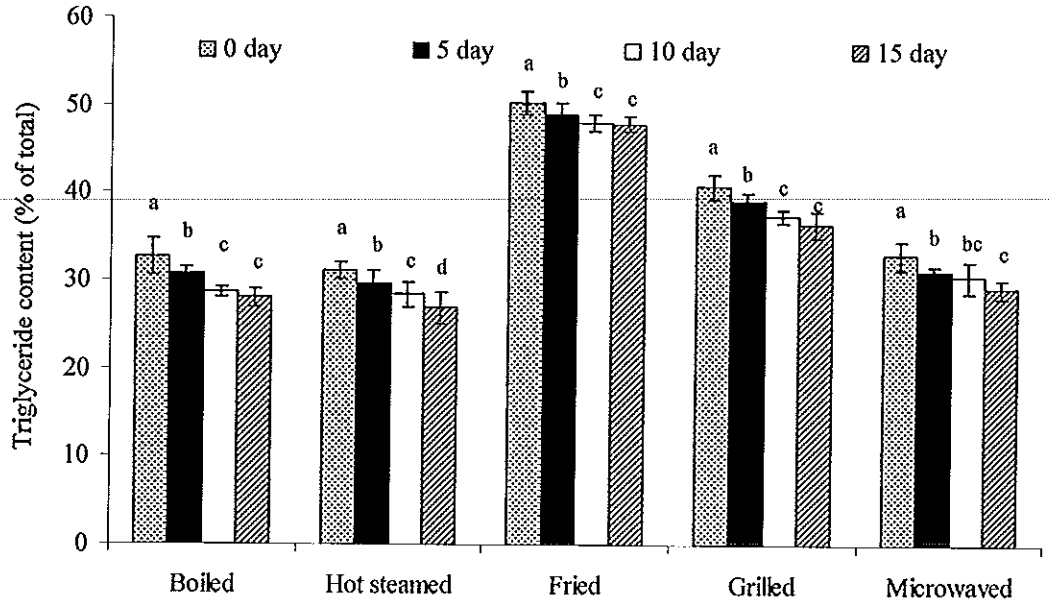


Figure 39 Change in triglyceride content (% of total) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

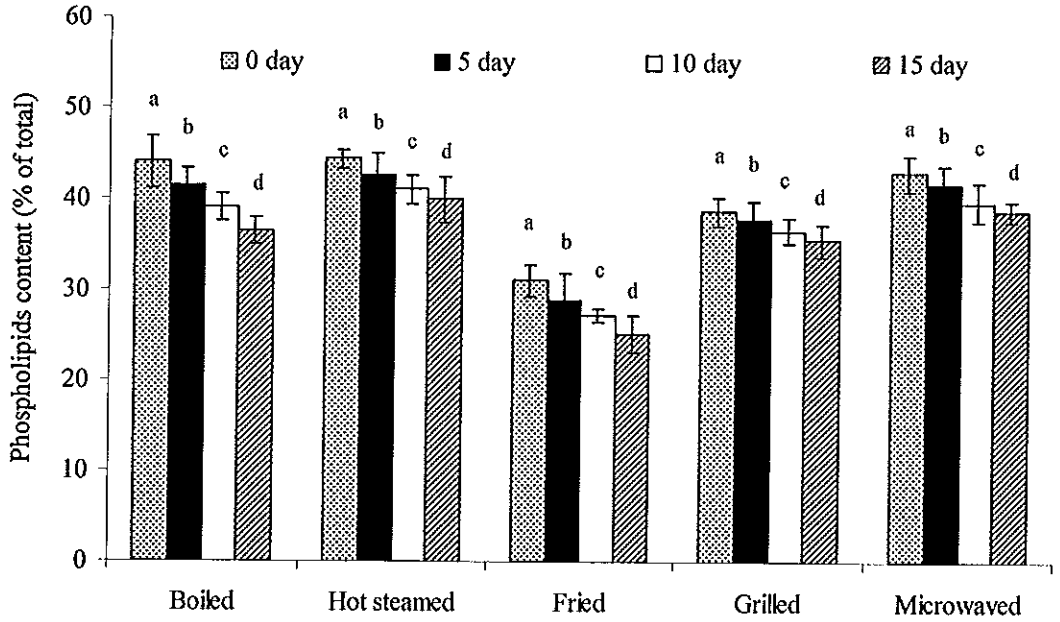


Figure 40 Change in phospholipids content (% of total) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

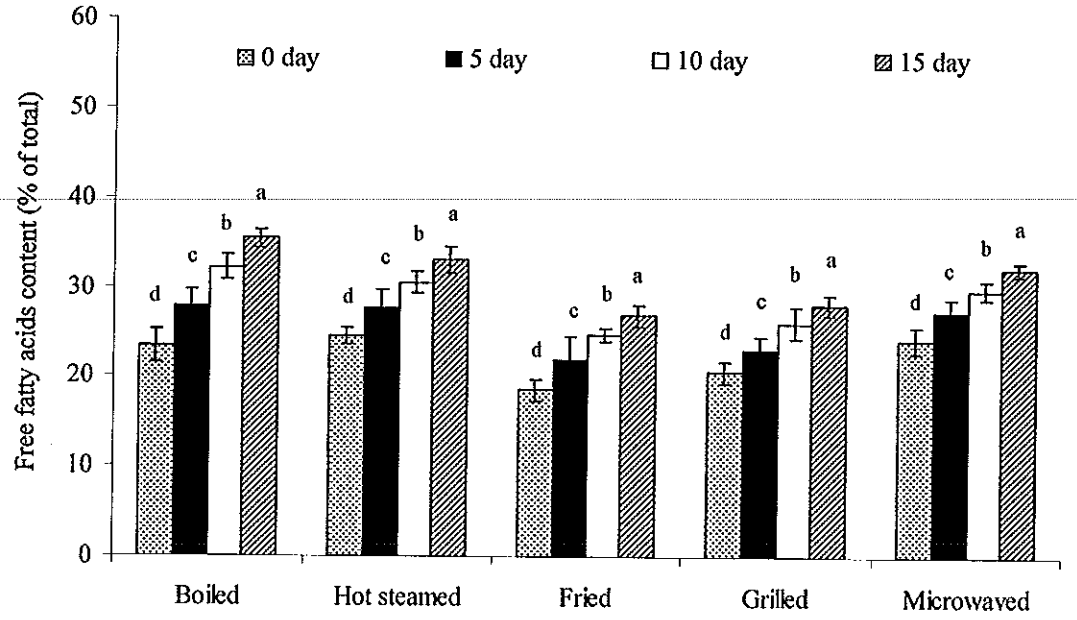


Figure 41 Change in free fatty acids content (% of total) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

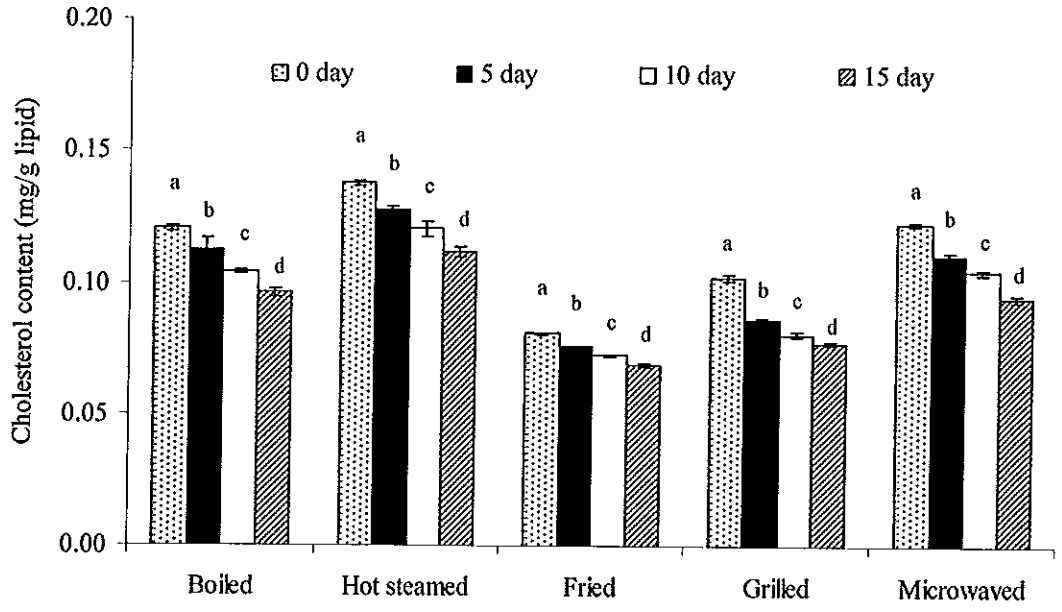


Figure 42 Change in cholesterol content (mg/g lipid) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from three replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

4.4 Changes in fatty acid composition

Tables 29-33 present the fatty acid compositions (% of total) in boiled, steamed, fried, grilled and microwaved broiler thigh meat during refrigerated storage, respectively. There were significantly differences ($P<0.05$) with different cooking methods (boiling, steaming, frying, grilling and microwave cooking). Boiled and steamed meat were found the decrease in SFAs and increase in MUFAs while fried, grilled and microwaved meat were found the decrease in SFAs and MUFAs at first 5 days of storage and then increase until 15 days however the PUFAs of all samples were decrease during storage for 15 days. Lipid oxidation and lipid hydrolysis in refrigerated cooked meat might be one reason that resulted in the decrease of PUFAs in meat samples when extended storage times. From this study, indicating to cooked meat with boiling, steaming, grilling, deep fat frying or microwave cooking could lead to modifications in fatty acid profiles of meat lipids (as shown in Table 29-33).

4.5 Lipid oxidation and COPs

The significantly increase in MDA content of cooked meat when extended storage time to 10 days was show in Figure 43. The increment of MDA content suggesting to the increase in lipid oxidation of cooked meat during refrigerated storage. The highest lipid oxidation of cooked meat was observed in steaming method and the lowest was found in frying method. However, cooking methods did not increase the oxidation of cholesterol at day 0. When extended storage time to 15 days, the increase in all COPs, 5-cholestane-3β-ol-7-one (cholestanetriol), α-epoxides, β-epoxides, 7β-hydroxy cholesterol and 25-hydroxycholesterol, of all cooked meat were observed. When determined at 5 days until 15 days of storage, it was found that steamed meat had more total COPs content than the other cooked meats. Cooking methods affected increase in the cholesterol oxidation in broiler meat with different rate. The highest increment rate of lipid and cholesterol oxidation was found in cooked meat with boiling and steaming methods (as shown in Figure 43 and Table 34).

Table 29 Fatty acid compositions (% of total) of boiled broiler thigh meat during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.87 ± 0.01^{a}	0.73 ± 0.01^{b}	0.61 ± 0.01^{c}	0.50 ± 0.02^{d}
C14:0	0.74 ± 0.01^{a}	0.60 ± 0.01^{b}	0.46 ± 0.01^{c}	0.35 ± 0.02^{d}
C16:0	12.53 ± 0.01^{c}	12.76 ± 0.01^{bc}	12.99 ± 0.02^{b}	13.23 ± 0.03^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	3.57 ± 0.01^{a}	3.52 ± 0.01^{a}	3.49 ± 0.01^{a}	3.47 ± 0.02^{a}
SFAs	**17.72 ± 0.04^{a}**	**17.61 ± 0.05^{a}**	**17.54 ± 0.06^{a}**	**17.55 ± 0.07^{a}**
C16:1	12.44 ± 0.01^{b}	12.62 ± 0.02^{b}	12.81 ± 0.02^{ab}	13.00 ± 0.02^{a}
C 18:1n-9c,t	33.13 ± 0.01^{d}	33.96 ± 0.02^{c}	34.78 ± 0.04^{b}	35.59 ± 0.06^{a}
C20:1	7.12 ± 0.02^{a}	7.15 ± 0.02^{a}	7.18 ± 0.01^{a}	7.22 ± 0.01^{a}
C24:1	5.25 ± 0.02^{a}	5.22 ± 0.02^{a}	5.20 ± 0.01^{a}	5.18 ± 0.01^{a}
MUFAs	**57.94 ± 0.05^{d}**	**58.95 ± 0.07^{c}**	**59.96 ± 0.09^{b}**	**60.99 ± 0.11^{a}**
C18:2n-6c,t	11.06 ± 0.01^{b}	11.20 ± 0.01^{ab}	11.33 ± 0.01^{a}	11.44 ± 0.01^{a}
C18:3n-6	4.14 ± 0.01^{a}	4.06 ± 0.01^{a}	3.97 ± 0.02^{ab}	3.86 ± 0.02^{b}
C18:3n-3	2.40 ± 0.01^{a}	2.23 ± 0.01^{a}	2.05 ± 0.02^{b}	1.85 ± 0.03^{c}
C20:3n-6	1.81 ± 0.01^{a}	1.65 ± 0.01^{b}	1.49 ± 0.02^{c}	1.31 ± 0.02^{d}
C20:3n-3	1.28 ± 0.01^{a}	1.10 ± 0.01^{b}	0.92 ± 0.02^{c}	0.72 ± 0.02^{d}
C22:2	1.84 ± 0.01^{a}	1.61 ± 0.02^{b}	1.38 ± 0.02^{c}	1.13 ± 0.03^{d}
C22:6n-3	1.80 ± 0.01^{a}	1.59 ± 0.01^{b}	1.38 ± 0.02^{c}	1.16 ± 0.03^{d}
PUFAs	**24.34 ± 0.08^{a}**	**23.44 ± 0.09^{b}**	**22.50 ± 0.13^{c}**	**21.46 ± 0.16^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 30 Fatty acid compositions (% of total) of steamed broiler thigh meat during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.73 ± 0.01^{a}	0.60 ± 0.01^{b}	0.45 ± 0.01^{c}	0.33 ± 0.01^{d}
C14:0	0.53 ± 0.01^{a}	0.40 ± 0.02^{b}	0.24 ± 0.01^{c}	0.10 ± 0.01^{d}
C16:0	11.47 ± 0.01^{b}	11.64 ± 0.02^{ab}	11.79 ± 0.02^{ab}	11.96 ± 0.02^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	3.24 ± 0.01^{a}	3.19 ± 0.01^{a}	3.12 ± 0.01^{a}	3.07 ± 0.02^{a}
SFAs	**15.96 ± 0.04^{a}**	**15.83 ± 0.06^{a}**	**15.59 ± 0.06^{a}**	**15.46 ± 0.07^{a}**
C16:1	13.26 ± 0.01^{d}	13.45 ± 0.01^{c}	13.64 ± 0.02^{b}	13.82 ± 0.03^{a}
C 18:1n-9c,t	31.80 ± 0.01^{c}	32.50 ± 0.02^{b}	33.23 ± 0.03^{a}	33.92 ± 0.04^{a}
C20:1	7.59 ± 0.02^{a}	7.63 ± 0.02^{a}	7.67 ± 0.02^{a}	7.71 ± 0.02^{a}
C24:1	5.95 ± 0.02^{a}	5.94 ± 0.02^{a}	5.94 ± 0.02^{a}	5.93 ± 0.02^{a}
MUFAs	**58.60 ± 0.05^{d}**	**59.52 ± 0.05^{c}**	**60.48 ± 0.08^{b}**	**61.38 ± 0.10^{a}**
C18:2n-6c,t	11.76 ± 0.01^{b}	11.90 ± 0.02^{b}	12.06 ± 0.01^{a}	12.19 ± 0.02^{a}
C18:3n-6	4.41 ± 0.01^{a}	4.34 ± 0.01^{ab}	4.27 ± 0.02^{b}	4.21 ± 0.02^{b}
C18:3n-3	2.48 ± 0.01^{a}	2.32 ± 0.01^{a}	2.18 ± 0.02^{b}	2.02 ± 0.03^{b}
C20:3n-6	1.83 ± 0.01^{a}	1.68 ± 0.01^{ab}	1.55 ± 0.02^{b}	1.40 ± 0.02^{b}
C20:3n-3	1.33 ± 0.01^{a}	1.17 ± 0.01^{b}	1.01 ± 0.02^{c}	0.85 ± 0.02^{d}
C22:2	1.86 ± 0.01^{a}	1.65 ± 0.02^{b}	1.46 ± 0.02^{c}	1.25 ± 0.03^{d}
C22:6n-3	1.77 ± 0.01^{a}	1.58 ± 0.02^{b}	1.41 ± 0.02^{c}	1.23 ± 0.03^{d}
PUFAs	**25.43 ± 0.01^{a}**	**24.65 ± 0.10^{b}**	**23.93 ± 0.12^{c}**	**23.16 ± 0.17^{c}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 31 Fatty acid compositions (% of total) of fried broiler thigh meat during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	2.01 ± 0.01^{a}	1.92 ± 0.02^{b}	1.77 ± 0.01^{c}	1.63 ± 0.01^{d}
C14:0	1.04 ± 0.01^{a}	0.91 ± 0.02^{b}	0.72 ± 0.01^{c}	0.54 ± 0.01^{d}
C16:0	14.18 ± 0.02^{d}	14.51 ± 0.01^{c}	14.83 ± 0.03^{b}	15.11 ± 0.01^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	5.32 ± 0.01^{a}	5.35 ± 0.02^{a}	5.34 ± 0.01^{a}	5.32 ± 0.01^{a}
SFAs	**22.54 ± 0.06^{a}**	**22.68 ± 0.06^{a}**	**22.65 ± 0.06^{a}**	**22.60 ± 0.04^{a}**
C16:1	10.16 ± 0.02^{b}	10.28 ± 0.03^{ab}	10.45 ± 0.04^{a}	10.56 ± 0.04^{a}
C 18:1n-9c,t	29.07 ± 0.01^{c}	29.86 ± 0.02^{bc}	30.74 ± 0.06^{b}	31.53 ± 0.04^{a}
C20:1	8.11 ± 0.02^{b}	8.17 ± 0.03^{b}	8.24 ± 0.04^{ab}	8.31 ± 0.04^{a}
C24:1	7.55 ± 0.02^{a}	7.59 ± 0.03^{a}	7.63 ± 0.04^{a}	7.69 ± 0.04^{a}
MUFAs	**54.90 ± 0.07^{d}**	**55.90 ± 0.10^{c}**	**57.03 ± 0.18^{b}**	**58.08 ± 0.15^{a}**
C18:2n-6c,t	9.80 ± 0.02^{a}	9.90 ± 0.02^{a}	10.04 ± 0.02^{a}	10.15 ± 0.02^{a}
C18:3n-6	3.24 ± 0.01^{a}	3.11 ± 0.02^{a}	2.98 ± 0.03^{b}	2.86 ± 0.02^{b}
C18:3n-3	2.69 ± 0.01^{a}	2.50 ± 0.02^{b}	2.31 ± 0.04^{c}	2.13 ± 0.03^{d}
C20:3n-6	1.76 ± 0.01^{a}	1.56 ± 0.02^{b}	1.37 ± 0.03^{bc}	1.20 ± 0.03^{c}
C20:3n-3	1.61 ± 0.01^{a}	1.41 ± 0.02^{b}	1.21 ± 0.03^{c}	1.02 ± 0.03^{d}
C22:2	1.88 ± 0.02^{a}	1.61 ± 0.03^{b}	1.34 ± 0.04^{c}	1.09 ± 0.03^{d}
C22:6n-3	1.58 ± 0.01^{a}	1.33 ± 0.02^{b}	1.08 ± 0.04^{c}	0.86 ± 0.03^{d}
PUFAs	**22.56 ± 0.10^{a}**	**21.42 ± 0.16^{b}**	**20.32 ± 0.24^{c}**	**19.31 ± 0.19^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 32 Fatty acid compositions (% of total) of grilled broiler thigh meat during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	1.04 ± 0.01[a]	0.95 ± 0.01[b]	0.82 ± 0.01[c]	0.71 ± 0.01[d]
C14:0	0.91 ± 0.01[a]	0.81 ± 0.01[b]	0.68 ± 0.01[c]	0.55 ± 0.01[d]
C16:0	14.20 ± 0.01[d]	14.37 ± 0.01[c]	14.63 ± 0.01[b]	14.91 ± 0.01[a]
C17:0	0.00	0.00	0.00	0.00
C18:0	3.85 ± 0.01[a]	3.82 ± 0.01[a]	3.79 ± 0.01[a]	3.76 ± 0.01[a]
SFAs	**20.01 ± 0.05[a]**	**19.96 ± 0.04[a]**	**19.92 ± 0.04[a]**	**19.94 ± 0.03[a]**
C16:1	11.84 ± 0.0[2a]	11.95 ± 0.02[a]	12.11 ± 0.03[a]	12.28 ± 0.04[a]
C 18:1n-9c,t	34.24 ± 0.01[c]	34.78 ± 0.02[c]	35.62 ± 0.03[b]	36.46 ± 0.04[a]
C20:1	7.01 ± 0.02[a]	7.03 ± 0.02[a]	7.05 ± 0.03[a]	7.08 ± 0.04[a]
C24:1	5.36 ± 0.02[a]	5.36 ± 0.02[a]	5.33 ± 0.03[a]	5.31 ± 0.04[a]
MUFAs	**58.45 ± 0.05[d]**	**59.12 ± 0.08[c]**	**60.12 ± 0.12[b]**	**61.12 ± 0.16[a]**
C18:2n-6c,t	9.72 ± 0.01[a]	9.78 ± 0.02[a]	9.88 ± 0.02[a]	9.96 ± 0.02[a]
C18:3n-6	4.30 ± 0.01[a]	4.25 ± 0.02[a]	4.17 ± 0.02[ab]	4.09 ± 0.02[b]
C18:3n-3	1.69 ± 0.01[a]	1.57 ± 0.02[b]	1.38 ± 0.02[c]	1.18 ± 0.03[d]
C20:3n-6	1.43 ± 0.01[a]	1.32 ± 0.02[b]	1.15 ± 0.02[c]	0.97 ± 0.02[d]
C20:3n-3	1.26 ± 0.01[a]	1.14 ± 0.02[b]	0.97 ± 0.02[c]	0.78 ± 0.02[d]
C22:2	1.36 ± 0.01[a]	1.20 ± 0.02[b]	0.97 ± 0.03[c]	0.72 ± 0.03[d]
C22:6n-3	1.78 ± 0.01[a]	1.65 ± 0.02[a]	1.46 ± 0.02[b]	1.25 ± 0.03[c]
PUFAs	**21.54 ± 0.08[a]**	**20.92 ± 0.13[b]**	**19.96 ± 0.15[c]**	**18.94 ± 0.18[d]**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

[a-d]Means with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 33 Fatty acid compositions (% of total) of microwaved broiler thigh meat during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.97 ± 0.01^{a}	0.90 ± 0.02^{a}	0.75 ± 0.02^{b}	0.63 ± 0.03^{c}
C14:0	0.88 ± 0.01^{a}	0.81 ± 0.02^{a}	0.65 ± 0.02^{b}	0.53 ± 0.03^{c}
C16:0	13.29 ± 0.01^{d}	13.45 ± 0.02^{c}	13.68 ± 0.03^{b}	13.95 ± 0.03^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	3.18 ± 0.01^{a}	3.15 ± 0.02^{a}	3.08 ± 0.02^{b}	3.04 ± 0.03^{b}
SFAs	**18.32 ± 0.04^{a}**	**18.31 ± 0.08^{a}**	**18.16 ± 0.10^{b}**	**18.15 ± 0.13^{b}**
C16:1	12.59 ± 0.01^{d}	12.71 ± 0.01^{c}	12.90 ± 0.01^{b}	13.07 ± 0.01^{a}
C 18:1n-9c,t	33.92 ± 0.01^{d}	34.42 ± 0.02^{c}	35.28 ± 0.02^{b}	36.12 ± 0.01^{a}
C20:1	6.65 ± 0.02^{a}	6.66 ± 0.01^{a}	6.67 ± 0.01^{a}	6.67 ± 0.01^{a}
C24:1	5.27 ± 0.02^{a}	5.26 ± 0.01^{a}	5.23 ± 0.01^{a}	5.19 ± 0.01^{a}
MUFAs	**58.43 ± 0.05^{d}**	**59.05 ± 0.03^{c}**	**60.08 ± 0.03^{b}**	**61.05 ± 0.03^{a}**
C18:2n-6c,t	11.02 ± 0.01^{b}	11.09 ± 0.02^{b}	11.23 ± 0.01^{a}	11.36 ± 0.02^{a}
C18:3n-6	3.94 ± 0.01^{a}	3.88 ± 0.01^{b}	3.79 ± 0.02^{c}	3.70 ± 0.02^{c}
C18:3n-3	2.04 ± 0.01^{a}	1.93 ± 0.02^{a}	1.75 ± 0.02^{b}	1.56 ± 0.02^{c}
C20:3n-6	1.76 ± 0.01^{a}	1.66 ± 0.01^{b}	1.50 ± 0.02^{c}	1.34 ± 0.02^{d}
C20:3n-3	1.20 ± 0.01^{a}	1.08 ± 0.01^{b}	0.90 ± 0.02^{c}	0.72 ± 0.02^{d}
C22:2	1.74 ± 0.01^{a}	1.60 ± 0.02^{b}	1.37 ± 0.02^{c}	1.13 ± 0.03^{d}
C22:6n-3	1.54 ± 0.01^{a}	1.41 ± 0.02^{b}	1.20 ± 0.02^{c}	0.99 ± 0.02^{d}
PUFAs	**23.24 ± 0.08^{a}**	**22.64 ± 0.11^{b}**	**21.75 ± 0.13^{c}**	**20.80 ± 0.15^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

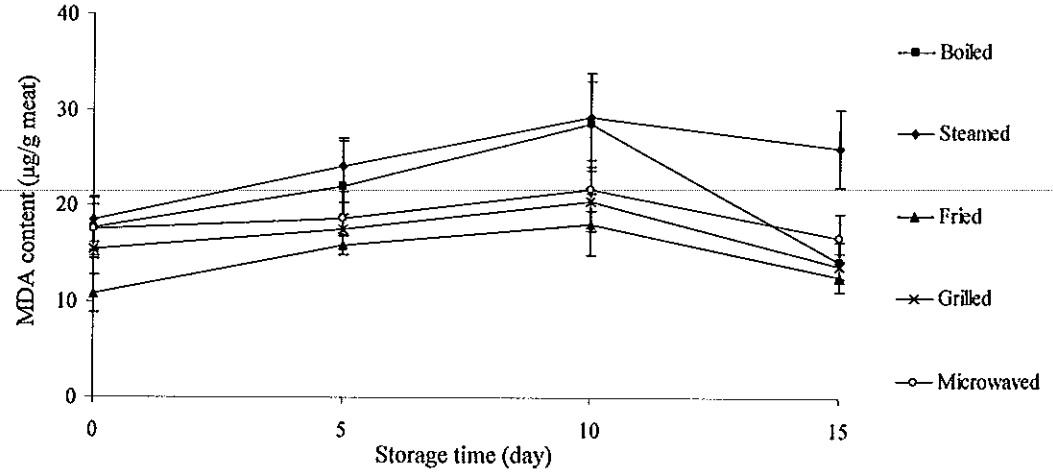


Figure 43 Change in MDA content (µg/g meat) of cooked broiler thigh meat during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

In the cooked meat, secondary COPs, which can be derived from primary COPs such as 5-cholestane-3β-ol-7-one (cholestanetriol), α-epoxides, β-epoxides, 7β-hydroxy cholesterol and 25-hydroxycholesterol, were detected. Cholestanetriol and 25-hydroxycholesterol were reported to be the most atherogenic among oxysterols studied (Taylor *et al.*, 1979). Peng *et al.* (1985) reported that a remarkably acute injury to the endothelium of rabbits resulted from 25-hydroxycholesterol and cholestanetriol. All thermally processed samples also showed no or low levels of COPs, because the temperature during thermal processing did not reach the values required to start the formation of COPs. According to several studies, such as those of Chien *et al.* (1998) and Kim and Nawar (1993), the conversion of cholesterol to COPs depends on the temperature. In fact, up to 120 °C, no or very little conversion is expected. The reduction of the level of cholesterol in thermally processed samples when compared with those in raw samples can be explained by the elution of this substance with fat released from food during processing, thermal degradation, association with other molecules or, probably, a combination of those facts.

According to several researches, such as those of Smith (1996); Paniangvait *et al.* (1995); Careri *et al.* (1998) and Lyons and Brown (1999), cholesterol can be oxidized by the action of enzymes (endogenous reactions) or several agents such as oxygen, heat, pro-oxidant ions, PUFAs and radiations (exogenous reactions), producing variable amounts of COPs. Rodriguez-Estrada *et al.* (1997) reported the presence of 7-ketocholesterol in hamburgers, but most of this oxidized derivative found in samples processed by heating.

The increase in TBARS values with extended storage time to 10 days in cooked broiler thigh meat had high correlation to the increase in lipid content, metmyoglobin formation, non-heme iron, free fatty acid and total COPs coincided with the decrease in moisture, myoglobin, heme iron content, triglyceride, phospholipids, SFAs, MUFAs and PUFAs. The increase of non-heme iron content was concomitant with the decrease in heme iron content due to non-heme iron was released from heme complex due to the breakdown of coordinate covalent bond between iron atom and porphyrin nitrogens during refrigerated storage. Possibility of converting of myoglobin to metmyoglobin was confirmed by the sharp decrease of myoglobin and redness index in chicken meat. Non-heme iron, metmyoglobin and free fatty acids might be accelerating the oxidation process in the muscle (Chan *et al.*, 1997; Lee *et al.*, 2003). Moreover, the radicals from autoxidation of myoglobin could be stimulated lipid oxidation. Alasnier *et al.* (2000) reported that refrigerated storage significantly affected free fatty acids amounts in muscle but the time course of free fatty acid formation during refrigerated storage was not linear and the increase in free fatty acid had high correlation with the increase in lipid oxidation when extended refrigerated storage time to 14 days.

Table 34 Cholesterol oxidation products (COPs) (μg/g lipid) of cooked broiler thigh meat during refrigerated storage for 15 days

Sample	Storage time (day)	COPs content (μg/g lipid)					
		7β -HC	β-CE	α-CE	CT	25-HC	Total
Boiled meat	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	1.85 ± 0.08^{i}	2.19 ± 0.09^{i}	1.74 ± 0.11^{i}	2.18 ± 0.14^{i}	1.44 ± 0.00^{i}	9.40 ± 0.08^{j}
	10	3.17 ± 0.09^{f}	2.75 ± 0.08^{h}	2.60 ± 0.20^{f}	2.62 ± 0.24^{g}	2.23 ± 0.02^{h}	13.37 ± 0.13^{g}
	15	4.39 ± 0.07^{c}	3.39 ± 0.02^{g}	4.11 ± 0.19^{b}	3.65 ± 0.06^{d}	3.40 ± 0.04^{c}	18.94 ± 0.08^{c}
Steamed meat	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	1.90 ± 0.14^{k}	2.72 ± 0.19^{h}	2.20 ± 0.04^{h}	2.75 ± 0.13^{g}	2.16 ± 0.08^{hi}	11.73 ± 0.12^{i}
	10	2.67 ± 0.08^{g}	3.38 ± 0.01^{g}	3.17 ± 0.02^{e}	3.67 ± 0.01^{d}	2.93 ± 0.12^{e}	15.83 ± 0.05^{f}
	15	3.14 ± 0.06^{c}	4.35 ± 0.09^{d}	4.50 ± 0.19^{a}	4.73 ± 0.18^{a}	3.36 ± 0.22^{c}	20.09 ± 0.15^{b}
Fried meat	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	2.69 ± 0.06^{g}	3.30 ± 0.05^{g}	2.21 ± 0.12^{h}	2.48 ± 0.05^{h}	1.93 ± 0.11^{i}	12.61 ± 0.08^{k}
	10	3.70 ± 0.07^{e}	4.15 ± 0.13^{e}	3.12 ± 0.10^{e}	3.20 ± 0.08^{e}	2.43 ± 0.14^{g}	16.60 ± 0.10^{e}
	15	4.39 ± 0.07^{c}	4.88 ± 0.06^{b}	4.02 ± 0.11^{c}	3.87 ± 0.21^{c}	3.35 ± 0.27^{c}	20.52 ± 0.14^{b}
Grilled meat	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	3.15 ± 0.05^{f}	3.24 ± 0.13^{g}	2.68 ± 0.02^{f}	2.39 ± 0.01^{h}	2.10 ± 0.10^{hi}	13.56 ± 0.06^{g}
	10	4.69 ± 0.11^{b}	3.68 ± 0.13^{f}	3.27 ± 0.09^{d}	3.07 ± 0.15^{f}	3.20 ± 0.14^{d}	17.91 ± 0.12^{d}
	15	5.19 ± 0.08^{a}	4.10 ± 0.07^{e}	4.16 ± 0.09^{b}	4.14 ± 0.10^{b}	4.55 ± 0.04^{a}	22.14 ± 0.08^{a}
Microwaved meat	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	3.65 ± 0.02^{e}	3.64 ± 0.03^{f}	2.46 ± 0.01^{g}	3.15 ± 0.20^{e}	2.63 ± 0.07^{f}	15.53 ± 0.06^{f}
	10	4.20 ± 0.10^{d}	4.59 ± 0.05^{c}	3.26 ± 0.09^{d}	3.57 ± 0.06^{d}	3.14 ± 0.13^{d}	18.77 ± 0.08^{c}
	15	4.78 ± 0.08^{b}	5.11 ± 0.03^{a}	4.14 ± 0.20^{b}	4.26 ± 0.23^{b}	3.72 ± 0.17^{b}	22.01 ± 0.14^{a}

Data are presented as mean ± standard deviation. N = 3.

[a-j]Means with differing superscripts in the same column are significantly different ($P<0.05$).

7β-HC = 7β-hydroxy cholesterol, β-CE = cholesterol 5β, 6β-epoxide, α-CE = cholesterol 5α, 6α-epoxide, CT = 5-cholestane-3β ol-7-one, 25-HC = 25-hydroxy cholesterol.

5. Effect of heating temperatures on changes in chemical and physical compositions and lipid oxidation of broiler thigh meat

The heating time of broiler thigh meat was recorded during heating the samples as shown in Table 35. All samples were heated and allowed to reach at different average end point internal temperature (70, 80 and 90 °C) of thigh meat. For study the effect of heating temperatures on cooked meat, the steaming and microwave cooking methods were selected for heating method due to the both methods affected high lipid oxidation in cooked meat as elucidated in previous past.

Table 35 Heating condition of broiler thigh meat to different end point internal temperature

Cooking method	Internal end point temperature (°C)	Convector/Conductor temperature (°C)	Overall time (min)
Steaming	70 ± 1	90 ± 1	18
	80 ± 1	90 ± 1	22
	90 ± 1	95 ± 1	26
Microwave cooking	70 ± 1	150 ± 1	21
	80 ± 1	170 ± 1	23
	90 ± 1	180 ± 1	28

Note: Individually meat sample size 146-160 grams with skin.

5.1 Changes in chemical properties and color value

Table 36 shows the amounts of water and crude fat before and after cooking including to cooking loss and water loss after cooking of broiler thigh meat. The loss of water in steamed meat at temperature of 70, 80 and 90 °C were 2.07, 8.31 and 19.97% of their initial water content (raw meat), respectively, while for microwaved meat were 8.73, 12.76 and 59.78 % of their initial water content (raw meat), respectively (Table 36). The loss of water in microwaved meat was higher ($P<0.05$) than that of steamed meat. When increased final temperature of cooked meat from both cooking methods, the water loss increased due to the leaching effect of water in combination with a longer cooking time. Notably, cooking meat to end point temperature at 90 °C caused sharply increase in water loss than those at other temperatures. Cooking loss of cooked thigh meat with both cooking methods increased with increasing internal temperature in concomitance with the increasing of water loss. The water and crude fat content of both cooked meats were decreased with increased internal

temperature of cooked meat, except lipid content of steamed meat at 80 °C end point temperature was higher than those at 70 °C end point temperature (Table 36). The decrease of lipid after thermal processing was probably due to lipid could be lost during cooking meat samples and the higher end point temperature of microwave cook leading to higher meat lipid lost and also when increased end point temperature of Steaming from 70 to 90 °C.

Table 35 Crude fat, water content and cooking loss (%) of raw, steamed and microwaved broiler thigh meat at 70, 80 and 90 °C internal end point temperature

Percentage	Raw meat	Steamed meat (°C)			Microwaved meat (°C)		
		70	80	90	70	80	90
Crude fat	1.87 ± 0.55^{ab}	1.88 ± 0.23^{ab}	2.11 ± 0.30^{a}	2.41 ±0.11^{bc}	1.78 ± 0.80^{b}	1.88 ± 0.19^{c}	2.32 ± 0.31^{d}
Water content	75.80 ± 1.58^{a}	74.23 ± 3.98^{a}	69.50 ± 10.19^{b}	60.66 ± 2.50^{d}	69.18 ± 11.13^{b}	66.13 ± 8.85^{c}	57.31 ± 7.19^{e}
Cooking loss	-	26.39 ± 2.20^{d}	28.07 ± 1.25^{c}	34.03 ± 3.28^{a}	24.1 ± 2.64^{e}	27.53 ± 3.06^{c}	30.49 ± 3.34^{b}
Water loss	-	2.07	8.31	19.97	8.73	12.76	59.78

Data are presented as mean ± standard deviation. N =8.

$^{a-e}$Means with differing superscripts in the same row are significantly different (P<0.05).

Changes in color of steamed and microwaved broiler thigh meat after heat treatment at different end point temperatures are shown in Table 37. The C.I.E. system values of lightness (L*), redness (a*) and redness index (a*/b*) of both methods cooked meat samples decreased significantly when increased internal meat temperatures from 70 to 90 °C while yellowness (b*) increased with increased meat temperatures. But the redness index (a*/b*) was not significantly different with increased end point temperatures to 90 °C. This suggesting to the detected changes in color values was not associated with visible or measured changes in external color. The increase in end point temperature led to a decrease in luminosity of samples and might be released the pigment from samples that resulted in lightness and redness values decreased. Changes in the surface reflection properties when the meat samples were heated for a long times to reach the recommended end point can provoke this luminosity decrease. Another reason, the L* value decreased might be due to the loss of water from denaturizing of muscle protein and not to be confined within meat surface so from this reason, light scattering in meat decreased and decrease in L* value of sample in this study.

Table 37 Color values of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C internal end point temperature

Sample	Meat temp. (°C)	L*	a*	b*	Redness index
Steamed meat	70	41.76 ± 2.89^{a}	-3.95 ± 0.48^{d}	17.54 ± 1.50^{b}	-0.22^{ab}
	80	39.12 ± 4.69^{c}	-4.25 ± 0.70^{e}	17.90 ± 2.53^{ab}	-0.24^{ab}
	90	35.24 ± 4.47^{d}	-4.73 ± 0.61^{f}	18.28 ± 0.94^{a}	-0.26^{b}
Microwaved meat	70	40.22 ± 1.99^{b}	-3.24 ± 0.56^{a}	15.23 ± 1.20^{d}	-0.21^{a}
	80	38.97 ± 3.80^{cd}	-3.40 ± 0.59^{b}	15.57 ± 1.17^{d}	-0.22^{a}
	90	35.68 ± 2.60^{d}	-3.79 ± 0.39^{c}	16.34 ± 1.30^{c}	-0.23^{ab}

Data are presented as mean ± standard deviation. N =16.

$^{a-f}$Means with differing superscripts in the same column are significantly different ($P<0.05$).

5.2 Changes in heme pigment

Myoglobin, heme iron, non-heme iron contents and metmyoglobin formation for steamed and microwaved broiler thigh meat are given in Table 38. The effect of heat temperatures on the myoglobin, heme and non-heme iron content and metmyoglobin formation of cooked thigh meat from both cooking methods were significantly different ($P<0.05$) when increased end point temperature. As shown in Table 38, the highest change in myoglobin, heme iron and non-heme iron content and metmyoglobin formation was found in steamed and microwaved meat at 70 °C end point temperature when compared with those at 80 and 90 °C end point temperatures. This result indicated that the higher end point temperature was not affected on the increase in the change in heme pigment. Heme pigments content of cooked meat increased significantly when it was heated to end point temperature of 90 °C ($P<0.05$). Kristensen and Purslow (2001) reported that heating temperatures has a gross effect on heme/non-heme iron with a decrease in heme iron content and increase in non-heme iron content when increase heating temperatures from 55 to 80 °C (increased 5 °C/step). The inverse relationship between heme and non-heme iron contents, as a function of heating temperatures, is in agreement with previous work where both heme and non-heme iron were measured in meat heat-treated at different temperatures (Buchowski *et al.*, 1998; Carpenter and Clark, 1995; Han *et al.*, 1993). Han *et al.* (1993) measured heme iron content in beef and chicken as a function of heating temperature and showed a large decrease in heme iron content between 55 and 70 °C. The major part of heme iron in meat is located in myoglobin and the thermal stability of myoglobin is highly dependent on an intact heme molecule

(Chanthai *et al.*, 1996a, b; Hargrove and Olson, 1996). The destruction of heme molecule will therefore easily lead to denaturation of myoglobin. Thus, loss of iron from the heme molecule will not occur in heat treated meat without denaturation of the myoglobin molecule. Geileskey *et al.* (1998) have investigated the kinetics of myoglobin denaturation in meat and found a very low denaturation rate at 55 °C. At temperatures above 55 °C, a large increase in denaturation rate was observed. An explanation for this study, at temperatures above 55 °C, the major part of myoglobin is denatured where the iron from heme can be liberated, which is the case at higher temperatures (70, 80 and 90 °C). The sharp increase in metmyoglobin formation and decrease in myoglobin content with increased heating temperatures suggested that myoglobin underwent more oxidation at high temperature.

Table 38 Myoglobin, heme iron, non-heme iron content and metmyoglobin formation of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature

	Steamed meat (°C)			Microwaved meat (°C)		
	70	80	90	70	80	90
Myoglobin (mg/g meat)	14.16 ± 0.54^{a}	12.04 ± 2.50^{b}	8.02 ± 0.73^{d}	13.71 ± 1.26^{ab}	10.80 ± 1.02^{c}	6.62 ± 0.85^{e}
Heme iron (µg/g meat)	51.51 ± 4.80^{a}	48.20 ± 9.75^{b}	43.06 ± 3.35^{c}	41.81 ± 3.45^{d}	38.75 ± 3.92^{e}	32.74 ± 2.80^{f}
Non-heme iron (µg/g meat)	1.97 ± 0.35^{cd}	2.21 ± 0.12^{c}	2.68 ± 0.12^{a}	1.73 ± 0.07^{d}	1.92 ± 0.17^{cd}	2.41 ± 0.33^{b}
Metmyoglobin formation (%)	77.45 ± 6.80^{d}	79.39 ± 3.39^{c}	90.47 ± 2.89^{a}	70.49 ± 8.35^{f}	74.26 ± 4.32^{e}	81.43 ± 3.79^{b}

Data are presented as mean ± standard deviation. N =8.

$^{a-f}$Means with different letters in the same row denote a statistical difference ($P<0.05$).

5.3 Changes in lipid fraction

Cooking led to a significant loss of triglyceride and phospholipids proportion, concomitant with the increase in free fatty acid when increase end point temperature (Table 39). With a higher end point temperature, the increase in free fatty acids proportion was observed due to a higher in lipolysis occurred when heating for longer times. Whereas the decrease in triglyceride and phospholipids proportion was probably due to the losses during heating to reach the higher final temperature and might be resulted by triglyceride and phospholipids could be occurred the lipolysis to formed free fatty acid. In this study, there was a higher phospholipids proportion than triglyceride proportion though generally meat had

a higher proportion of triglyceride than phospholipids. Ono *et al.* (1985) suggested that most of lipid losses in meat containing mainly triacylglycerols of adipose tissues with relatively more saturated than unsaturated fatty acids and corresponding to Maranesi *et al.* (2005), who also found a reduction of the proportion of saturated fatty acid in meat after cooking. Igene *et al.* (1981) found that drip collected during the cooking of beef and chicken contained primarily triglycerides, whereas phosphatidylethanolamine, a major phospholipid in meat, was essentially absent in the drip, indicating that the phospholipids remained bound to the membranes. Therefore, in this study, thigh meat sample probably consist of membrane tissues more than adipose tissue.

Table 39 Triglyceride, phospholipids and free fatty acid content (% of total lipid) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature

Sample	End point temp. (°C)	Triglyceride (%)	Phospholipids (%)	Free fatty acid (%)
Steamed meat	70	34.45 ± 1.33^{a}	47.23 ± 1.70^{a}	18.33 ± 0.73^{d}
	80	32.20 ± 0.96^{bc}	46.28 ± 1.99^{ab}	21.53 ± 1.69^{c}
	90	29.32 ± 1.49^{d}	42.06 ± 3.21^{d}	28.62 ± 2.54^{a}
Microwaved meat	70	33.96 ± 1.40^{a}	45.39 ± 1.69^{ab}	20.66 ± 1.50^{c}
	80	33.13 ± 1.36^{ab}	44.46 ± 2.08^{bc}	22.42 ± 1.76^{c}
	90	31.17 ± 0.77^{c}	42.68 ± 1.86^{cd}	26.15 ± 2.11^{b}

Data are presented as mean ± standard deviation. N = 8.

[a-d]Means with differing superscripts in the same column are significantly different ($P<0.05$).

5.4 Changes in fatty acid compositions

Figure 44 shows the difference in fatty acid compositions of lipid from steamed and microwaved meat. When increased end point temperatures, the decrease ($P<0.05$) in proportion of SFAs, MUFAs and PUFAs of cooked meat were observed especially MUFAs and PUFAs. At the 70 and 80 °C end point temperatures, steamed meat had a higher ($P<0.05$) in MUFAs proportion but lower ($P<0.05$) in SFAs and PUFAs proportion when compared with those of microwaved meat. While at the 90 °C end point temperature, steamed meat was not significant difference in SFAs and MUFAs proportions when compared with those of microwaved meat, excepted PUFAs proportion of steamed meat had a higher ($P<0.05$) than that of microwaved meat. When increased the end point temperatures both cooking methods, the total of SFAs, MUFAs and PUFAs decreased

significantly because of the higher in melting of fat during cooking to reach the higher end point temperature. Some researcher reported that the advantage of roasting with microwave oven over to other cooking methods such as pan broiling and broiling in water is larger fat retention in semimembranosus muscle of beef (John *et al.*, 1992; Badiani *et al.*, 2002).

Hamm and Deatherage (1960) suggested that cells were dispersed in a protein matrix without showing fat coalescence. Denaturation of protein during heating caused matrix (tissue) to break down and resulted in the fat separation during cooking. Fat distribution was altered by using various heat treatments. Generally, uneven distribution of fat in muscle system influenced fat loss during cooking and the fat cells which are located in the interior of muscle, were lost more slowly compared to the fat cells located near the surface of the muscle. During heating of meat to reach the highest end point temperature (90 °C), overall migration of fat globules was higher than other end point temperature (80 and 70 °C) and also higher in saturated, mono- and polyunsaturated fatty acid loss were observed (Figure 44 and Table 40).

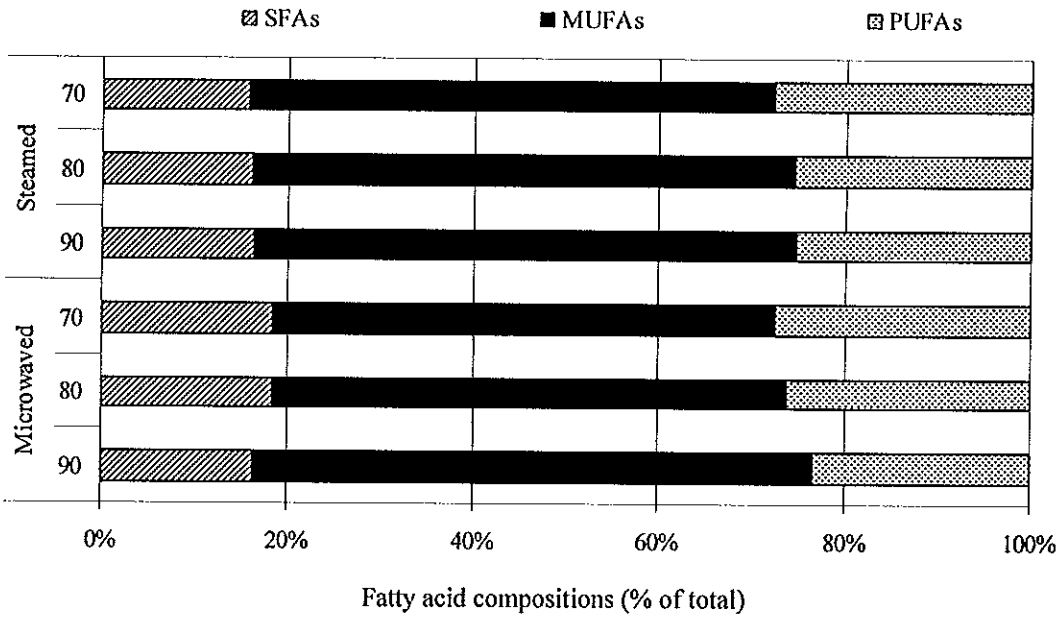


Figure 44 Fatty acid compositions (% of total) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature.

SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids.

Table 40 Fatty acid compositions (% of total) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature

Fatty acid	Steamed meat (°C)			Microwaved meat (°C)		
	70	80	90	70	80	90
C12:0	0.77 ± 0.02^{c}	0.70 ± 0.03^{d}	0.73 ± 0.01^{d}	0.97 ± 0.01^{a}	0.86 ± 0.01^{b}	0.81 ± 0.02^{c}
C14:0	0.66 ± 0.02^{bc}	0.59 ± 0.03^{c}	0.52 ± 0.01^{c}	0.89 ± 0.01^{a}	0.77 ± 0.01^{b}	0.73 ± 0.02^{b}
C16:0	11.07 ± 0.04^{c}	11.61 ± 0.05^{d}	11.90 ± 0.02^{c}	12.42 ± 0.03^{b}	12.73 ± 0.03^{a}	11.94 ± 0.03^{c}
C17:0	0.00	0.00	0.00	0.00	0.00	0.00
C18:0	3.10 ± 0.02^{b}	3.18 ± 0.02^{b}	3.16 ± 0.01^{b}	3.91 ± 0.02^{a}	3.91 ± 0.01^{a}	2.80 ± 0.02^{c}
SFAs	**15.59 ± 0.10^{c}**	**16.08 ± 0.10^{b}**	**16.31 ± 0.06^{b}**	**18.19 ± 0.07^{a}**	**18.27 ± 0.06^{a}**	**16.27 ± 0.10^{b}**
C16:1	12.74 ± 0.02^{c}	13.11 ± 0.05^{b}	13.96 ± 0.02^{a}	12.34 ± 0.04^{d}	12.54 ± 0.04^{cd}	12.78 ± 0.04^{c}
C 18:1n-9c,t	28.94 ± 0.05^{c}	30.28 ± 0.13^{b}	30.21 ± 0.02^{b}	29.97 ± 0.05^{b}	30.84 ± 0.05^{b}	35.55 ± 0.05^{a}
C20:1	7.98 ± 0.02^{a}	8.08 ± 0.04^{a}	7.99 ± 0.02^{a}	6.96 ± 0.03^{ab}	6.97 ± 0.04^{ab}	6.40 ± 0.04^{b}
C24:1	7.01 ± 0.02^{a}	7.05 ± 0.04^{a}	6.28 ± 0.03^{b}	5.10 ± 0.03^{d}	5.03 ± 0.04^{d}	5.65 ± 0.04^{c}
MUFAs	**56.67 ± 0.10^{c}**	**58.51 ± 0.24^{b}**	**58.44 ± 0.07^{b}**	**54.37 ± 0.14^{e}**	**55.38 ± 0.15^{d}**	**60.37 ± 0.15^{a}**
C18:2n-6c,t	12.09 ± 0.01^{a}	12.32 ± 0.03^{a}	11.82 ± 0.02^{b}	10.60 ± 0.02^{c}	10.74 ± 0.02^{c}	11.14 ± 0.02^{b}
C18:3n-6	4.30 ± 0.02^{b}	4.05 ± 0.04^{c}	4.33 ± 0.02^{b}	4.64 ± 0.03^{a}	4.54 ± 0.02^{a}	4.12 ± 0.03^{c}
C18:3n-3	2.79 ± 0.03^{a}	2.36 ± 0.05^{b}	2.61 ± 0.02^{a}	2.49 ± 0.03^{b}	2.26 ± 0.03^{b}	2.00 ± 0.03^{c}
C20:3n-6	2.08 ± 0.02^{b}	1.68 ± 0.05^{c}	1.85 ± 0.02^{bc}	2.22 ± 0.03^{a}	2.03 ± 0.02^{b}	1.75 ± 0.03^{c}
C20:3n-3	2.17 ± 0.02^{a}	1.76 ± 0.05^{b}	1.30 ± 0.02^{bc}	2.29 ± 0.03^{a}	2.09 ± 0.03^{a}	1.17 ± 0.03^{c}
C22:2	2.30 ± 0.03^{c}	1.75 ± 0.06^{cd}	1.67 ± 0.02^{d}	2.80 ± 0.04^{a}	2.53 ± 0.03^{b}	1.69 ± 0.04^{d}
C22:6n-3	2.00 ± 0.03^{b}	1.50 ± 0.06^{cd}	1.66 ± 0.02^{c}	2.40 ± 0.03^{a}	2.16 ± 0.03^{ab}	1.48 ± 0.04^{d}
PUFAs	**27.74 ± 0.16^{a}**	**25.41 ± 0.32^{c}**	**25.25 ± 0.12^{c}**	**27.44 ± 0.20^{a}**	**26.35 ± 0.18^{b}**	**23.35 ± 0.22^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-c}$Means with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

5.5 Changes in cholesterol and COPs

The results show that there were considerable losses of cholesterol from steamed and microwaved meat when increased the end point temperature to 90 °C (Table 41). The results are in agreement with values found in other studies (Sheard *et al.*, 1998; Chappell, 1986). Sheard *et al.* (1998) reported fat and cholesterol losses for cooked pork chop and Chappell (1986) found fat and cholesterol losses for cooked sirloin steak at 90 °C as high as 53%. Cooking methods also had an influence on the magnitude of fat and cholesterol losses: the longer the cooking time, the higher the fat and cholesterol losses. In this study, there was a longer times for heating meat sample to reach the higher end point temperature. Similar results were found by Sheard *et al.* (1998). Regarding beef brisket (boiling 1 h) the losses due to cooking were expected to be higher because of the long cooking time.

Table 41 shows the undetectable of COPs in steamed and microwaved meat after heating. In both cooked meat without storage, secondary COPs, which can be derived from primary COPs such as 5-cholestane-3β-ol-7-one (cholestanetriol), 25-hydroxycholesterol α-epoxides, β-epoxides and 7β-hydroxy cholesterol were also not detected at all heating temperatures.

Table 41 Cholesterol content (mg/g lipid) and COPs (μg/g lipid) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature

Sample	End point temp. (°C)	Cholesterol (mg/g lipid)	COPs (μg/g lipid)
Steamed meat	70	169.76 ± 2.87^{a}	ND
	80	149.38 ± 0.81^{c}	ND
	90	122.54 ± 4.39^{e}	ND
Microwaved meat	70	152.34 ± 2.23^{b}	ND
	80	139.25 ± 0.29^{d}	ND
	90	109.88 ± 6.20^{f}	ND

Data are presented as mean ± standard deviation. N =3. ND = not detected.

$^{a\text{-}f}$Means with different letters in the same column are significantly different ($P<0.05$).

5.6 Lipid oxidation

Table 42 shows the lipid oxidation of steamed and microwaved meat with difference end point temperatures. In fact, the lipid oxidation may be increased with increasing the end point temperature of cooked meat. The highest lipid oxidation was found in steamed and microwaved meat at 90 °C internal temperature. Wattanachant *et al.* (2005) reported that the fibre diameter of chicken muscles was expanded when heated to end-point temperatures above 60 °C suggesting to the protein denaturation occurred when heating. The protein denaturation by cooking, which can lead to the loss of antioxidant enzyme activity or the release of catalytically-active iron from metallo-proteins (mainly myoglobin); disruption of cell membranes, which bring polyunsaturated fatty acids into contact with pro-oxidants: transformation of myoglobin from an antioxidant to a pro-oxidant species; and thermal decomposition of hydroperoxides to pro-oxidant species, such as alkoxyl hydroxyl radicals. Thus, cooking lead to significantly increased oxidation, as reflected by TBARS values (Grau *et al.*, 2001).

Table 42 TBARS value (μg MDA/g meat DW) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature

Sample	End pint temperature (°C)	TBARS (μg MDA/g meat)
Steamed meat	70	13.62 ± 0.98^{d}
	80	16.13 ± 2.08^{c}
	90	21.44 ± 1.16^{a}
Microwaved meat	70	13.70 ± 0.78^{d}
	80	14.96 ± 2.15^{cd}
	90	19.52 ± 1.54^{b}

Data are presented as mean ± standard deviation. N = 8.

[a-d]Means with different letters in the same column are significantly different ($P<0.05$).

6. Effect of heating temperatures on changes in chemical and physical compositions and lipid and cholesterol oxidation of cooked broiler thigh meat during refrigerated storage

6.1 Changes in chemical compositions and color values

When storage times extended to 15 days, moisture content of all sample gradually decreased ($P<0.05$) with concomitant increase ($P<0.05$) in lipid content (Figure 45 and 46, respectively). The change in lipid content of chicken meat conversely to the change of moisture content during refrigerated storage for 15 days. The decrease in moisture content due to drip loss occurred in meat during refrigerated storage and the decrease in lipid content was found in cooked meat especially steamed meat at 80 °C (Figure 45 nd 46) while microwaved meat at 90 °C rather no change during storage.

The a* value and redness index in cooked meat decreased when extended storage time to 15 days coincided to the increase in L* and b* value (Figure 47). The redness index (a*/b* ratio) of cooked meat decreased when increased the storage time (Figure 48). At day 0, the redness index of microwaved meat was higher than that of steamed meat at three end point temperatures. Boulianne and King (1998) showed a strong positive correlation between total pigment concentration and a* value. The decrease in the a* value and redness index was associated with the darkening of meats, resulting from the formation of metmyoglobin (Love, 1983). Renerre and Labas (1987) reported that dark coloration in meat was also associated with the total pigment concentration.

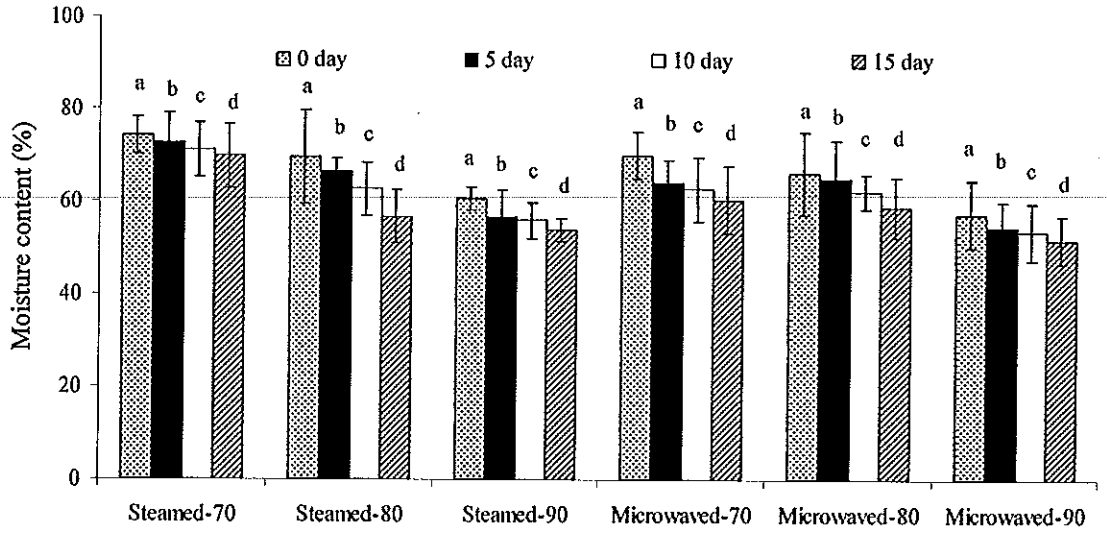


Figure 45 Change in moisture (%) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

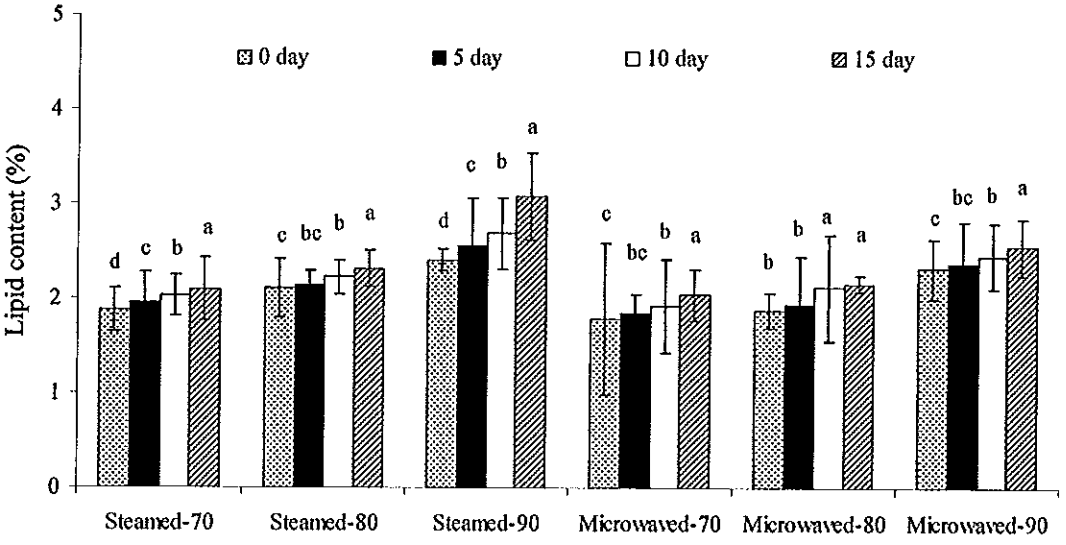
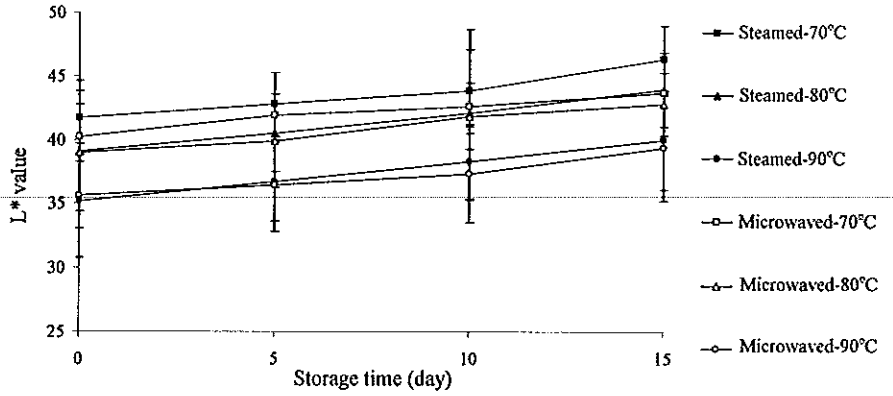
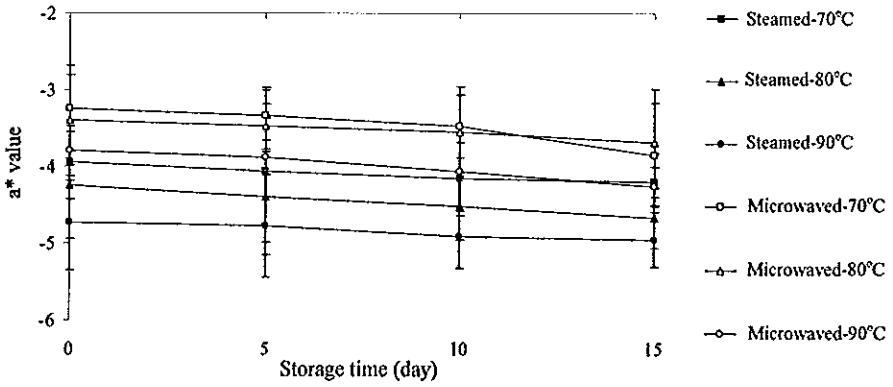


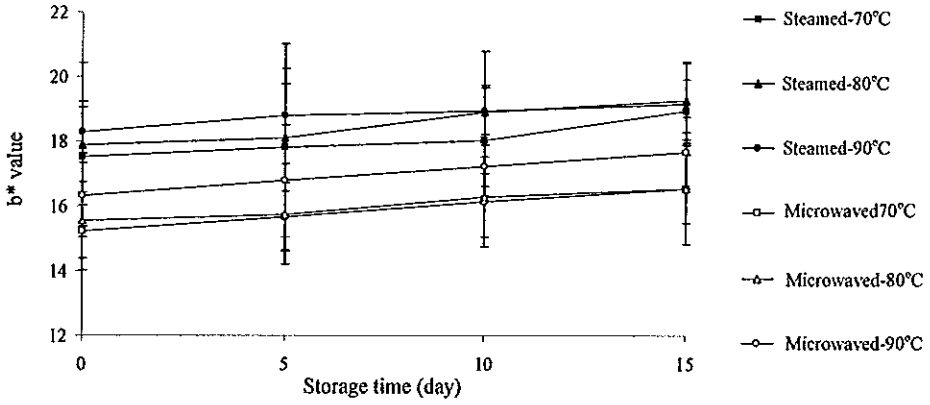
Figure 46 Change in lipid (%) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).



(a)



(b)



(c)

Figure 47 Change in color values; lightness value (L*) (a), redness (a*) (b) and yellowness (b*) (c), of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from sixteen replicate determinations.

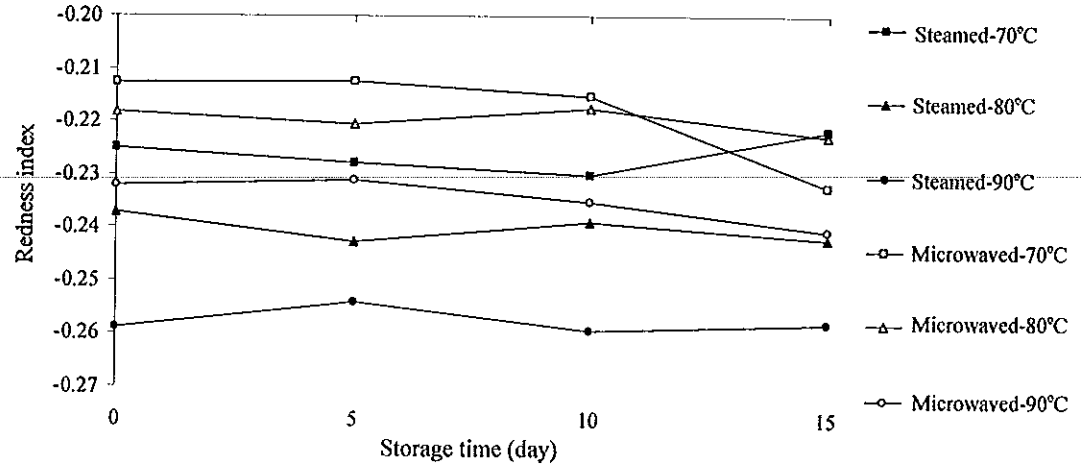


Figure 48 Change in redness index (a*/b*) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days.

6.2 Changes in heme pigment

Fig 49-51 and Table 43, show the change of myoglobin, heme iron, and non-heme iron content metmyoglobin formation, respectively, of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature when extended refrigerated storage times. The decrease in myoglobin and heme iron content was observed while non-heme iron content and metmyoglobin formation were increased. The decrease in myoglobin and heme iron content coincided with the increase in non-heme iron content and metmyoglobin formation in cooked meat during storage have been described in the previous section. The highest changing rate in heme pigment was found in steamed and microwaved meat at 70 and 90 °C end point temperatures while the lowest was found in steamed and microwaved meat at 80 °C end point temperature. The decreased myoglobin and heme iron content at first 10 day of storage is presumably due to the release of free iron from heme. The increment of this content at 15 days storage might be due to the higher soluble heme pigment in fresh meat caused by autolysis (Chaijan *et al.*, 2005). Additionally, the lowered heme pigment extractability with increasing storage time also resulted in the lower iron content of the heme extracted.

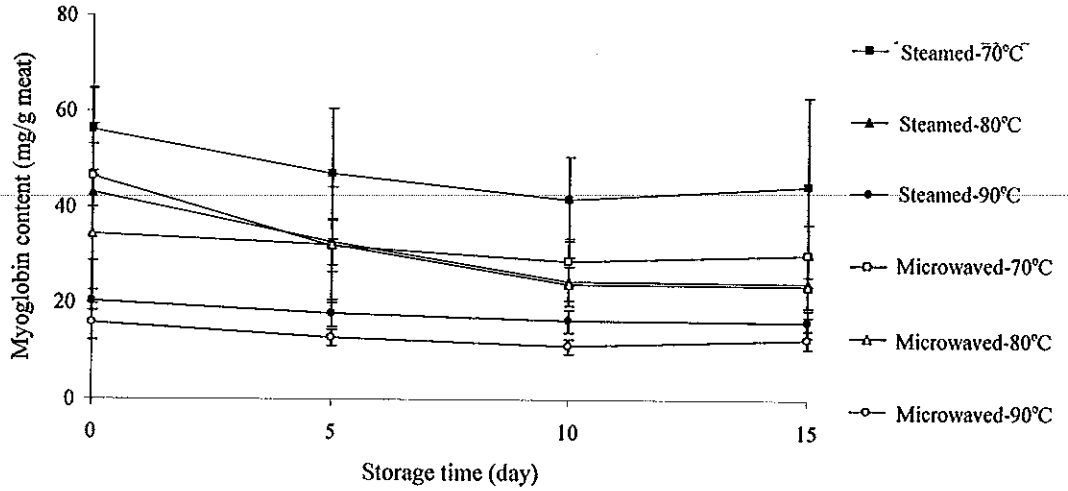


Figure 49 Change in myoglobin content (mg/g meat) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

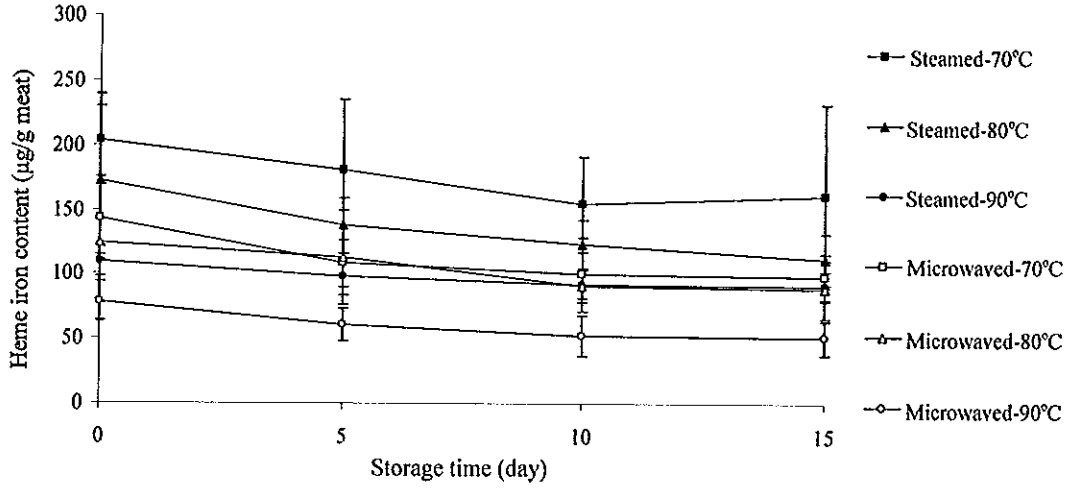


Figure 50 Change in heme iron content (µg/g meat) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

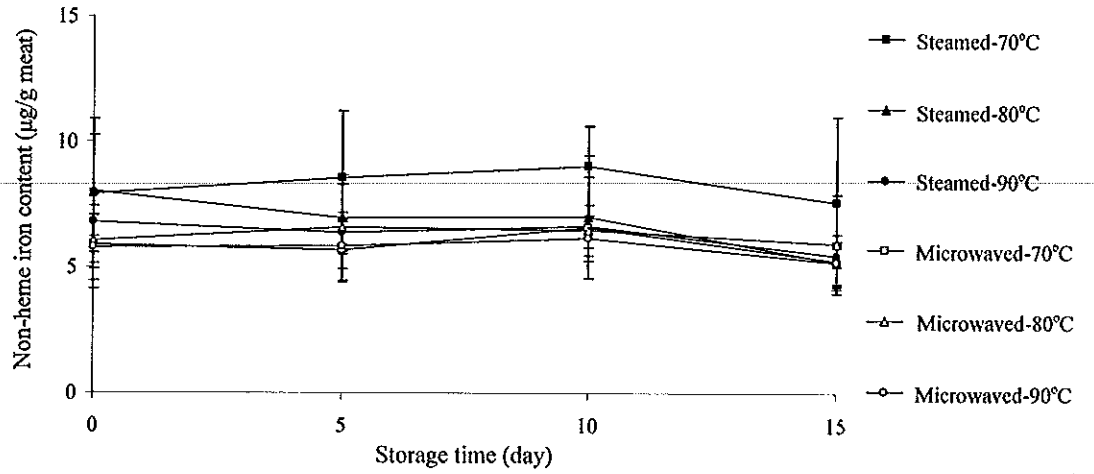


Figure 51 Change in non-heme iron content (µg/g meat) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

Table 43 Metmyoglobin formation of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage at 4 °C for 15 days

Storage time (days)	Steamed meat (°C)			Microwaved meat(°C)		
	70	80	90	70	80	90
0	77.45 ± 6.80^{a}	79.39 ± 3.39^{a}	90.47 ± 2.89^{a}	70.49 ± 8.35^{a}	74.26 ± 4.32^{a}	81.43 ± 3.79^{a}
5	79.07 ± 3.98^{ab}	80.57 ± 3.71^{a}	93.45 ± 4.41^{ab}	72.57 ± 8.51^{a}	77.37 ± 7.29^{a}	85.06 ± 8.60^{a}
10	82.39 ± 1.51^{b}	81.65 ± 2.77^{a}	94.88 ± 3.37^{b}	73.77 ± 4.76^{a}	78.42 ± 3.77^{a}	88.80 ± 4.84^{a}
15	80.62 ± 3.71^{ab}	77.54 ± 5.09^{a}	91.69 ± 4.76^{ab}	69.93 ± 5.62^{a}	74.18 ± 9.28^{a}	83.68 ± 0.67^{a}

Data are presented as mean ± standard deviation. N =8.

$^{a-b}$Means with different letters in the same column denote a statistical difference (P<0.05).

6.3 Changes in lipid fraction and cholesterol

The changes in triglyceride, phospholipids and free fatty acid content of cooked broiler thigh meat are shown in Figure 52-54, respectively. These results showed the significant decrease of triglyceride and phospholipids content when extended storage time to 15 days, coincided with the increase of free fatty acids content (P<0.05). The alteration of lipid fractions content in different heating temperatures of steamed and microwaved meat at 70, 80 and 90 °C end point temperatures during refrigerated storage resulted by the same reason as described those in cooked meat with different cooking methods at 85 °C end point

temperatures. The increase of free fatty acids content might be due to lipolysis from triglyceride and phospholipids and release free fatty acid during refrigerated storage, moreover, form the lipolysis also resulted in the decrease of triglyceride and phospholipids content.

Cholesterol levels were analyzed in cooked meat with different heating temperatures (70, 80 and 90 °C) during refrigerated storage. The change of cholesterol content among all sample tended to similarly to the change of cholesterol content in cooked meat with different cooking methods as described in previous section. When extended storage time to 15 days, the decrease of cholesterol content of steamed and microwaved meat at 70, 80 and 90 °C were observed. At the end of storage, the cholesterol content of all samples had a lower (P<0.05) when compared those at the initial of storage time (Figure 55). The decrease in cholesterol content might be due to cholesterol loss with the oxidation of cholesterol during refrigerated storage. This could be confirmed with the occurred of cholesterol oxidation products in cooked meat sample as reported in the next section.

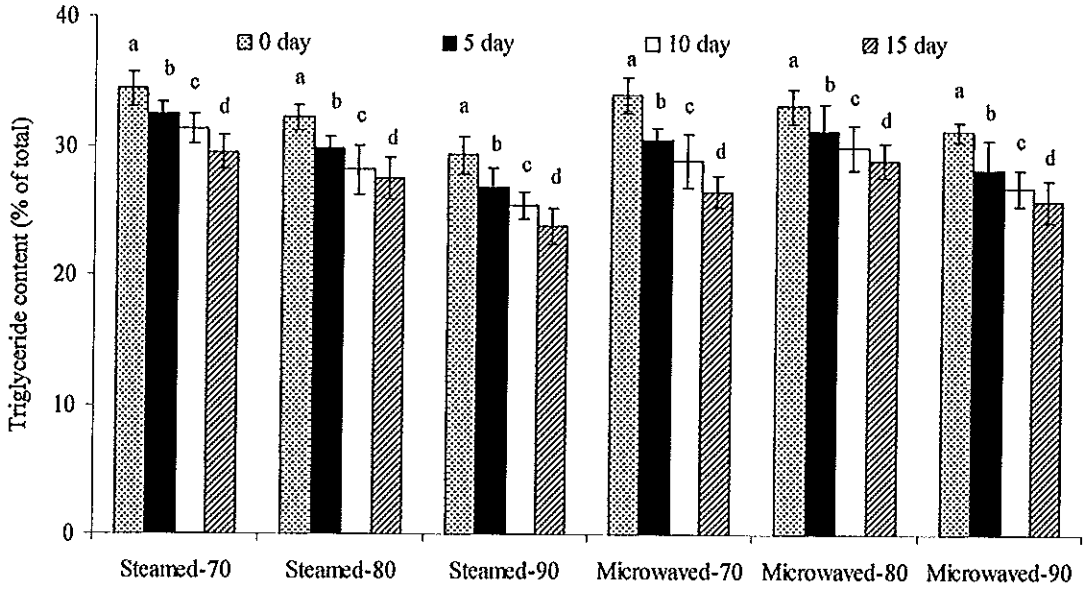


Figure 52 Change in triglyceride content (% of total) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

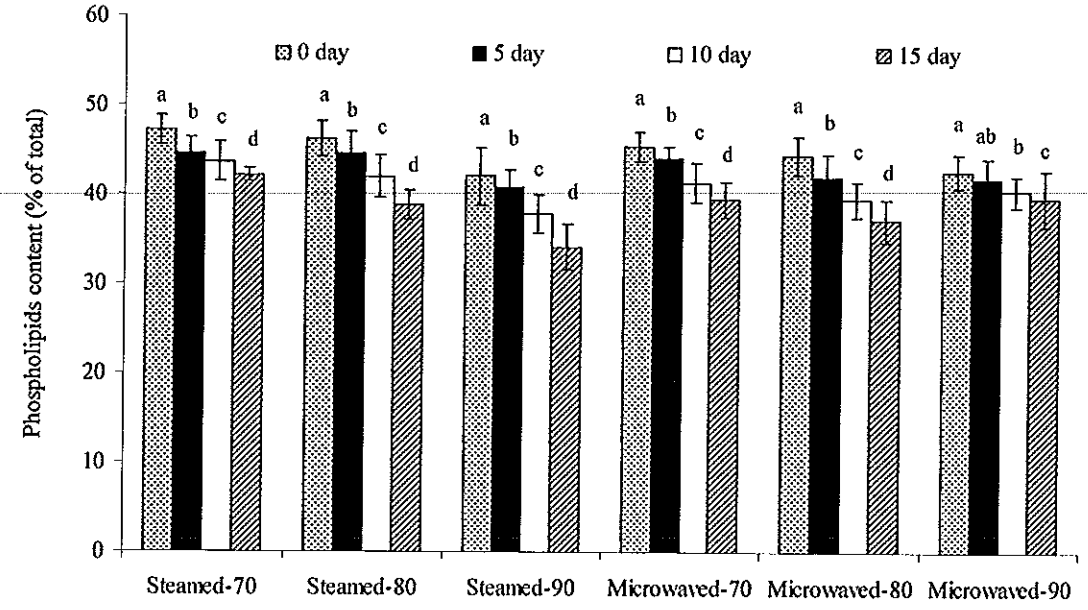


Figure 53 Change in phospholipids content (% of total) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

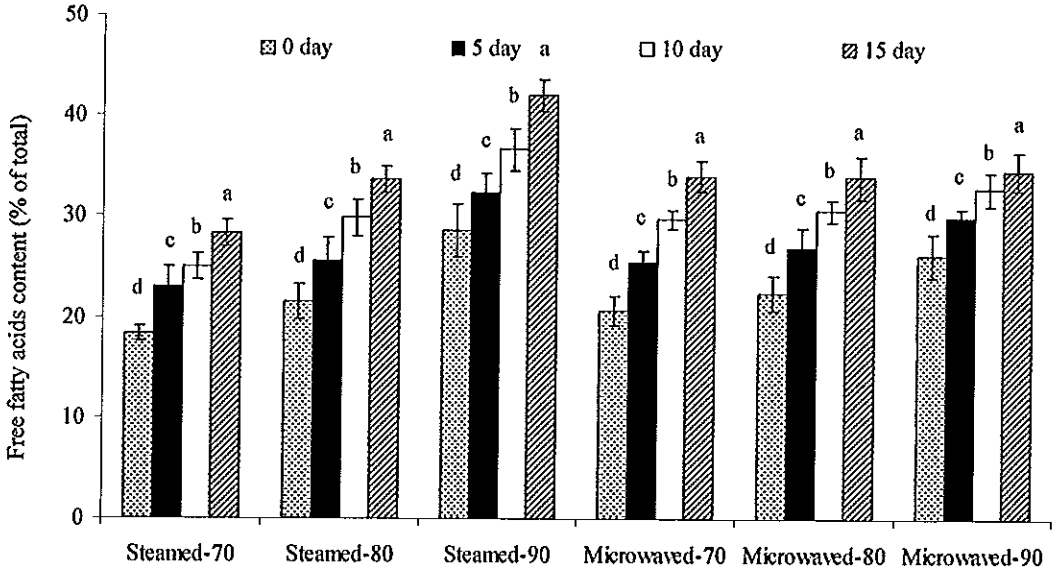


Figure 54 Change in free fatty acids content (% of total) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

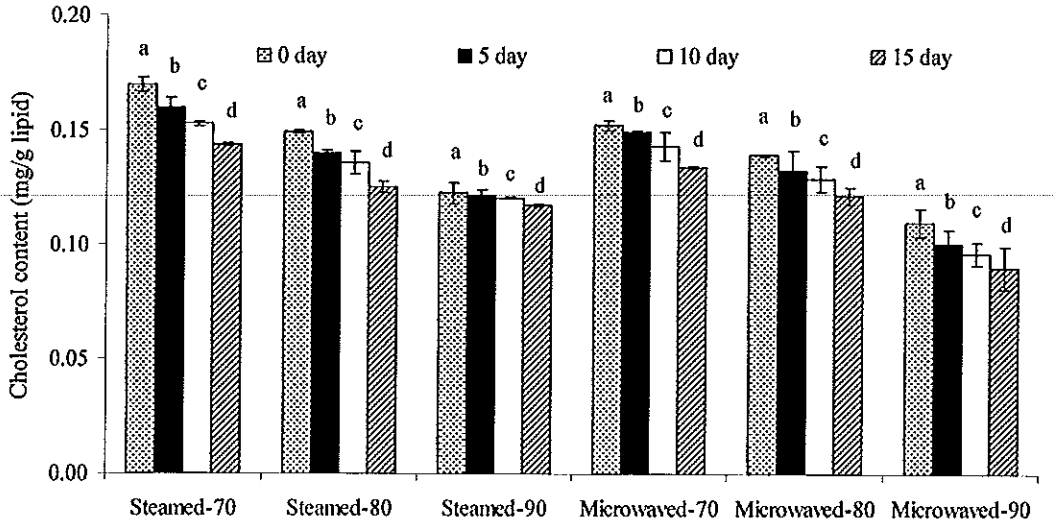


Figure 55 Change in cholesterol content (mg/g lipid) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from three replicate determinations. Different letters under the same cooking methods indicate significant differences (P<0.05).

6.4 Changes in fatty acid composition

Tables 44-49 present the fatty acid compositions (% of total) in steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperatures during refrigerated storage, respectively. There were significant difference (P<0.05) with different end point temperatures (70, 80 and 90 °C). At 15 days of storage, SFAs, MUFAs and PUFAs content gradually decreased with increased storage times among all samples were observed. The changes in fatty acid compositions in cooked meat similarly to those changes have been described in the previous section. This results indicating to heating temperatures to reach the end point temperatures at 70, 80 and 90 °C could lead to modifications in fatty acid profiles of meat lipids. The highest change of fatty acid compositions was observed in steamed meat at 90 °C and microwaved meat at 70 °C end point temperature when compared with those at other end point temperature. These results indicated cooking with steam at 70 °C and microwave at 70 °C end point temperature leading to highest affected on fatty acid in cooked meat.

Table 44 Fatty acid compositions (% of total) of steamed broiler thigh meat at 70 °C end point temperature during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.77 ± 0.02^{a}	0.67 ± 0.02^{b}	0.55 ± 0.03^{c}	0.46 ± 0.04^{d}
C14:0	0.66 ± 0.02^{a}	0.56 ± 0.03^{b}	0.44 ± 0.03^{c}	0.34 ± 0.04^{d}
C16:0	11.07 ± 0.04^{b}	11.19 ± 0.05^{b}	11.31 ± 0.05^{ab}	11.45 ± 0.07^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	3.10 ± 0.02^{a}	3.05 ± 0.03^{a}	3.00 ± 0.03^{a}	2.96 ± 0.04^{a}
SFAs	**15.59 ± 0.10^{a}**	**15.47 ± 0.12^{a}**	**15.29 ± 0.15^{b}**	**15.20 ± 0.18^{b}**
C16:1	12.74 ± 0.02^{a}	12.87 ± 0.02^{a}	13.01 ± 0.02^{a}	13.16 ± 0.03^{a}
C 18:1n-9c,t	28.94 ± 0.05^{c}	29.42 ± 0.05^{b}	29.93 ± 0.04^{b}	30.46 ± 0.07^{a}
C20:1	7.98 ± 0.02^{a}	8.01 ± 0.02^{a}	8.05 ± 0.02^{a}	8.10 ± 0.03^{a}
C24:1	7.01 ± 0.02^{a}	7.02 ± 0.02^{a}	7.04 ± 0.02^{a}	7.07 ± 0.03^{a}
MUFAs	**56.67 ± 0.10^{c}**	**57.32 ± 0.09^{b}**	**58.02 ± 0.08^{a}**	**58.78 ± 0.13^{a}**
C18:2n-6c,t	12.09 ± 0.01^{b}	12.21 ± 0.01^{ab}	12.34 ± 0.01^{ab}	12.46 ± 0.02^{a}
C18:3n-6	4.30 ± 0.02^{a}	4.25 ± 0.02^{a}	4.20 ± 0.02^{a}	4.13 ± 0.03^{a}
C18:3n-3	2.79 ± 0.03^{a}	2.68 ± 0.03^{a}	2.57 ± 0.03^{ab}	2.44 ± 0.05^{b}
C20:3n-6	2.08 ± 0.02^{a}	1.98 ± 0.03^{ab}	1.87 ± 0.03^{b}	1.75 ± 0.04^{b}
C20:3n-3	2.17 ± 0.02^{a}	2.06 ± 0.03^{a}	1.96 ± 0.03^{ab}	1.83 ± 0.04^{b}
C22:2	2.30 ± 0.03^{a}	2.15 ± 0.04^{ab}	2.01 ± 0.04^{b}	1.84 ± 0.05^{c}
C22:6n-3	2.00 ± 0.03^{a}	1.87 ± 0.03^{ab}	1.74 ± 0.04^{b}	1.58 ± 0.05^{c}
PUFAs	**27.74 ± 0.16^{a}**	**27.21 ± 0.18^{b}**	**26.69 ± 0.20^{c}**	**26.02 ± 0.28^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 45 Fatty acid compositions (% of total) of steamed broiler thigh meat at 80 °C end point temperature during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.70 ± 0.03^{a}	0.58 ± 0.03^{b}	0.43 ± 0.02^{c}	0.32 ± 0.03^{d}
C14:0	0.59 ± 0.03^{a}	0.46 ± 0.03^{b}	0.31 ± 0.02^{c}	0.18 ± 0.03^{d}
C16:0	11.61 ± 0.05^{b}	11.77 ± 0.06^{b}	11.92 ± 0.06^{b}	12.09 ± 0.06^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	3.18 ± 0.02^{a}	3.12 ± 0.03^{a}	3.05 ± 0.03^{a}	3.01 ± 0.03^{a}
SFAs	**16.08 ± 0.10^{a}**	**15.94 ± 0.13^{a}**	**15.72 ± 0.13^{ab}**	**15.59 ± 0.14^{b}**
C16:1	13.11 ± 0.05^{a}	13.28 ± 0.06^{a}	13.46 ± 0.06^{a}	13.63 ± 0.07^{a}
C 18:1n-9c,t	30.28 ± 0.13^{c}	30.91 ± 0.14^{c}	31.55 ± 0.14^{b}	32.17 ± 0.15^{a}
C20:1	8.08 ± 0.04^{a}	8.13 ± 0.04^{a}	8.18 ± 0.04^{a}	8.23 ± 0.05^{a}
C24:1	7.05 ± 0.04^{a}	7.08 ± 0.04^{a}	7.10 ± 0.04^{a}	7.12 ± 0.05^{a}
MUFAs	**58.51 ± 0.24^{d}**	**59.39 ± 0.26^{c}**	**60.29 ± 0.28^{b}**	**61.14 ± 0.31^{a}**
C18:2n-6c,t	12.32 ± 0.03^{a}	12.46 ± 0.03^{a}	12.62 ± 0.03^{a}	12.77 ± 0.03^{a}
C18:3n-6	4.05 ± 0.04^{a}	3.97 ± 0.04^{a}	3.90 ± 0.05^{a}	3.83 ± 0.05^{a}
C18:3n-3	2.36 ± 0.05^{a}	2.21 ± 0.06^{a}	2.07 ± 0.07^{b}	1.92 ± 0.07^{b}
C20:3n-6	1.68 ± 0.05^{a}	1.54 ± 0.05^{ab}	1.40 ± 0.06^{b}	1.27 ± 0.06^{c}
C20:3n-3	1.76 ± 0.05^{a}	1.62 ± 0.06^{b}	1.48 ± 0.06^{c}	1.34 ± 0.07^{d}
C22:2	1.75 ± 0.06^{a}	1.55 ± 0.07^{b}	1.36 ± 0.08^{c}	1.17 ± 0.09^{d}
C22:6n-3	1.50 ± 0.06^{a}	1.32 ± 0.07^{b}	1.15 ± 0.07^{c}	0.97 ± 0.08^{d}
PUFAs	**25.41 ± 0.32^{a}**	**24.67 ± 0.37^{b}**	**23.99 ± 0.40^{bc}**	**23.26 ± 0.43^{c}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 46 Fatty acid compositions (% of total) of steamed broiler thigh meat at 90 °C end point temperature during refrigerated storage for 15 days.

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.73 ± 0.01^{a}	0.62 ± 0.02^{b}	0.45 ± 0.01^{c}	0.31 ± 0.01^{d}
C14:0	0.52 ± 0.01^{a}	0.39 ± 0.02^{b}	0.22 ± 0.01^{c}	0.06 ± 0.01^{d}
C16:0	11.90 ± 0.02^{c}	12.11 ± 0.02^{b}	12.30 ± 0.02^{b}	12.47 ± 0.01^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	3.16 ± 0.01^{a}	3.12 ± 0.02^{a}	3.05 ± 0.01^{a}	2.97 ± 0.01^{a}
SFAs	**16.31 ± 0.06^{a}**	**16.24 ± 0.07^{ab}**	**16.02 ± 0.06^{b}**	**15.81 ± 0.05^{c}**
C16:1	13.96 ± 0.02^{b}	14.17 ± 0.03^{b}	14.41 ± 0.04^{a}	14.62 ± 0.04^{a}
C 18:1n-9c,t	30.21 ± 0.02^{c}	30.90 ± 0.03^{c}	31.65 ± 0.06^{b}	32.33 ± 0.04^{a}
C20:1	7.99 ± 0.02^{a}	8.03 ± 0.03^{a}	8.09 ± 0.03^{a}	8.14 ± 0.04^{a}
C24:1	6.28 ± 0.03^{a}	6.28 ± 0.03^{a}	6.28 ± 0.03^{a}	6.28 ± 0.04^{a}
MUFAs	**58.44 ± 0.07^{d}**	**59.38 ± 0.10^{c}**	**60.42 ± 0.17^{b}**	**61.37 ± 0.15^{a}**
C18:2n-6c,t	11.82 ± 0.02^{a}	11.96 ± 0.02^{a}	12.13 ± 0.02^{a}	12.28 ± 0.02^{a}
C18:3n-6	4.33 ± 0.02^{a}	4.25 ± 0.02^{ab}	4.18 ± 0.03^{b}	4.11 ± 0.02^{b}
C18:3n-3	2.61 ± 0.02^{a}	2.45 ± 0.03^{b}	2.29 ± 0.04^{bc}	2.14 ± 0.03^{c}
C20:3n-6	1.85 ± 0.02^{a}	1.69 ± 0.02^{b}	1.54 ± 0.03^{c}	1.40 ± 0.03^{d}
C20:3n-3	1.30 ± 0.02^{a}	1.12 ± 0.02^{b}	0.95 ± 0.03^{c}	0.79 ± 0.03^{d}
C22:2	1.67 ± 0.02^{a}	1.45 ± 0.03^{b}	1.22 ± 0.04^{c}	1.02 ± 0.03^{d}
C22:6n-3	1.66 ± 0.02^{a}	1.46 ± 0.03^{b}	1.26 ± 0.04^{c}	1.08 ± 0.03^{d}
PUFAs	**25.25 ± 0.12^{a}**	**24.38 ± 0.17^{b}**	**23.57 ± 0.23^{c}**	**22.82 ± 0.19^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 47 Fatty acid compositions (% of total) of microwaved broiler thigh meat at 70 °C end point temperature during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.97 ± 0.01[a]	0.90 ± 0.02[a]	0.79 ± 0.01[b]	0.70 ± 0.01[b]
C14:0	0.89 ± 0.01[a]	0.81 ± 0.02[a]	0.70 ± 0.01[b]	0.60 ± 0.01[c]
C16:0	12.42 ± 0.03[a]	12.52 ± 0.03[a]	12.70 ± 0.03[a]	12.88 ± 0.02[a]
C17:0	0.00	0.00	0.00	0.00
C18:0	3.91 ± 0.02[a]	3.89 ± 0.02[a]	3.86 ± 0.02[a]	3.84 ± 0.01[a]
SFAs	**18.19 ± 0.07[a]**	**18.12 ± 0.07[a]**	**18.05 ± 0.07[a]**	**18.02 ± 0.05[a]**
C16:1	12.34 ± 0.04[a]	12.44 ± 0.04[a]	12.58 ± 0.05[a]	12.73 ± 0.06[a]
C 18:1n-9c,t	29.97 ± 0.05[b]	30.35 ± 0.06[b]	30.93 ± 0.07[ab]	31.51 ± 0.08[a]
C20:1	6.96 ± 0.03[a]	6.90 ± 0.04[a]	7.00 ± 0.04[a]	7.02 ± 0.05[a]
C24:1	5.10 ± 0.03[a]	5.09 ± 0.04[a]	5.06 ± 0.04[a]	5.04 ± 0.05[a]
MUFAs	**54.37 ± 0.14[c]**	**54.86 ± 0.17[c]**	**55.57 ± 0.20[b]**	**56.29 ± 0.23[a]**
C18:2n-6c,t	10.60 ± 0.02[a]	10.67 ± 0.02[a]	10.76 ± 0.02[a]	10.85 ± 0.03[a]
C18:3n-6	4.64 ± 0.03[a]	4.60 ± 0.03[a]	4.54 ± 0.03[a]	4.48 ± 0.04[a]
C18:3n-3	2.49 ± 0.03[a]	2.40 ± 0.04[a]	2.26 ± 0.04[b]	2.12 ± 0.04[b]
C20:3n-6	2.22 ± 0.03[a]	2.14 ± 0.03[a]	2.03 ± 0.04[b]	1.90 ± 0.04[b]
C20:3n-3	2.29 ± 0.03[a]	2.21 ± 0.03[a]	2.09 ± 0.04[b]	1.97 ± 0.04[b]
C22:2	2.80 ± 0.04[a]	2.69 ± 0.04[b]	2.53 ± 0.05[c]	2.37 ± 0.05[d]
C22:6n-3	2.40 ± 0.03[a]	2.31 ± 0.04[a]	2.16 ± 0.04[ab]	2.01 ± 0.05[b]
PUFAs	**27.44 ± 0.20[a]**	**27.02 ± 0.24[a]**	**26.38 ± 0.26[b]**	**25.70 ± 0.28[c]**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

[a-c]Means with differing superscripts in the same row are significantly different ($P<0.05$).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 48 Fatty acid compositions (% of total) of microwaved broiler thigh meat at 80 °C end point temperature during refrigerated storage for 15 days

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.86 ± 0.01^{a}	0.80 ± 0.02^{a}	0.66 ± 0.02^{b}	0.56 ± 0.03^{c}
C14:0	0.77 ± 0.01^{a}	0.70 ± 0.02^{a}	0.56 ± 0.02^{b}	0.45 ± 0.03^{c}
C16:0	12.73 ± 0.03^{b}	12.86 ± 0.03^{ab}	13.05 ± 0.04^{a}	13.26 ± 0.04^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	3.91 ± 0.01^{a}	3.90 ± 0.02^{a}	3.86 ± 0.02^{a}	3.85 ± 0.03^{a}
SFAs	**18.27 ± 0.06^{a}**	**18.26 ± 0.08^{a}**	**18.13 ± 0.10^{a}**	**18.12 ± 0.13^{a}**
C16:1	12.54 ± 0.04^{c}	12.63 ± 0.03^{b}	12.80 ± 0.03^{a}	12.94 ± 0.04^{a}
C 18:1n-9c,t	30.84 ± 0.05^{c}	31.23 ± 0.05^{b}	31.90 ± 0.05^{b}	32.54 ± 0.05^{a}
C20:1	6.97 ± 0.04^{a}	6.98 ± 0.03^{a}	7.00 ± 0.03^{a}	7.01 ± 0.03^{a}
C24:1	5.03 ± 0.04^{a}	5.02 ± 0.03^{a}	4.99 ± 0.03^{a}	4.94 ± 0.03^{a}
MUFAs	**55.38 ± 0.15^{b}**	**55.86 ± 0.14^{b}**	**56.68 ± 0.13^{a}**	**57.43 ± 0.15^{a}**
C18:2n-6c,t	10.74 ± 0.02^{a}	10.80 ± 0.02^{a}	10.92 ± 0.02^{a}	11.02 ± 0.02^{a}
C18:3n-6	4.54 ± 0.02^{a}	4.49 ± 0.02^{a}	4.44 ± 0.02^{a}	4.37 ± 0.03^{a}
C18:3n-3	2.26 ± 0.03^{a}	2.16 ± 0.03^{a}	2.01 ± 0.03^{b}	1.86 ± 0.04^{c}
C20:3n-6	2.03 ± 0.02^{a}	1.94 ± 0.03^{ab}	1.81 ± 0.03^{b}	1.67 ± 0.03^{c}
C20:3n-3	2.09 ± 0.02^{a}	2.00 ± 0.03^{ab}	1.87 ± 0.03^{b}	1.73 ± 0.03^{c}
C22:2	2.53 ± 0.03^{a}	2.42 ± 0.04^{a}	2.25 ± 0.04^{b}	2.06 ± 0.04^{c}
C22:6n-3	2.16 ± 0.03^{a}	2.06 ± 0.03^{ab}	1.90 ± 0.03^{b}	1.73 ± 0.04^{c}
PUFAs	**26.35 ± 0.18^{a}**	**25.87 ± 0.20^{b}**	**25.19 ± 0.20^{c}**	**24.45 ± 0.23^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-c}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Table 49 Fatty acid compositions (% of total) of microwaved broiler thigh meat at 90 °C end point temperature during refrigerated storage for 15 days.

Fatty acid	Storage time (day)			
	0	5	10	15
C12:0	0.81 ± 0.02^{a}	0.73 ± 0.03^{b}	0.56 ± 0.04^{c}	0.43 ± 0.04^{d}
C14:0	0.73 ± 0.02^{a}	0.64 ± 0.03^{b}	0.47 ± 0.04^{c}	0.33 ± 0.05^{d}
C16:0	11.94 ± 0.03^{c}	12.08 ± 0.04^{b}	12.29 ± 0.05^{b}	12.53 ± 0.06^{a}
C17:0	0.00	0.00	0.00	0.00
C18:0	2.80 ± 0.02^{a}	2.77 ± 0.03^{a}	2.67 ± 0.04^{a}	2.62 ± 0.04^{a}
SFAs	**16.27 ± 0.10^{a}**	**16.22 ± 0.14^{a}**	**15.99 ± 0.16^{b}**	**15.91 ± 0.19^{b}**
C16:1	12.78 ± 0.04^{c}	12.90 ± 0.03^{bc}	13.11 ± 0.03^{b}	13.31 ± 0.03^{a}
C 18:1n-9c,t	35.55 ± 0.05^{d}	36.13 ± 0.05^{c}	37.11 ± 0.05^{b}	38.07 ± 0.06^{a}
C20:1	6.40 ± 0.04^{a}	6.40 ± 0.03^{a}	6.41 ± 0.03^{a}	6.40 ± 0.03^{a}
C24:1	5.65 ± 0.04^{a}	5.64 ± 0.03^{a}	5.62 ± 0.03^{a}	5.59 ± 0.03^{a}
MUFAs	**60.37 ± 0.15^{d}**	**61.07 ± 0.13^{c}**	**62.25 ± 0.12^{b}**	**63.37 ± 0.15^{a}**
C18:2n-6c,t	11.14 ± 0.02^{d}	11.22 ± 0.03^{c}	11.38 ± 0.02^{b}	11.53 ± 0.03^{a}
C18:3n-6	4.12 ± 0.03^{a}	4.06 ± 0.03^{a}	3.98 ± 0.03^{ab}	3.88 ± 0.04^{b}
C18:3n-3	2.00 ± 0.03^{a}	1.87 ± 0.04^{b}	1.68 ± 0.04^{c}	1.47 ± 0.05^{d}
C20:3n-6	1.75 ± 0.03^{a}	1.64 ± 0.03^{b}	1.47 ± 0.04^{c}	1.29 ± 0.04^{d}
C20:3n-3	1.17 ± 0.03^{a}	1.04 ± 0.04^{b}	0.85 ± 0.04^{c}	0.64 ± 0.04^{d}
C22:2	1.69 ± 0.04^{a}	1.54 ±0.05^{a}	1.29 ± 0.05^{b}	1.03 ± 0.06^{c}
C22:6n-3	1.48 ± 0.04^{a}	1.34 ± 0.04^{ab}	1.12 ± 0.04^{b}	0.88 ± 0.05^{c}
PUFAs	**23.35 ± 0.22^{a}**	**22.71 ± 0.25^{b}**	**21.76 ± 0.26^{c}**	**20.72 ± 0.30^{d}**
Total	**100.00**	**100.00**	**100.00**	**100.00**

Data are presented as mean ± standard deviation. N =3.

$^{a-d}$Means with differing superscripts in the same row are significantly different (P<0.05).

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs= polyunsaturated fatty acids.

6.5 Lipid oxidation and COPs

The significant increase in MDA content of steamed and microwaved meat at 70, 80 and 90 °C end point temperatures when extended storage time to 10 days show in Figure 56. The increase of MDA content suggesting to the increase in lipid oxidation of cooked meat during refrigerated storage. The highest lipid oxidation rate of steamed and microwaved meat were observed at 90 °C end point temperature and the lowest were observed at 70 °C end point temperature. In additionally, Table 50, heating temperatures did not increase the oxidation of cholesterol at day heating without storage. After 5 days until 15 days of storage, however, steamed and microwaved meat at 90 °C end point temperatures had more total COPs content than those at 70 °C end point temperatures.

When extended storage time to 15 days, the increase in all COPs, 5-cholestane-3β-ol-7-one (cholestanetriol), α-epoxides, β-epoxides, 7β-hydroxy cholesterol and 25-hydroxycholesterol, of cooked meat for all end point temperatures were observed (Table 50). These resulted suggesting to the increase in cholesterol oxidation of cooked meat with different heating temperatures during refrigerated storage. The increase in COPs has been described in the previous section. Similar increases in COPs contents during refrigerated storage were reported by Park and Addis (1987) in pork, De Vore (1988) in beef and Zubillaga and Maerker (1991) in veal, beef and pork. Pie *et al.* (1991) also reported increases in COPs contents of frozen beef, veal and pork which they attributed to the oxidation of cholesterol and its products at lower temperatures in the presence of oxygen as well as free radical initiators, such as hydroperoxides, formed during the oxidation of polyunsaturated fatty acids. However, Nam *et al.* (2001) suggesting to cholesterol was oxidized in beef without fatty acid oxidation, but fatty acid oxidation accelerated the oxidation of cholesterol in turkey and pork. COPS increased in processed chicken during refrigerated storage.

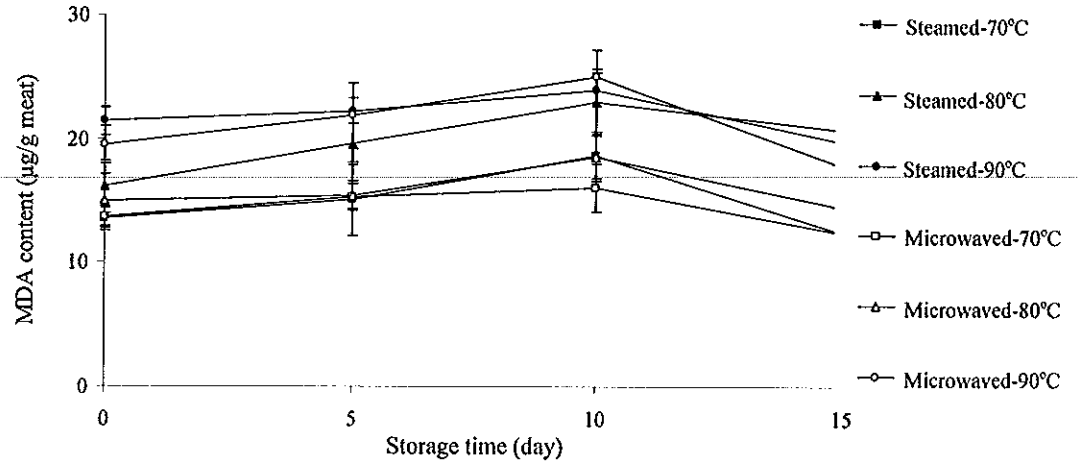


Figure 56 Change in MDA content (µg/g meat) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage with skin (4 °C) for 15 days. Bars indicate SD from eight replicate determinations.

The increase of TBARS values with extended storage time to 10 days in steamed and microwaved thigh meat at 70, 80 and 90 °C end point temperature had correlation to lipid content, metmyoglobin formation, non-heme iron, free fatty acid and total COPs coincided with the decrease in moisture content, myoglobin content, heme iron content, triglyceride, phospholipids, SFAs, MUFAs and PUFAs. The increase of non-heme iron content was concomitant with the decrease of heme iron content due to non-heme iron was released from heme complex due to the breakdown of coordinate covalent bond between iron atom and porphyrin nitrogens during refrigerated storage. Possibility of converting of myoglobin to metmyoglobin was comfirmed by the sharp decrease of myoglobin and redness index in chicken meat. Non-heme iron, metmyoglobin and free fatty acids might accelerate the oxidation process in the muscle (Chan *et al.*, 1997; Lee *et al.*, 2003). Moreover, the radicals from autoxidation of myoglobin could be stimulated lipid oxidation. Alasnier *et al.* (2000) reported that refrigerated storage significantly affected free fatty acids amounts in muscle but free fatty acid formation during refrigerated storage was not linear and the increase in free fatty acid had high correlation with the increase in lipid oxidation when extended refrigerated storage time to 14 days. Moreover, Nam *et al.* (1997) suggested that the higher susceptibility of PUFAs to oxidation was reflected in cooked samples, as the more unsaturated the fat source, the higher the increase in TBARS values due to cooking.

Table 50 Cholesterol oxidation products (COPs) (μg/g lipid) of steamed and microwaved broiler thigh meat at 70, 80 and 90 °C end point temperature during refrigerated storage for 15 days

Sample	Storage time (day)	COPs content (μg/g lipid)					
		7β -HC	β-CE	α-CE	CT	25-HC	Total
Steamed meat 70 °C	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	1.32 ± 0.07^{c}	2.09 ± 0.06^{c}	1.54 ± 0.09^{c}	2.00 ± 0.07^{c}	1.60 ± 0.02^{c}	8.55 ± 0.32^{c}
	10	1.64 ± 0.05^{b}	2.46 ± 0.05^{b}	1.98 ± 0.01^{b}	2.57 ± 0.21^{b}	2.21 ± 0.05^{b}	10.87 ± 0.37^{b}
	15	2.08 ± 0.01^{a}	2.93 ± 0.06^{a}	2.51 ± 0.06^{a}	3.47 ± 0.07^{a}	2.86 ± 0.12^{a}	13.85 ± 0.33^{a}
Steamed meat 80 °C	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	1.61 ± 0.11^{c}	2.41 ± 0.10^{c}	1.93 ± 0.08^{c}	2.48 ± 0.05^{c}	1.97 ± 0.00^{c}	10.40 ± 0.35^{c}
	10	2.13 ± 0.04^{b}	2.92 ± 0.06^{b}	2.31 ± 0.02^{b}	2.96 ± 0.07^{b}	2.26 ± 0.01^{b}	12.59 ± 0.20^{b}
	15	2.78 ± 0.07^{a}	3.44 ± 0.05^{a}	2.93 ± 0.10^{a}	3.41 ± 0.02^{a}	2.92 ± 0.11^{a}	15.48 ± 0.35^{a}
Steamed meat 90 °C	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	2.67 ± 0.07^{c}	3.18 ± 0.04^{c}	2.99 ± 0.01^{c}	3.19 ± 0.03^{c}	2.68 ± 0.14^{c}	14.71 ± 0.30^{c}
	10	3.08 ± 0.11^{b}	3.65 ± 0.03^{b}	3.40 ± 0.18^{b}	3.87 ± 0.16^{b}	3.27 ± 0.09^{b}	17.27 ± 0.57^{b}
	15	3.78 ± 0.01^{a}	4.31 ± 0.05^{a}	3.94 ± 0.02^{a}	4.26 ± 0.10^{a}	3.92 ± 0.16^{a}	20.21 ± 0.34^{a}
Microwaved meat 70 °C	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	2.46 ± 0.04^{c}	3.08 ± 0.03^{c}	1.56 ± 0.02^{c}	2.25 ± 0.06^{c}	1.76 ± 0.24^{c}	11.11 ± 0.40^{c}
	10	2.85 ± 0.12^{b}	3.67 ± 0.03^{b}	2.09 ± 0.06^{b}	2.94 ± 0.01^{b}	2.38 ± 0.10^{b}	13.93 ± 0.32^{b}
	15	3.32 ± 0.12^{a}	4.42 ± 0.16^{a}	2.68 ± 0.04^{a}	3.40 ± 0.13^{a}	3.10 ± 0.17^{a}	16.92 ± 0.61^{a}
Microwaved meat 80 °C	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	2.98 ± 0.01^{c}	3.53 ± 0.04^{c}	2.12 ± 0.10^{c}	2.78 ± 0.01^{c}	2.11 ± 0.03^{c}	13.52 ± 0.19^{c}
	10	3.45 ± 0.03^{b}	4.15 ± 0.18^{b}	2.73 ± 0.10^{b}	3.28 ± 0.02^{b}	2.77 ± 0.16^{b}	16.38 ± 0.50^{b}
	15	3.89 ± 0.07^{a}	4.85 ± 0.10^{a}	3.27 ± 0.12^{a}	4.09 ± 0.06^{a}	3.32 ± 0.03^{a}	19.41 ± 0.38^{a}
Microwaved meat 90 °C	0	0.00	0.00	0.00	0.00	0.00	0.00
	5	4.15 ± 0.03^{c}	4.34 ± 0.09^{c}	2.92 ± 0.10^{c}	3.58 ± 0.06^{c}	3.18 ± 0.04^{c}	18.17 ± 0.38^{c}
	10	4.65 ± 0.03^{b}	4.86 ± 0.06^{b}	3.65 ± 0.12^{b}	4.22 ± 0.05^{b}	3.89 ± 0.14^{b}	21.27 ± 0.40^{b}
	15	5.26 ± 0.04^{a}	5.29 ± 0.01^{a}	4.32 ± 0.24^{a}	4.93 ± 0.13^{a}	4.26 ± 0.04^{a}	24.06 ± 0.46^{a}

Data are presented as mean ± standard deviation. N = 3.

$^{a-c}$Means with differing superscripts in the same column are significantly different (P<0.05).

7β-HC = 7β-hydroxy cholesterol, β-CE = cholesterol 5β, 6β-epoxide, α-CE = cholesterol 5α, 6α-epoxide, CT = 5-cholestane-3β-ol-7-one, 25-HC = 25-hydroxy cholesterol.

CHAPTER 4
CONCLUSIONS

Fresh thigh meat contained higher lipid (phospholipids, cholesterol, free fatty acids and unsaturated fatty acids) and heme pigment (myoglobin, heme iron, non-heme iron and metmyoglobin) related to more lipid and cholesterol oxidation compared to fresh breast meat. Focus the attention on heme and non-heme iron, for breast meat, broiler had the highest content among three chicken breeds while for thigh meat, the highest of these content were found in spent hen. When emphasize on lipid that susceptible to oxidation including to unsaturated fatty acid, free fatty acids, phospholipids and cholesterol, broiler meat had the highest content of these when compared with other chicken breeds meat within either breast or thigh meat. Consequence, broiler thigh meat showed the highest lipid oxidation than that of other chicken breeds meat while no significant difference for breast meat was observed. Surprisingly, both spent hen meat had the highest total COPs content than those of other chicken breed meat at 6 and 9 days storage. No significant difference in chemical compositions and lipid oxidation included COPs of chicken meat between stored with and without skin were observed.

The highest content in crude fat, heme pigment and free fatty acids which coincided with the highest lipid oxidation was found in boiled, steamed and microwaved broiler thigh meat. The highest cooking loss was found in fried samples and the lowest were found in grilled samples. The decrease in myoglobin, moisture and heme iron content of cooked meat was in coincidental with the increase in non-heme iron and lipid content and metmyoglobin formation compared to those in raw meat.

The highest water and lipid loss was found in steamed and microwaved meat at 90 °C end point temperatures when compared with those at 80 and 70 °C, respectively. Also, cooked meat at 70 °C end point temperatures had a higher change in heme pigment than those of 80 and 90 °C while changes in lipid fractions, fatty acid compositions, lipid and cholesterol oxidation of steamed meat at 90 °C and microwaved meat at 70 °C end point temperatures had a higher change than those at other end point temperature within cooking method. The increase of lipid oxidation with extended storage time to 10 days in steamed and microwaved thigh meat at 70, 80 and 90 °C end point temperatures had high correlation to lipid content, metmyoglobin formation, non-heme iron, free fatty acid, and total COPs coincided with the decrease in moisture content, myoglobin content, heme iron content, triglyceride, phospholipids.

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List of Publication and Proceedings

Liwa, J. and Wattanachant, S. 2008. Lipid oxidation of different muscle types during refrigerated storage as related to heme pigment in the broiler muscle. The 6th Regional IMT-GT UNINET Conference, August 28-30, 2008, Penang, Malaysia.

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