EFFECT OF POTASSIUM IODIDE AND TUNA OIL FORTIFICATION ON FRESHWATER FISH SAUSAGE QUALITIES

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วัตถุประสงค์ของงานวิจัยเพื่อศึกษาความสามารถในการเป็นอิมัลซิไฟเออร์ของโปรตีนจากปลาน้ำจืด และศึกษาผลของการเติมโปแตสเซียมไอโอไดด์ต่อความสามารถในการเป็นอิมัลซิไฟ-เออร์ของโปรตีนจากปลาน้ำจืด นอกจากนี้ศึกษาผลของการเติมโปแตสเซียมไอโอไดด์และน้ำมันปลาทูน่าต่อการเกิดออกซิเดชันของไขมันในระบบอิมัลชันแบบน้ำมันในน้ำ (Oil in water emulsion) และในระบบไส้กรอกอิมัลชัน และผลของการเติมสารทั้งสอง ต่อการเปลี่ยนแปลงสีและเนื้อสัมผัสของไส้กรอกอิมัลชัน จากการศึกษาความสามารถในการเป็นอิมัลซิไฟเออร์ของโปรตีนจากปลาน้ำจืด 5 ชนิด ได้แก่ ปลานิล (Nile tilapia, NT : *Oreochromis niloticus*), ปลายี่สกเทศ (Rohu, RH : *Labeo rohita*) ปลานวลจันทร์ (Small scale mud carp, SM : *Cirrhina microlepis*) ปลาดุกบิ๊กอุย (Hybrid catfish, HC : *Clarias macrocephalus* × *Clarias garieepinus*) และ ปลาดุกแอฟริกัน (African walking catfish, AF : *Clarias gariepinus*) พบว่า โปรตีนจากปลาทุกชนิดมีความสามารถในการเป็นอิมัลซิไฟเออร์ไม่แตกต่างกันที่อุณหภูมิห้อง (28 องศาเซลเซียส) ($p>0.05$) โปรตีนมัยโอฟิบริลลาร์ (Myofibrillar protein) จากปลาทุกชนิด ให้ค่าความเสถียรของอิมัลชัน (Emulsion stability, ES) มากกว่าโปรตีนจากสารละลายเนื้อปลาบด (Mince protein) ที่ 50 องศาเซลเซียส โปรตีนจากปลายี่สกเทศให้ค่าความเสถียรของอิมัลชันสูงที่สุดเมื่อเทียบกับปลาชนิดอื่น ส่วนโปรตีนจากปลาดุกแอฟริกันให้ค่าความเสถียรของอิมัลชันต่ำที่สุด ค่าดัชนีบ่งชี้ความสามารถในการเป็นอิมัลซิไฟเออร์ (Emulsifying activity index, EAI) และค่าความเสถียรของอิมัลชันที่ 50 องศาเซลเซียส มีค่าต่ำกว่าที่อุณหภูมิห้อง อนุภาคเม็ดไขมัน (Oil droplet diameter, $d_{3,2}$) ของตัวอย่างบ่มที่อุณหภูมิ 50 องศาเซลเซียส มีขนาดใหญ่กว่าเมื่อบ่มที่อุณหภูมิห้อง การเติมโปแตสเซียมไอโอไดด์เข้มข้น 10-100 มิลลิโมลาร์ ไม่มีผลต่อความสามารถในการละลายของโปรตีนมัยโอฟิบริลลาร์จากปลายี่สก-เทศ อย่างไรก็ตามค่าพื้นผิวไฮโดรฟอบิก (Surface hydrophobic, S_0-ANS) เพิ่มขึ้นเมื่อความเข้มข้นของโปแตสเซียมไอโอไดด์มากกว่า 50 มิลลิโมลลาร์ ซึ่งสอดคล้องกับการเพิ่มขึ้นของค่าดัชนีบ่งชี้ความสามารถในการเป็นอิมัลซิไฟเออร์ ค่าความเสถียรของอิมัลชันและขนาดอนุภาคของเม็ดไขมัน มีค่าคงที่ในช่วงความเข้มข้นของโปแตสเซียมไอโอไดด์ที่ศึกษา (10 ─ 100 มิลลิโมลาร์) เมื่อเตรียมอิมัลชันแบบน้ำมันในน้ำ (น้ำมันร้อยละ 10) โดยเติมโปแตสเซียมไอโอไดด์ (50 และ 100 มิล

มิลลิโมลาร์) ร่วมกับน้ำมันปลาทูน่า (ร้อยละ 5 และ 10) โดยใช้โปรตีนมัยโอฟิบริลลาร์เป็นอิมัลซิ-ไฟเออร์ (1 มิลลิกรัมต่อมิลลิลิตร) พบว่าค่าไฮโดรเปอร์ออกไซด์ (Hydroperoxide value, HPV) และค่าทีบาร์ส (Thiobarbituric reactive substances, TBARS) เพิ่มขึ้นตามระดับความเข้มข้นของน้ำมันปลาทูน่า ($p<0.05$) การเติมโปแตสเซียมไอโอไดด์ที่ความเข้มข้น 100 มิลลิโมลาร์ มีผลเร่งการเกิดออกซิเดชันของไขมันในระบบอิมัลชันแบบน้ำมันในน้ำ

เมื่อผลิตไส้กรอกอิมัลชันจากปลาดุกแอฟริกัน และปลายี่สกเทศ โดยเติมน้ำมันปลาทูน่า 3 ระดับ คือร้อยละ 2 6 และ 10 และโปแตสเซียมไอโอไดด์ 150 ไมโครกรัมต่อ 100 กรัมตัวอย่าง ส่วนตัวอย่างควบคุม เติมน้ำมันพืชร้อยละ 10 โดยไม่เติมโปแตสเซียมไอโอไดด์ เก็บตัวอย่างในสภาวะสุญญากาศที่ 4 องศาเซลเซียส ตลอดการทดลอง พบว่า ไส้กรอกปลาดุกแอฟริกันมีค่าความแข็งแรงของเจล (Gel strength) สูงกว่าไส้กรอกจากปลายี่สกเทศ แต่มีค่าความสว่าง (Lightness, L^*) ต่ำกว่า ($p<0.05$) การเติมน้ำมันปลาทูน่าและโปแตสเซียมไอโอไดด์ที่ทุกระดับความเข้มข้นไม่มีผลต่อค่าความแข็งแรง และค่าความสว่างของไส้กรอก ($p>0.05$) ปริมาณไอโอดีนในไส้กรอกปลาทั้ง 2 ชนิด มีค่าลดลงร้อยละ 14 เมื่อผ่านกระบวนการแปรรูป แต่มีค่าคงที่ตลอดระยะเวลาการเก็บ 4 สัปดาห์ การเพิ่มระดับน้ำมันปลาทูน่าในไส้กรอก ส่งผลให้ปริมาณกรดไขมันโอเมกา 3 โดยเฉพาะ อีพีเอ (EPA, Eicosapentaenoic acid) และ ดีเอชเอ (DHA, Docosahexaenoic acid) เพิ่มขึ้น แต่ปริมาณกรดไขมันโอเมกา 6 มีค่าลดลง อัตราส่วนของกรดไขมันโอเมกา 6 ต่อกรดไขมันโอเมกา 3 (n6/n3 ratio) ของไส้กรอกจากปลาทั้ง 2 ชนิดมีค่าลดลง เมื่อเพิ่มระดับน้ำมันปลาทูน่าและมีค่าคงที่ตลอดระยะเวลาการเก็บ ปริมาณกรดไขมันลิโนเลอิก (Linoleic acid) ลิโนเลนิก (Linolenic acid) อีพีเอ และ ดีเอชเอ มีค่าลดลงในสัปดาห์ที่ 3 และ 4 ของการเก็บในทุกๆ ตัวอย่าง การเพิ่มระดับของการเติมน้ำมันปลาทูน่าส่งผลให้ค่าไฮโดรเปอร์ออกไซด์ และค่าทีบาร์สเพิ่มขึ้น ($p<0.05$) การเติมโปแตสเซียมไอโอไดด์ในระดับ 150 ไมโครกรัม ต่อ 100 กรัมตัวอย่าง ไม่มีผลต่อการเกิดออกซิเดชันของไขมันในไส้กรอกปลาทั้ง 2 ชนิด ระดับการเกิดออกซิเดชันของไขมันจากไส้กรอกปลายี่สกเทศ สูงกว่าไส้กรอกปลาดุกแอฟริกัน ($p<0.05$)

สาขาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา_____

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FRESHWATER FISH/EMULSIFYING PROPERTIES/OIL IN WATER EMULSION/POTASSIUM IODIDE/TUNA OIL/SAUSAGE/LIPID OXIDATION

The objectives of this study were to investigate emulsifying properties of various freshwater fish species and to study the effect of potassium iodide (KI) concentration on emulsifying properties of freshwater fish muscle protein. In addition, the effects of KI and tuna oil fortification on lipid oxidation of oil in water (O/W) emulsion model system and emulsion sausages and on changes of color and textural properties of emulsion sausage were evaluated. Five freshwater fish species investigated were Nile tilapia (NT : *Oreochromis niloticus*), rohu (RH : *Labeo rohita*), small scale mud carp (SM: *Cirrhina microlepis*), hybrid catfish (HC : *Clarias macrocephalus* × *Clarias gariepinus*), and African walking catfish (AF : *Clarias gariepinus*). Myofibrillar protein stabilized emulsions showed higher emulsion stability (ES) than mince protein in all species. RH proteins exhibited the highest ES, while AF proteins showed the lowest ES at 50 °C. Emulsifying activity index (EAI) and ES values of all samples incubated at 50 °C were lower than those incubated at room temperature (28 °C). Changes of average oil droplet diameter ($d_{3,2}$) were higher at 50 °C than at 28 °C. EAI and surface hydrophobicity (S_0-ANS) of RH myofibrillar

mM KI. ES and $d_{3,2}$ were not affected by addition of 10–100 mM KI. Fortification of 100 mM KI and tuna oil (5 and 10%) resulted in an increase of hydroperoxide (HPV) and thiobarbituric reactive substances (TBARS) values ($p<0.05$).

AF and RH were selected for emulsion sausages production. Samples were fortified with three levels of tuna oil (2, 6, and 10%) and 150 μg KI/ 100g sample. The controls were prepared using 10% soybean oil without KI. Samples were vacuum-packed and stored at 4 °C. AF sausages showed higher gel strength and lower lightness than RH sausages ($p<0.05$). Tuna oil and KI concentration had no effect on gel strength and lightness ($p>0.05$). Iodine content of sausages decreased about 14% after cooking (week 0) and remained constant during storage up to 4 weeks. Sausages fortified with tuna oil had higher level of omega 3 fatty acids (eicosapentaenoic acid; EPA and docosahexaenoic acid; DHA), but lower levels of omega 6 fatty acids ($p<0.05$) than the control (10% soybean oil). The ratio of omega 6 to omega 3 fatty acids (n6/n3 ratio) of both sausages decreased with an increase of tuna oil addition and was stable throughout 4 weeks of storage. LA (linoleic acid), LNA (linolenic acid), EPA, and DHA content decreased at the 3rd and 4th week of storage in all samples. HPV and TBARS values increased as addition level of tuna oil increased ($p<0.05$). KI had no effect on lipid oxidation of fish sausages. HPV and TBARS values of AF sausages were lower than those of RH sausages ($p<0.05$).

School of Food Technology Student' Signature _____

Academic Year 2005 Advisor' Signature _____

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CHAPTER I

INTRODUCTION

1.1 Introduction

Fish play a very important role in human diet as a good source of high quality protein, essential lipid, mineral and trace elements. Fish protein contains all essential amino acids and it is highly digestible with the protein efficiency ratio between 2.7 to 3.2 (Haard, 1995). Both freshwater and marine fish are rich source of protein and lipid. Protein content of raw freshwater fish ranges from 17- 20 g/100 g and that of raw marine fish ranges from 18 – 22 g/100 g. In addition, fat content of freshwater fish is about 0.6 - 14 g/100 g and 0.5 – 9.2 g/100 g for marine fish (Puwastien, Judprasong, Keltwan, Vasanachitt, Nakngamanong, and Bhattacharjee, 1999).

World fishery production was amount 39.8 million tones with the value of US$ 53.8 billions in 2002 (FAO, 2005). Total catch of marine fish was 15.9 million tones, while the production quantity of freshwater fish was 23.8 million tones. The value of freshwater fish was US$ 27.2 billions and that of marine fish was US$ 26.5 billions (FAO, 2005). The major problem of freshwater fish in the world is its low value. To increase the value of freshwater fish, a variety of fishery products should be developed. Fish flesh can be used as a raw material for sausage production because muscle proteins can form gel and act as an emulsifying agent (Yada and Jackman, 1994). Muscle proteins mainly consist of myofibrillar proteins, which is about 65 – 80% of total muscle protein (Hall, 1997). The proportion of myofibrillar protein is higher in fish than in land animal (Ruiter, 1995). Myofibrillar proteins, particularly

actin and myosin, are essential for textural properties of emulsion meat products because they are macromolecular surfactants with amphiphilic property (Nakai and Modler, 1996). The ability of unfolded proteins to move to interface is different depending on species (Nakai and Modler, 1996). Therefore, emulsifying properties in fish species should be studied.

Freshwater fish contain less nutritional values than marine fish, especially iodine and omega 3 fatty acids. Iodine content of freshwater fish ranged between 21 to 56 mg/ 100 g. Omega 3 fatty acids content is insignificant (Judprasong, Sungpuang, Puwastein, and Charoenkiatkul, 2002). Therefore, to improve nutritional value of freshwater fish product, iodine and omega-3 fatty acid could be added. These compounds are essential for metabolism of human body.

Iodine, a non metabolic trace element, is required by human for synthesis of thyroid hormones, triodothyronine (T3) and thyroxine (T4). It is, therefore, essential for normal thyroid function. The thyroid gland traps iodine from the blood and converts it to thyroid hormones that are stored and released to the circular when needed (Tiwari, Godbole, Chattopadhyay, Mandal, and Mithal, 1996). In liver and brain, T3 which is the physiologically active thyroid hormone can bind to thyroid receptors in the cells and regulate gene expression. T4 is the most abundant circulating thyroid hormone. It can be converted to T3 by an enzyme known as deiodinase in target organs (Insel, Tunrmer, and Ross, 2001). In this manner, thyroid hormones regulate a number of physiological processes, including growth, metabolism, and reproductive function. About 1 mg of iodine per week is needed to ensure normal level for the synthesis of thyroid hormones (Pethes, 1980). An urinary iodine excretion below 40 μg per day (80 μg iodide per day) indicated iodine

deficiency in man, if renal clearance is normal (Koutras, 1968). A daily excretion of less than 60 μg iodide per day is an indicator of the existence of endemic goiter in a community (Stanbury, Hermans, Hetzel, Pretell, and Querido, 1974).

Iodine deficiency disorder (IDD) is a public health problem, especially in developing countries. At least 2225 million people or 38% of the world's population live in area at risk of iodine deficiency and 740 million people are affected by goiter (13% of world's population) (WHO, 2000). School children with iodine deficiency showed poorer school performance, lower IQ, and higher incidence of learning disabilities (Tiwari et al., 1996). In Thailand, iodine deficiency disorder is a critical problem, especially in the North and Northeast regions. Leenawat, Warasai, Limsiritong, and Kittidlokkul (2000) reported that iodine deficiency was about 2.24% of Thai population in 1999.

Omega-3 fatty acids are found in vegetable oils, marine fish, and seafoods. The major omega-3 fatty acids found in vegetable oils is ∝ - linolenic acid (18:3, ω3) whereas eicosapentaenoic acid (EPA, 20:5, ω3) and docosahexaenoic acid (DHA, 22:6, ω3) are predominant in fish and seafoods (Gunstone, 1996). In human, EPA and DHA can be formed by desaturation and elongation of ∝ - linolenic acid (Chow, 1992). Omega- 3 fatty acids play an important role in improving blood pressure and renal function (Holm et al., 2001), reducing the risk of cardiovascular disorder (Alexander, 1998), and decreasing cholesterol and triglycerides in blood. Moreover, omega-3 fatty acids are reported to inhibit carcinogenic process. Several studies reported the anti-carcinogenic function of omega-3 fatty acids. Cave (1996) reviewed the effect of omega-3 fatty acids on breast cancer. He found that omega-3 fatty acids delayed or reduced tumor development. Similarly, Dommels, Alink, Bladeren, and

Ommen (2002) found that fish oil supplemented with high amount of omega-3 fatty acids can protect against colorectal carcinogenesis (cancer of the colon or rectum) in animal model and human. In addition, Josyula and Schut (1999) studied the potential of omega-3 fatty acids in inhibiting DNA adduct formation in mice and rat. They concluded that dietary omega-3 fatty acids inhibited PhIP – DNA adduct formation in a spleen as well as in liver and gastrointestinal tract of mice but had no effect in rats.

Polyunsaturated fatty acids, such as omega 3 fatty acids, are extremely sensitive to lipid oxidation (Nuchi, McClements, and Decker, 2001). Lipid oxidation results in off-flavor. Addition of omega 3 fatty acids to improve nutritional value of food products could promote lipid oxidation and lessen storage stability. Thus, the effect of fish oil fortification on lipid oxidation of food products should be evaluated.

According to Verditti, Deleo, and Dimeo (1997), lipid peroxidation in blood can occur in patients with hyperthyroidisms. In addition, lipid peroxidation increased in rodents with goiter treated iodine (Allen, 1996). However, there are few studies about the effect of iodine on lipid oxidation in food products, particularly those containing high level of unsaturated fatty acids. Therefore, the effect of potassium iodide (KI) addition on lipid oxidation of freshwater fish products containing fish oil should be elucidated.

1.2 Research objectives

(1) To investigate emulsifying property of various freshwater fish species

(2) To evaluate the effect of iodide salt on flocculation of oil in water (O/W) emulsion model system

(3) To investigate the effect of fortified iodide salt and tuna oil on lipid oxidation of O/W emulsion

(4) To investigate the effect of iodine and tuna oil at various concentrations on physical and chemical properties of freshwater fish emulsion sausage

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CHAPTER II

LITERATURE REVIEWS

2.1 Chemical composition of fish muscle

Freshwater and marine fish supply protein equivalent to other animal but contain less fat (Table 2.1). Freshwater and marine fish are rich source of protein, fat, and ash. Freshwater fish flesh contains protein ranging from 17-20 g/100g, while protein of marine fish flesh is about 18-22 g/100 g. Fish protein contains all essential amino acids, such as lysine and methionine and it is highly digestible. In general, freshwater fish contain less fat content than marine fish (Table 1). Marine fish oil is a rich source of omega 3 polyunsaturated fatty acids, particularly eicosapentaeonic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are considered to be beneficial for growth and development throughout the life cycle and play an important role in the prevention of cardiovascular disease and hypertension. Lipid in freshwater fish contains high amount of saturated fatty acids, especially palmitic and strearic acids.

2.2 Lipids in fish muscle

Fish lipids contain high concentration of unsaturated fatty acids, especially omega 3 fatty acids. Omega 3 fatty acids are dominated by two members, eicosapentaenoic acid (EPA, 20:5n3), and docosahexaenoic acid (DHA, 22:6n3). EPA

Table 2.1 Proximate composition of raw freshwater and marine fish (per 100 g)

Fish	Moisture (g)	Protein (g)	Fat (g)	Ash (g)
Freshwater fish				
Common silver carp (*Cyprinus carpio*)	73.4	17.9	7.4	1.1
Nile tilapia (*Tilapia niloticus*)	78.1	19.8	1.8	1.0
Snake skin gourami (*Trichogaster pectoralis*)	55.5	19.8	5.9	8.7
Spotted featherback (*Notopterus chitala*)	80.3	16.7	1.2	1.6
Striped catfish (*Pangasius sutchi*)	71.6	17.0	8.9	1.0
Striped snake head (*Channa striatus*)	70.4	19.9	8.5	1.2
Walking catfish (*Clarias batrachus*)	65.1	17.8	14.7	1.1
Black – banded trevally (*Seriola nigrofasciata*)	71.1	18.1	9.2	1.5
Black – pomfret (*Parastromateus nigeo*)	74.3	20.4	3.6	1.2
Marine fish				
Giant seaperch (*Lates calcarifer*)	73.0	22.0	3.2	1.2
Malabar red snapper (*Lutjanus malabaricus*)	79.7	18.1	0.5	1.1
Mackerel (*Scomber scombrus*)				
Spanish	73.3	21.5	3.6	1.5
Short-bodied	79.7	18.18.1	0.5	1.1
Silver pomfert (*Pampus argenteus*)	72.7	19.4	6.8	1.0

Source: Modified from Puwastein Judprasong, Keltwan, Vasanachitt, Nakngamanong, and Bhattacharjee, 1999

and DHA are synthesized mainly by both uni- and multi- cellular marine plants, such as phytoplankton and algae (Kinsella, 1990). They are eventually transferred through the food web and are incorporated into lipids of aquatic species, such as fish and marine mammals, particularly those living in the cold water of the Atlantic region, at low temperatures. This was probably due to the ability of these fatty acids to maintain

fluidity in such environments (Pigott and Tucker, 1987). Fish species that are rich in omega 3 fatty acids are anchovy (European), bass (striped), bluefish, mackerel (Atlantic), herring (Atlantic and Pacific), pompano (Florida), salmon (Atlantic, Chinook, Pink, and Sockeye), sardines, trout (Lake and Rainbow), sablefish, and tuna (Sidhu, 2003) (Table 2.2).

Table 2.2 Omega 3 fatty acids content of various fish species (g/100g)

Fish (100g edible protein, raw)	Fat (g)	Omega 3 fatty acids (g)
Anchovy, European	4.8	1.4
Bass, striped	2.3	0.8
Blue fish	6.5	1.2
Mackerel, Atlantic	13.9	2.5
Pompano, Florida	9.5	0.6
Herring		
Atlantic	9.0	1.6
Pacific	13.9	1.7
Salmon		
Atlantic	5.4	1.2
Chinook	10.4	1.4
Pink	3.4	1.0
Sockeye	8.6	1.2
Sardine, in sardine oil	15.5	3.3
Trout		
Lake	9.7	1.6
Rainbow	3.4	0.5
Sablefish	15.3	1.4
Tuna	2.5	0.5

Source: Sidhu, 2003

2.2.1 Health benefits of omega 3 fatty acids consumption

The beneficial effects of omega 3 fatty acids might be due to its inhibitory action on the cyclooxygenase pathway (Figure 2.1). This pathway metabolizes arachidonic acid to the 2-series of prostaglandin (PGs), especially PGE_2, $PGF_2\propto$, and thomboxane A2. EPA is also an excellent substrate for the enzyme 5-lipoxygenase.

PGs occur at very low concentration in all mammalian tissues. They were first isolated from human and animal semen and initially assumed to be secreted by the prostate gland. PGs have been found to exert a wide variety of pharmacological effects on humans and animals, such as contraction and relaxation of smooth muscle of the tissues. PGE_2 is used in obstetrics to induce absorptions during the early to middle stages of pregnancy. PGE_1 also has vasodilatory properties and it is used for maintaining newborn infants with congenital heart defects of facilitating blood oxygenation prior to corrective surgery. It is also used to achieve erection. PGI_2 reduces blood pressure and inhibits platelet aggregation by reducing calcium concentrations. It is employed to inhibit blood clotting during renal dialysis and inhibit gastric acid secretion.

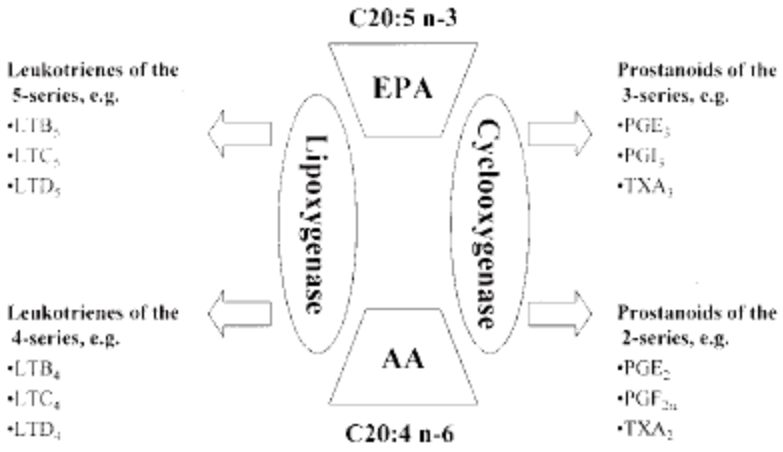

Figure 2.1 Eicosanoids derived from EPA and arachidonic acid

Source: Furst and Kuhn, 2000

Omega 3 fatty acids play a role in the reduction of thombotic tendencies and hypertriaclyglycerides (Harris, Rothrock, and Fanning, 1990). DHA levels in both the brain and the retina are crucial for proper nervous system and vision development

(Salem and Paloskey, 1992; Jorgensen, Hernell, Hughes, and Michaleson, 2001). This is especially true in infant development, where it has been shown that sight develops slowly in infants fed conventional DHA-free infant formula (Jorgensen et al., 2001; Carlson, 1995). EPA is believed to protect cardiovascular disease, immune disorder, inflammation, allergies, and diabetes (Sardesai, 1992; Terano, Salmon, Higgs, and Moncada, 1986). In the Netherland, taking 30g of fish daily consumption was found to be associated with 50% fewer deaths from coronary heart disease (Kromhout, Bosschieter, and de Lezenne Coulander, 1985)

However, omega 3 fatty acids are extremely susceptible to oxidation in both biological and food systems because of their high degree of unsaturation. Lipid oxidation is undesirable in most foods, causing the development of off-flavors and potentially toxic products (Kubow, 1993).

2.2.2 Lipid oxidation

2.2.2.1. Chemistry of lipid oxidation

Lipid oxidation is one of the major causes of quality deterioration in meat products (Gray, 1978; Fennema, 1993). Autooxidative degradation of lipids gives products that change food quality, including color, aroma, flavor, texture, and even nutritive value (Fennema, 1993). Lipid oxidation usually causes a decrease in consumer acceptance. The two major components involved in lipid oxidation are unsaturated fatty acids and oxygen. In this process, oxygen from atmosphere is added to certain fatty acids creating unstable intermediates that eventually break down to form flavor and aroma compounds. Although enzymatic and photogenic oxidation

may play a role important process by which unsaturated fatty acids and oxygen interact. This process is a free radical mechanism characterized by three main phases:

(1) Initiation:

$$RH + O_2 \longrightarrow R\bullet + \bullet OOH$$

(2) Propagation:

$$R\bullet + O_2 \longrightarrow ROO\bullet$$

$$RH + ROO\bullet \longrightarrow ROOH + R\bullet$$

$$ROOH \longrightarrow RO\bullet + \bullet OH$$

(3) Termination:

$$R\bullet + R\bullet \longrightarrow R - R$$

$$R\bullet + ROO\bullet \longrightarrow ROOR$$

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2$$

Initiation occurs as hydrogen is abstracted from unsaturated fatty acids, resulting in a lipid free radical, which in turn reacts with molecular oxygen to form a lipid peroxyl radical. The propagation phase of oxidation is fostered by lipid – lipid interactions, whereby the lipid peroxyl radical abstracts hydrogen from an adjacent molecule, resulting in a lipid hydroperoxide and a new lipid free radical. Such reactions continue 10 to 100 times before two free radicals combine to terminate process. However, lipid oxidation also occurs in branching reaction (secondary initiation): $Fe^{2+} + LOOH \longrightarrow LO\bullet + OH\bullet$. The radicals will proceed to abstract hydrogen from unsaturated fatty acids.

By themselves, lipid hydroperoxides are not considered harmful to food quality; however, they are further degraded into compounds that are responsible for off – flavor. The main mechanism for the formation of aldehydes from lipid

hydroperoxides is hemolytic scission (β cleavage) of the two C – C bonds on either side of the hydroperoxy group (Frankel, 1982). This reaction proceeds via the lipid alkoxyl radical, with the two odd electrons produced on neighboring atoms forming a carbonyl double bond. Two types of aldehydes are formed from the cleavage of the carbon bond: aliphatic aldehydes derived from the methyl terminus of the fatty acid chain and aldehydes still bound to the parent lipid molecule. Since unsaturated aldehydes can be oxidized further, additional volatile products may be formed (Frankel, 1982).

2.2.2.2 Lipid oxidation measurement

Numerous analytical procedures for the measurement of lipid oxidation in meat and meat products have been described. There are chemical methods, such as peroxide value, Kreis test, total and volatile carbonyl compounds, and thiobarbituric acid reactive substance (TBARS). In addition, physical methods that can be employed are polarography, infrared spectroscopy, refractrometry, fluorescence, and conjugated diene method. Currently, the techniques most widely used to measure lipid oxidation are:

2.2.1.2.1 Hydroperoxide value (HPV)

Hydroperoxides formed by the reaction between oxygen and unsaturated fatty acids are the primary products of lipid oxidation. Hydroperoxides have no flavor or odor but can break down rapidly to form aldehydes, which have a strong, unpleasant flavor and odor. Although a linear relationship has been observed between HPV and flavor scores during the initial stage of lipid oxidation, this method alone is not a very

good flavor quality indicator because the HPV increases to maximum and then decreases as storage time increases.

2.2.1.2.2 Thiobarbituric reactive substances (TBARS)

TBARS is another empirical method widely used to evaluate lipid oxidation in meat products. Malonaldehyde (MDA), a secondary decomposition product of polyunsaturated fatty acids with three or more double bonds, reacts with TBA to form a stable pink chromophore with maximal absorbance at 532 nm. The reaction with TBA occurs by attacking of monoenolic form of MDA on the active methylene groups of TBA (Figure 2.2).

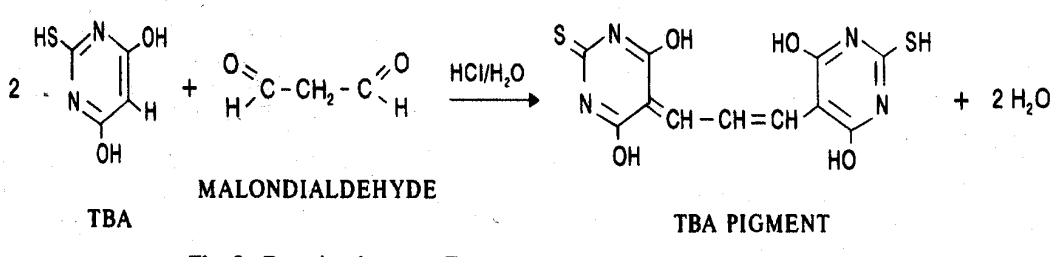

Figure 2.2 Reaction between TBA and MDA to form TBA pigment

Source: Fernandez, Perez-Alvarez, and Fernandez-Lopez, 1997

The intensity of color is a measure of MDA concentration and has been correlated with the rancidity warmed-over flavor (W.O.F.) (Fernandez, Perez-Alvarez, and Fernandez-Lopez, 1997). TBA test compares the absorbance of a MDA – TBA complex with a standard made from 1,1,3,3 tetraethoxypropane (TEP) or 1,1,3,3 tetramethoxypropane (TMP), because the MDA can be obtained from acid hydrolysis of TMP or TEP in an equimolecular reaction.

2.2.1.2.3 Substrate (Unsaturated fatty acids) measurement

Determination of the fatty acid composition as their fatty acids methyl esters (FAMEs) by gas chromatography (GC) is a commonly used technique. Since fatty acids are the basic substrate of lipid oxidation, it would seem logical that their consumption should correspond to the progress of the oxidation.

2.2.1.2.4 Determination of volatile flavor compound

Volatile hydrocarbons released during the decomposition of the oxidized intermediates can be used to monitor the extent of lipid oxidation. GC is the most powerful tool available for determining volatile oxidation products due to its high resolution and the possibility of coupling a mass spectrometer for compound identification. The product can be analyzed directly (e.g. by headspace or simultaneous distillation extraction (SDE) extract). Pentane is one of the main hydrocarbon gases released through thermal decomposition of the oxidized intermediates. The amount of pentane correlated with flavor scores.

2.3. Protein in fish muscle

2.3.1 Protein composition

Proteins of muscle can be divided into three main groups depending on their solubility in salt solution (Table2.3).

2.3.1.1 Sarcoplasmic proteins: The sarcoplasmic or ‘myogen’ fraction of muscle is the large family of proteins that is soluble in water and diluted salt solution. Sarcoplamic proteins content in fish are more or less like those from land animals (Table 2.3). Sarcoplasmic proteins included myoglobin, hundreds of enzymes, and other albumins. The muscles of fish and lower vertebrates differ from those of land

animals in that they contain large quantities of Ca^{2+} - binding protein called parvalbumins (Gazzaz and Rasco, 1993).

Table 2.3 Skeletal muscle proteins in the flesh of fish and mammals

Protein group	Content (%)	
	Fish	Mammals
Sarcloplasmic	20 - 35	30 - 35
Myofibrillar	65 - 75	52 - 56
Connective tissue	3 - 10	10 - 15

Source: Hettiarachchy, 1994

2.3.1.2 Myofibrillar protein: Myofibrillar proteins are extractable in 0.5 M NaCl. Myofibrillar proteins, myosin and actin and their product of interaction, actomyosin, are the major proteins of skeletal muscle. Myosin is the main protein component of the thick filament, while actin together with tropomyosin and troponin constitute the thin filament. Myosin has the molecular weight of 500,000 Da varying with species. It comprises two heavy chains of molecular weight 200,000 Da. And four light chains of molecular weight 20,000 Da. The two heavy chains exist in a supercoiled helical transformation (Figure 2.3) and at the end of both chains are folded into globular structure. The twin globular heads contain the site involved in the contractile mechanism and ATPase activity. Fish myosins, particularly those of white fish species from temperate water, are the least stable of all myosins and are in general more susceptible to the action of proteolytic enzymes. Actin from fish appears to be identical to that from mammals. As the monomer, actin has a molecular weight of 42,000 and it exists in the thin filament as the polymeric form, F actin, a helical

double strand of monomer units. The proportion of myofibrillar protein in fish was higher in mammals (Table 2.3).

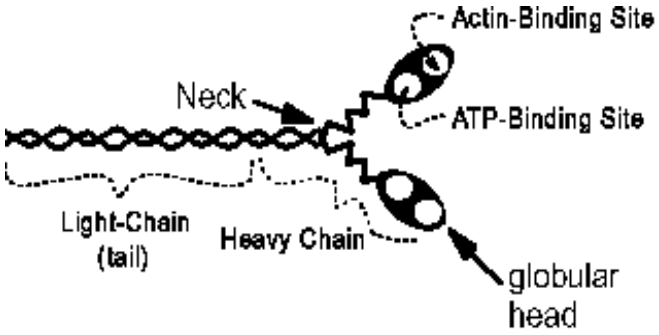

Figure 2.3 Schematic structure of myosin

Source: http://www.neuro.wustl.edu/neuromuscular, 2004

2.3.1.3 Connective tissue: The insoluble matter remaining after removing sarcoplasmic and myofibrillar proteins from the muscle contain the connective tissue. The two major types of these proteins are collagen and elastin. Connective tissues are located primarily in the extracellular matrix and contain few living cells composed of carbohydrates and lipids as well as proteins. The connective tissue contains small amount of other proteins. Fish contain smaller content of connective tissue than mammals (Table 3).

2.3.2 Emulsifying properties

Emulsification is one of the most important processes in the manufacturing of many formulated foods. Food emulsions are classified as macroemulsions with droplet size of 0.2 to 50 μm (McClements, 1999). Emulsion represents a heterogeneous mixture of fat globulars. Food emulsion can be of the oil in water

(O/W) or water in oil (W/O) type. Emulsifying activity is the ability of protein to participate in emulsion formation and to stabilize the newly created emulsion. Emulsifying activity of proteins depend on shape, charge, and hydrophobicity of protein molecules, hydration of polar groups. Emulsion stability depends on the magnitude of these interactions (Voutsinas and Nakai, 1983). To produce stable emulsions, soluble protein should absorb rapidly at the interface and form a strong cohesive film (Zayas, 1997).

2.3.2.1 Interfacial film formation and properties

The interfacial film formation at an oil-water interface has been studied. The surface activity of proteins is expressed as the protein ability to migrate, absorb, unfold and form a layer of the interface as a result of rearrangement. Proteins form membranes or film around the fat droplets and lower the interfacial tension between water and oil. As a result, proteins retard coalescence of fat droplets. Surface film formation is a result of diffusion and absorption of protein molecule at the interface. Formation of protein film is facilitated if protein is solubilized, and is influenced by protein capacity to diffuse to the interface, followed by partial unfolding and reorientation at the interface (Kinsella and Whitehead, 1987). The important property of proteins for their surface activity is molecular flexibility and conformation. Formation of the interfacial film includes:

1). Diffusion of the proteins to interface: During emulsification the soluble protein diffuses and concentrates at the interface. The diffusion is influenced by protein concentration, molecular size, temperature, pH, ionic strength, and solubility.

2). Protein adsorption at the interface: Formation of stable interfacial films and emulsion is a result of rapid adsorption of the surface-active protein at the oil-water interface. Polar and non-polar sites of proteins control the adsorption and reorientation of protein surfactants at the interface. The non-polar groups contribute to hydrophobicity of proteins and affinity of the proteins for the oil phase. Proteins with an excess of hydrophobic groups have a higher potential for adsorption at the interface than hydrophilic proteins. The adsorption and layer formation at the surface is affected by the amino acid composition, protein conformation, pH, ion concentration, viscosity, temperature, hydrophilic-hydrophobic balance, and intensity of homogenization (Zayas, 1997).

3). Changes of protein conformation resulted from unfolding and reorientation of molecules: Protein molecules already adsorbed at the interface are exposed to conformational changes, rearrangement, and expansion. Protein molecules unfold at the interface depending on their molecular properties and spread to cover the surface area, forming an extended film formed. Protein rearrangement is affected by the nature of protein, molecular flexibility, and protein concentration at the interface.

2.3.2.2 Measurement of emulsifying properties

A variety of methods have been applied to measure emulsifying capacity (EC) and emulsion stability (ES). For this reason, there are difficulties in comparing the measurement carried out by different methods. Emulsifying property is influenced by the procedure of emulsion preparation, and there is no accepted standard procedure.

EC and ES can be measured by various methods: change of the particle size distribution by static light scattering, change in the separated oil fraction, change in the drained water phase over the time, and change in the turbidity by spectrophotometer. The spectroturbidity method was widely used to measure EC and the data was expressed as the emulsifying activity index (EAI). The EAI indicates the area of interface stabilized per unit weight of protein. EAI is also influenced by the conditions of emulsification and emulsifying equipment. The best method of measuring ES is dispersion analysis to register the size distribution of fat particles as a function of different factors (Zayas, 1997). The most reliable method of emulsion dispersivity measurment is static light scattering observation to assess changes in the dimensions of the individual droplets. Emulsion breakdown can be determined by the increase in the large oil particle.

Static light scattering is used for emulsion stability measurement. The principle of this technique is determination of droplet size distribution. Knowledge of the particle size of an emulsion is useful for predicting the long-term stability creaming, flocculation, and coalescence. Static light scattering is used to determine particle size between 0.1 and 1000 μm and is therefore suitable for characterizing oil droplet in most food emulsions. When a beam of light is directed through an emulsion, it is scatted by the droplets. A measurement of the degree of scattering can be used to provide information about the droplet size distribution and concentration. Theories that relate light scattering data to droplet size distribution are based on a mathematical analysis of the propagation of an electromagnetic wave through droplet particle (McClements, 1999).

Most modern particle sizing instruments based on static light scattering measure the angular dependence of the scattered light (Mikula, 1992). The sample to be analyzed is diluted to an appropriate concentration and then placed in a glass cell. A laser beam is generated by a helium-neon laser (λ = 632.8 nm) and directed through the cell, where it is scattered by the emulsion droplets. The intensity of the scatted light is measured as a function of the scattering angle using an array of detectors located around the sample. The scattering pattern recorded by the detectors is sent to a computer. Before analyzing a sample, the instrument is usually blanked by measuring the scattering profile from the continuous phase in the absence of emulsion droplets. This scattering pattern is then subtracted from that of the emulsion to eliminate extraneous scattering from background sources other than the droplets.

2.3.2.3 Emulsifying properties of fish protein

Salt soluble fish proteins are considered to have emulsifying properties superior to water soluble proteins. Borderias, Jimenenez-Colmenero and Tejada (1985) reported the emulsifying capacity (EC) of myofibrillar and sarcoplasmic proteins of various fish species. They found that EC decreased with an increased protein concentration, resulting in fewer molecules at an interface in the emulsion. In addition, as protein concentration increases, the emulsion system is over-saturated with proteins, resulting in decreased EC. Variations among fish species were related to different concentrations of soluble proteins. Sarcoplasmic proteins of all fish species exhibited the same emulsifying capacity. At low protein concentrations (5mg/ml), EC of sarcroplasmic proteins was higher than that of myofibrillar proteins and the muscle homogenates. This was contradicted to the finding of Gaska and

Regenstein (1982) who concluded that sarcoplasmic proteins did not take part in emulsion formation. Sarcoplasmic proteins had lower molecular weight and less molecular flexibility than myofibillar protein. Therefore, protein unfolding and alignment of sarcoplasmic proteins at the oil interface took place to a less extent than those of myofibrillar proteins resuling in a lower EC.

EC of hake collagen increased as concentration increased (Montero and Borderias, 1991). The EC of muscle collagen was higher than that of skin. EC values of hake collagen remained low and stable at 1 and 2% NaCl, and increased notably at 3% NaCl. This was due to an increased solubility at 3% NaCl. EC values were low but stable at pH 1 - 5 and increased markedly at pH 5-6. This was probably due to a disruption of intermolecular cross-links at pH 5-6. The EC was generally higher for the collagen from hake than from trout. The EC of minced fish meat decreased during ice and frozen storage (Vidya Sarga Reddy and Srikar, 1991). A marked decrease in EC occurred during the first 90 and 120 days of frozen storage and 3-day ice stored sample. This might be explained by a concurrent decrease in muscle protein solubility due to protein denaturation induced by freezing and frozen storage.

2.4. Iodine in fish muscle

Iodine, non metallic trace elements, is required by human for the synthesis of thyroid hormones. Fish is one of the rich sources of iodine, especially marine fish (Table 2.4). Marine fish contain large amount of iodine because they adsorb iodine from seawater. Iodine is one of the destabilizing salts in the Hofmeister series. Iodide (I^-) can increase surface tension of water and their ability was lower than chloride (Cl^-).

Table 2.4 Iodine concentration of some fish species

Species	Iodine content (μg/100g sample)
Changu	88.4
Tasi	12.2
Pawale	29.1
Dagaa mchete	22.8
Dagaa papa	34.7
Dagaa kavu	36.8
Prawns	138
Barbus	0.5
African catfish	2.0
Nile tilapia	1.4
Carp	3.1

Source:Eckhoff and Maage, 1997

2.4.1 Function

Iodine is an essential component for synthesis of thyroid hormones. In the thyroid cells, iodide is actively transported from the blood to thyroid cells by thyroid-stimulating hormone (TSH or thyrotropin). Iodide is oxidized to iodine by the enzyme iodide peroxidase. Thyroid hormones also influence physical and mental growth and differentiation or maturation of tissues; affect other endocrine glands, especially the hypophysis and the gonads; influence neuromuscular functioning; have an effect on the integument and its outgrowths, hair, fur, and feathers; influence the metabolism of nutrients, including various minerals and water (McDowell, 1992).

2.4.2 Iodine deficiency

Iodine deficiency disorder (IDD) is a critical worldwide public health problem, especially in developing countries. World Health Organization (WHO), International council for the Control of Iodine deficiency disorder (ICCIDD), and United Nations International Children' Emergency Fund (UNICEF) reported that

2225 million people of the world are living in iodine deficiency areas (38% of world population) and 740 million people are affected by goiter (13% of world population) (WHO, 2000).

Goiter is most visible signs of iodine deficiency. Goiter is response to persistent stimulation by TSH. In mild iodine deficiency, this adaptation response may be enough to provide the body with sufficient thyroid hormone. However, more severe cases of iodine deficiency result in hypothyroidism. Adequate iodine intake will generally reduce the size of goiters, but the reversibility of the effects of hypothyroidism depends on an individual's stage of development. Iodine deficiency has an adverse effect on stages of development, but is most damaging to the developing brain. In addition to regulating many aspects of growth and development, thyroid hormone is important for the myelination of the central nervous system, which is most active before and shortly after birth.

Iodine deficiency during infancy may result in abnormal brain development and consequently, impaired intellectual development (Hetzel, 2000). School children with iodine deficient show poorer school performance, lower IQs, and a higher incidence of learning disabilities (Tiwari, Godbole, Chattopadhyay, Mandal, and Mithal, 1996). The effects of hypothyroidism are more subtle in the brains of adults than children. Hetzel and Clugston (1999) suggested that hypothyroidism resulted in slower response times and impaired mental function in adult. Iodine deficiency during pregnancy has been associated with increased incidence of miscarriage, stillbirth, and birth defects. Moreover, severe iodine deficiency during pregnancy may result in congenital hyperthyroidism (cretinism) in the offspring (Lavander and Whanger, 1996). Iodine deficient woman who are breastfeeding may not be able to provide

sufficient iodine to their infants who are particularly vulnerable to iodine deficiency (Hetzel and Clugston, 1999).

2.4.3 Iodine requirements

To ensure an adequate supply of thyroid hormones, the thyroid must trap about 60 μg of iodine per day (Underwood, 1977). The daily iodine requirement for prevention of goiter in adults is 50 – 75 μg, or approximately 1 μg/kg of body weight (Food and Nutrition Board, 1970). To provide a margin of safety, an allowance of 150 μg is recommended for adolescents and adults (National Academy of Sciences, 1980). The recommended allowances are 40 – 50 μg/ day for infants and 70 – 120 μg/day for children of 1 to 10 years old (National Academy of Sciences, 1980). Additional allowances are rise about 25 and 50 μg/day for pregnant and lactating women, respectively.

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CHAPTER III

EMULSIFYING PROPERTIES OF SOME FRESHWATER FISH MUSCLE PROTEINS

Abstract

Emulsifying activity index (EAI), emulsion stability (ES), and changes of oil droplet diameter ($d_{3,2}$) of muscle protein from various freshwater fish species were investigated. The effect of temperature (28 and 50 °C) on EAI, ES, and $d_{3,2}$ was also evaluated. The studied species were Nile tilapia (NT: *Oreochromis niloticus*), rohu (RH: *Labeo rohita*), small scale mud carp (SM: *Cirrhina microlepis*), hybrid catfish (HC: *Clarias macrocephalus* × *Clarias gariepinus*), and African walking catfish (AF: *Clarias gariepinu*). EAI and ES of emulsion stabilized by either myofibrillar and mince proteins of various species were similar at 28 °C. EAI and ES values of samples incubated at 50 °C were lower than those incubated at 28 °C. Changes of $d_{3,2}$ were higher at 50 °C than at 28 °C. Myofibrillar and mince proteins of rohu exhibited the highest ES at 50 °C. Emulsions prepared from myofibrillar protein were more stable than those prepared from mince protein in all species.

Key words: Emulsification activity index (EAI), emulsion stability (ES), $d_{3,2,}$

3.1 Introduction

Fish is an important source of protein for human. World fishery production was reported to be 93.2 million metric tons in 2002 (FAO, 2005). Freshwater and marine fish production was 20 and 15.9 million metric tons with the value of US$ 27.2 billion and US$ 26.5 billon, respectively (FAO, 2005). Protein content of raw freshwater fish was 17-20 g/100 g meat and that of marine fish was 18-22 g/100 g meat. Proteins play an important role in functional properties of food, such as gel forming ability, water holding capacity, foaming ability, and emulsifying property.

Muscle proteins are widely used to produce emulsion based food products, such as emulsion sausage. Muscle proteins play an important role in gel formation and emulsion of meat products. Many research works used an oil in water (O/W) emulsion model system to study the emulsifying behavior of muscle proteins to deputize for real food. The ability to stabilize O/W emulsion is one of the most important functional properties of muscle proteins. The dispersion of oil into water greatly increases the interfacial area, thus increases the free energy of the interface between the two phases (Jackman, Yada, and Paulson, 1989). The surface-active properties of protein serve to lower the free energy of the oil/water interface. Protein stabilized emulsion by forming a viscoelastic-adsorbed layer, which are thought to influence the stability of emulsion (Murray and Dickinson, 1996). The ability of proteins to generate repulsive interactions (e.g. steric and electrostatic) between oil droplets and to form an interfacial membrane that is resistant to rupture are critical for stabilizing droplets against flocculation and coalescence during long term storage (Bos and van Vliet, 2001; Wilde, Mackie, Husband, Gunning, and Morris, 2004; Tcholakova, Dankov, Ivanov, and Campbell, 2002). It is known that the extent of protein

adsorption at the interface is influenced by surface hydrophobicity (Li-Chan, Nakai, and Wood, 1984; Nakai and Modler, 1996). Once proteins are adsorbed, they unfold and rearrange their secondary and tertiary structure to expose hydrophobic residues to the hydrophobic phase of oil droplet (Nakai and Modler, 1996). Emulsifying properties of protein-stabilized emulsion are influenced by various factors, such as pH, ionic strength, and heating. Heating is an integral part of comminuted meat manufacture. Thus, the effect of heating on emulsifying properties of emulsion stabilized by muscle proteins should be investigated.

Most research works thus far have focused on emulsifying properties of muscle protein from marine fish. Very few studies have investigated emulsifying properties of freshwater fish protein. Thus, the objectives of this study were to elucidate the effect of freshwater fish species, protein types (myofibrillar and mince protein), and protein concentration on emulsion activity and stability.

3.2 Materials and Methods

3.2.1 Sample preparation

Fresh freshwater fish were obtained from a local fish market at Nakhon Ratchasima. These included Nile tilapia *(*NT: *Oreochromis niloticus*), rohu (RH: *Labeo rohita*:), hybrid catfish (HC: *Clarias macrocephalus* × *Clarias garieepinus*), small-scale mud carp (SM*: Cirrhina mrigala*), and African walking catfish (AF: *Clarias gariepinus*). Fish were headed, degutted and washed in cold water. Skin and bones were manually removed. Fish fillet were comminuted using a grinder (Biro 8-22, Biro Manufacturing, Marblehead, Ohio, USA). Minced fish was used to prepare myofibrillar and mince protein solution.

Fish minces were suspended in 6 volumes of 0.6 M NaCl in 10 mM phosphate buffer (pH 7). The homogenate was filtered through double layers of cheesecloth and centrifuged at 11,000×g for 30 min at 4 °C. The supernatant was referred as mince protein.

Myofibrillar protein was prepared by homogenizing mince in 10 volumes of 0.1 M NaCl in 10 mM phosphate buffer (pH 7) at 4 °C. The homogenate was centrifuged at 11,000×g for 30 min at 4 °C. The supernatant was discarded and the precipitates were washed with the same buffer. The second washed precipitates were suspended in 6 volumes of 0.6 M NaCl in 10 mM phosphate buffer (pH 7) at 4 °C and homogenized using a high-speed homogenizer (Nissei AM-8, NSS Nihonseiki Kaisha Co., Ltd., Kyoto, Japan) for about 30 s. Connective tissue and other non-solubilized proteins were removed by centrifugation at 11,000×g for 30 min 4 °C. The supernatant was used as myofibrillar protein.

3.2.2 Emulsifying properties of fish protein

Oil in water (O/W) emulsion was prepared by a slight modification of Fomuso, Correding, and Akoh (2002) method. Corn oil (10%, v/v) and protein solutions were mixed to contain final protein content of 1, 3.5, and 5 mg/ml using a homogenizer (Ystral, scientific, T25, Eastleigh, Hampshire, England) at 60,000 rpm for 15 s. Emulsion activity index (EAI) and emulsion stability (ES) were evaluated by the method of Pearce and Kinsella (1978). Emulsions were diluted in 41 volumes of 0.1% sodium dodecyl sulfate (SDS) and stirred for 20 s using a vortex mixer before taking absorbance measurement at 500 nm. EAI of myofibrillar and mince proteins was calculated from:

$$EAI\ (m^2/g\ protein) = \frac{2 \times 2.303 \times A}{L \times C \times \Phi} \times (k)$$

When A = Absorbance at 500 nm L = path length of the cuvette

C = protein concentration in aqueous phase (mg/ml) Φ = oil ratio

K = dilution factor of protein in SDS

ES was calculated from:

$$ES\ (\%) = \frac{\text{Absorbance at 1 h} \times 100}{\text{Absorbance at initial time}}$$

3.2.3 Particle size measurement

Emulsions were diluted about 500 folds using 0.6 M NaCl in 10 mM phosphate buffer solution (pH 7) to avoid multiple scattering effects. The particle size of the emulsion was measured using a laser light scattering instrument (Mastersizer S Malvern Instruments, Malvern, Worcestershire, UK). The instrument measures the angular dependence of the intensity of laser light (λ=632.8 nm) scattered by a diluted emulsion. Particle size measurements were reported as the volume average mean diameter ($d_{3,2}$).

3.2.4 Surface hydrophobicity

Surface hydrophobicity (S_0) of mince and myofibrillar protein solution (in 0.6 M NaCl in 10 mM phosphate buffer, pH 7) was measured using 8-anilino-1-naphthalene-sulfonic acid (ANS) as described by Hayakawa and Nakai (1985). The protein-ANS complex was measured at excitation and emission wavelengths at 375 and 485 nm, respectively. The fluorescence intensity of the protein – ANS complex was plot against protein concentration (0 – 0.1%). S_0 of each sample was calculated

from the slope of the fluorescence intensity vs percentage of protein concentration (w/v).

3.2.5 Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used to monitor proteolysis of muscle proteins. Raw myofibrillar and mince proteins and those incubated at 50 °C for 1 h were homogenized with 10 volume of 5% SDS. The homogenate were centrifuged at 13000×g for 30 min. Supernatants were mixed with the treatment buffer (0.125 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol) and heated in boiling water for 5 min. Protein was loaded at 20 μg. Molecular weight standards ranging from 4500 to 205000 Da were used (Bio-Rad Laboratories, Hercules, CA, USA). SDS-PAGE was performed on 4% stacking gel and 10% running gel (Laemmli, 1970).

3.2.6 Statistical analyses

The whole experiment was carried out in two replications. Mean values ± standard deviations were presented. The effects of freshwater fish species, protein type, and protein concentration on EAI, ES, and changes of oil droplet diameter were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 1993; SAS Institute Inc., Carry, NC, USA) as a factorial design in randomized complete block design (RCBD). Significant difference of mean values was determined by using the Duncan multiple ranges test (DMRT). Level of significance was set at $p \leq 0.05$.

3.3 Results and discussion

3.3.1 Emulsifying activity of freshwater fish muscle proteins

EAI values of myofibrillar and mince protein were comparable among species studied ($p>0.05$, Figure 3.1). EAI values of myofibrillar and mince proteins at any protein concentration were also similar ($p>0.05$). Majority of mince protein is myofibrillar protein, resulting in similar EAI of myofibrillar and mince protein. These results suggested that myofibrillar protein was a major component contributing to emulsifying property. EAI of both proteins tended to decrease with protein concentration in all species (Figure 3.1). This was in agreement with Montero and Guillen (1998) who studied emulsifying property of freeze-dried wash water protein from krill processing. Huidobro, Montero, and Borderias (1998) described the same effect in emulsifying capacity of freeze-dried sardine wash water. Montero and

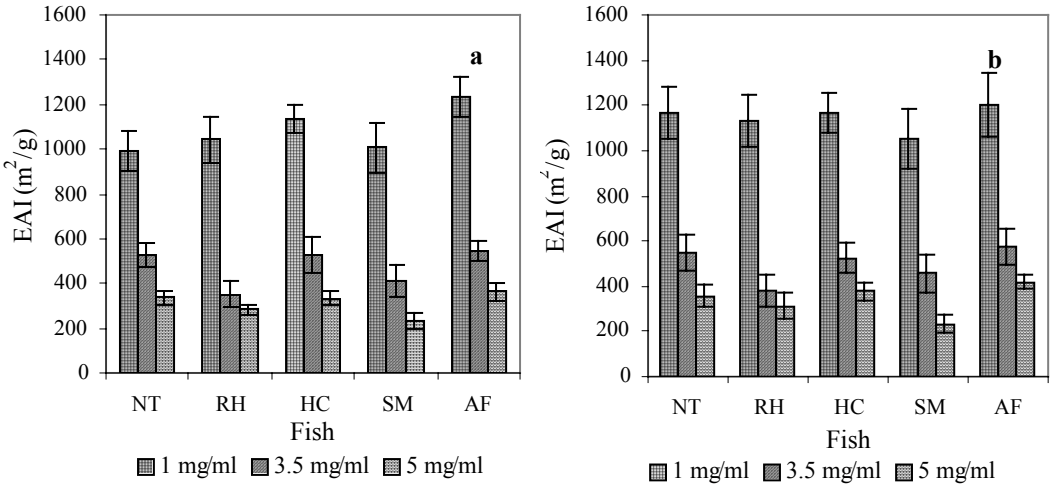

Figure 3.1 Emulsifying activity index (EAI) of myofibrillar (a) and mince protein (b) of various freshwater fish species; NT, Nile tilapia: RH, rohu: HC, hybrid catfish: SM, small scale mud carp: AF,African walking catfish

Borderias (1991) also found that emulsifying activity of collagen decreased with concentrations. Kinsella (1976) indicated that an increase in protein concentration led to protein aggregation in the aqueous phase, subsequently a fewer molecules acted at the interface of emulsion.

The effect of fish species and protein types (myofibrillar and mince protein) on EAI corresponded with surface hydrophobicity of proteins (Table 3.1). Surface hydrophobicity values of mince and myofibrillar protein were comparable among species at 4 °C ($p>0.05$). Protein adsorption and orientation at the oil water interface is affected mostly by surface hydrophobicity (Phillips, 1981). Li-Chan, Nakai, and Wood, 1985) reported that emulsifying property of crude salt soluble muscle proteins correlated with their surface hydrophobicity. They also found that EAI of beef top round and rockfish increased with surface hydrophobicity. In addition, Kristisson and Hultin (2003) showed that EAI of emulsion stabilized by acid treated cod myosin increased with increasing of surface hydrophobicity.

Table 3.1 Surface hydrophobicity of freshwater fish protein at 4 °C and incubated at 50 °C for 1 h

Fish	Surface hydrophobicity			
	Myofibillar protein		Mince protein	
	4 °C	50 °C	4 °C	50 °C
NT	1092.1[a]	2381[b]	958.7 [a]	1514.6[b]
SM	973.1 [a]	2397.1[b]	753.4 [a]	1449.5[b]
HC	895.2 [a]	2394.2[b]	906.3 [a]	1406.6[b]
RH	978.4 [a]	2591.7[a]	1045.7 [a]	1897.6[a]
AF	741.7 [a]	1986.9[c]	855.9 [a]	960.5[c]

** [a, b] in the same column indicated statistical difference at $p<0.05$

Incubation of proteins at 50 °C for 1 h resulted in higher surface hydrophobicity than at 4 °C in all samples ($p<0.05$, Table 3.1). Fish muscle proteins underwent conformational changes when subjected to 50 °C. Intramolecular linkages including ionic interactions, hydrogen bonds, and hydrophobic interactions were disrupted, resulting in partial unfolding and exposure of hydrophobic groups. Surface hydrophobicity of RH proteins was the highest at 50 °C while that of AF proteins was the lowest ($p<0.05$). AF proteins appeared to possess more molecular rigidity than other species. Surface hydrophobicity of myofibrillar protein was higher than that of mince at 50 °C in all species (Table 3.1). Mince solution composed of both sarcoplasmic and myofibrillar proteins. Myofibrillar protein fraction in the mince solution was lower than in the myofibrillar protein solution. In addition, thermal denaturation temperatures of sarcloplasmic proteins (67.2 °C) were higher than those of myosin (40-45 °C) (Paredi, Tomas, Crupkin, and Anon, 1996). For this reason, surface hydrophobicity of mince protein was lower than that of myofibrillar protein at 50 °C.

3.3.2 Emulsion stability (ES) of freshwater fish muscle proteins

ES of mince and myofibrillar protein among various species were similar ($p>0.05$, Table 3.2). ES of myofibrillar protein was not different from that of mince solution ($p>0.05$). ES was similar at all concentrations studied ($p>0.05$, Table 2). This was in agreement with Cofrades, Carballo, Careche, and Jimenez-Colmenero (1996) who reported that protein concentration did not affect ES of hake actomyosin. In addition, Huidobro et al. (1998) found that ES of freeze-dried sardine wash water at

concentrations of 5–25 mg/ml was similar. When protein concentration increased to excessive level, the oil-water interface became saturated, resulting in the constant ES. Changes of oil droplet diameter were not affected by fish species and protein types ($p>0.05$, Figure 3.2). Protein concentration at 1 to 5 mg/ml did not affect the changes of oil droplet diameter within 10 min (Figure 3.2). These results corresponded with ES values.

Table 3.2 Emulsion stability (ES) of myofibrillar and mince proteins from various freshwater fish species at 28 °C

Fish	Protein type	ES (%)		
		1 mg/ml	3.5 mg/ml	5 mg/ml
NT	Mince	50.9	56.64	60.3
	Myo	53.97	57.78	52.28
RH	Mince	57.14	52.89	48.85
	Myo	56.61	48.39	47.37
SM	Mince	60.43	56.14	55.35
	Myo	59.23	50.06	55.35
HC	Mince	54.6	57.42	56.41
	Myo	55.73	61.36	56.32
AF	Mince	53.6	60.51	67.96
	Myo	52.15	57.4	63

- Myo, myofibrillar protein

3.3.3 Effect of incubating temperature on emulsifying activity

Sausages are typically smoked at 40 to 50 °C for 40 min before cooking. Thus, the effect of incubating at 50 °C on emulsifying properties of freshwater fish protein

was studied. Both proteins from all species showed similar EAI at 50 °C ($p>0.05$, Figures 3.3). A decrease in EAI was observed in all species when heated at 50 °C as

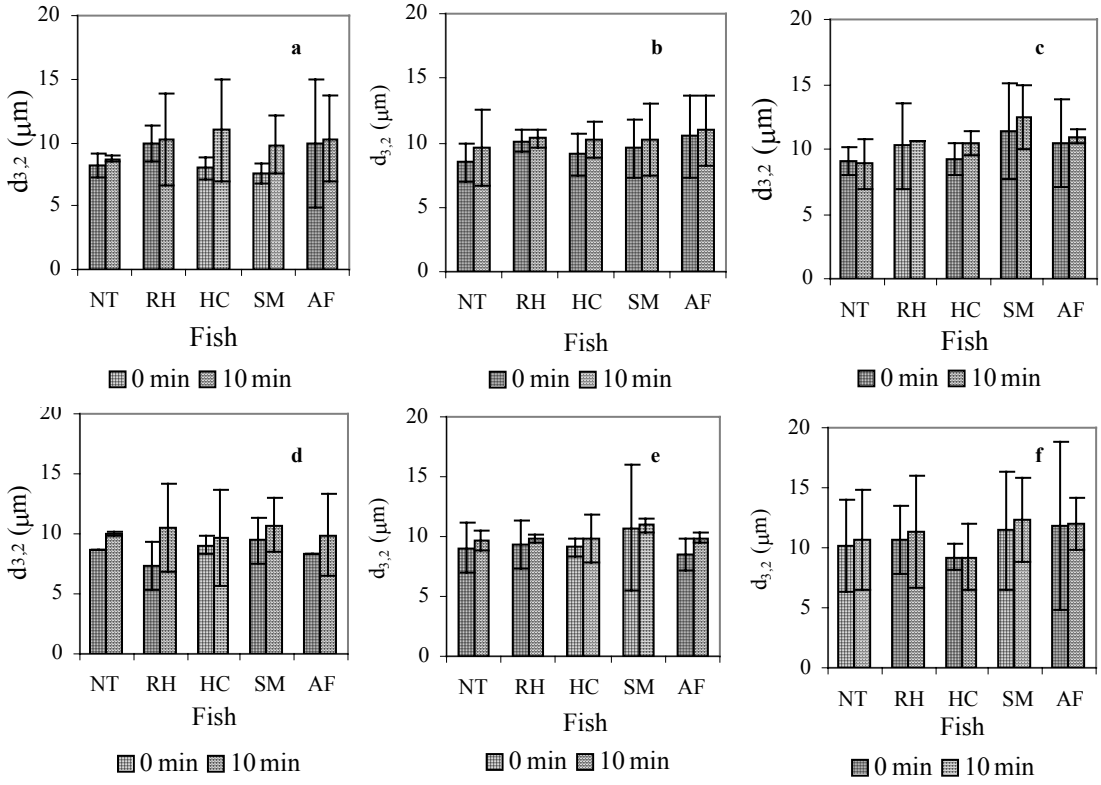

Figure 3.2 Changes of oil droplet diameter stabilized by myofibrillar (a-c) and mince protein (d-f) of various species at 28 °C; a,d; 1 mg/ml, b,e: 3.5 mg/ml, c,f: 5mg/ml

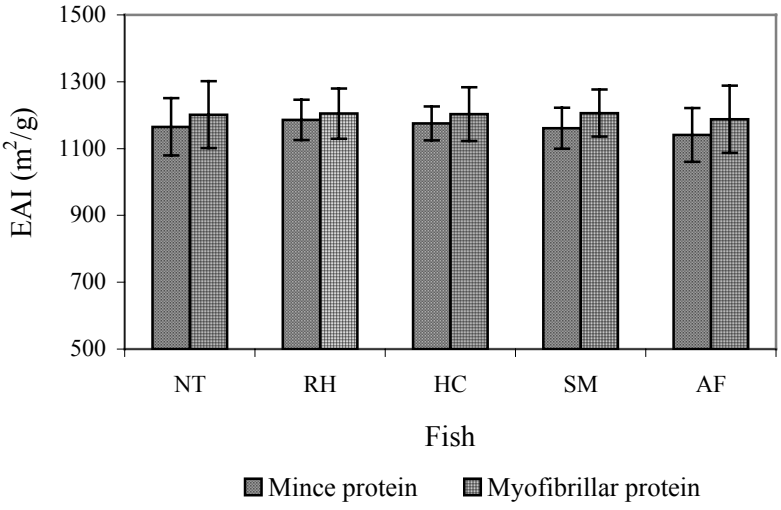

Figure 3.3 Effect of incubation at 50 °C on EAI of myofibrillar and mince proteins of various freshwater fish species at 1 mg protein/ml emulsion

compared to at 28 °C (Figure 3.1). This was probably related to excessive unfolding of proteins around oil droplet, resulting in a destabilization of the film at the oil-water interface and the coalescence of emulsion. Witt (1989) suggested that emulsifying properties of whey protein concentrate was reduced when heating emulsion at temperature above 60-70 °C. Emulsifying activity of egg yolk LDL decreased as temperature increased from 55 to 100 °C (Tsutsui, 1988). In addition, Li-Chan et al (1985) reported that EAI of beef top round and rockfish decreased as a function of heating temperature (35-75 °C). It should be noted that EAI of mince protein was lower than that of myofibrillar protein in all species at 50 °C ($p<0.05$, Figure 3.3). This might be due to the greater extent of unfolding of myofibrillar protein as seen by S_0-ANS values (Table 3.1).

3.3.4 Effect of incubating temperature on ES and change of oil droplet diameter

Myofibrillar protein extracted from RH exhibited the highest ES at 50 °C (Table 3.3). These results were in consistent with surface hydrophobicity (Table 3.1). The greater exposure of hydrophobic amino acids could favor hydrophobic interaction of RH myofibrillar protein at the interface, yielding a higher ES. ES values of samples heated at 50 °C were lower than those at 28 °C in all species (Table 3.2, 3.3). It was possible that heating induced conformational changes of protein around oil droplet, resulting in a disruption of protein and lipid interactions. Changes of protein conformation during heating at 50 °C were confirmed by the results of surface hydrophobicity (Table 3.1). Li-Chan et al. (1985) also reported that emulsifying

stability index (ESI) of rockfish protein decreased when heating from 35 to 85 °C. The lower ES at 50 °C observed in this study was in agreement with the changes of oil droplet diameter (Figure 3.4). Diameter of oil droplet stabilized by either myofibrillar or mince proteins increased dramatically at 50 °C (Figure 3.2), indicating that high temperature activated flocculation/coalescence of oil droplet, resulting in a decrease of ES. Likewise, Petursson et al. (2004) reported that emulsion stabilized by acid extracted protein from cod underwent flocculation at > 40 °C, resulting in an

Table 3.3 Effect of incubation at 50 °C on ES of muscle proteins from various freshwater fish species

Fish	ES (%)	
	Mince protein	Myofibrillar protein
NT	39.58[c]	41.18[c]
RH	45.87[a]	50.47[a]
SM	40.37[c]	41.93[c]
HC	42.23[b]	43.36[b]
AF	37.73[d]	40.76[d]

**a,b,c,d, in the same column indicated statistical difference at $p<0.05$

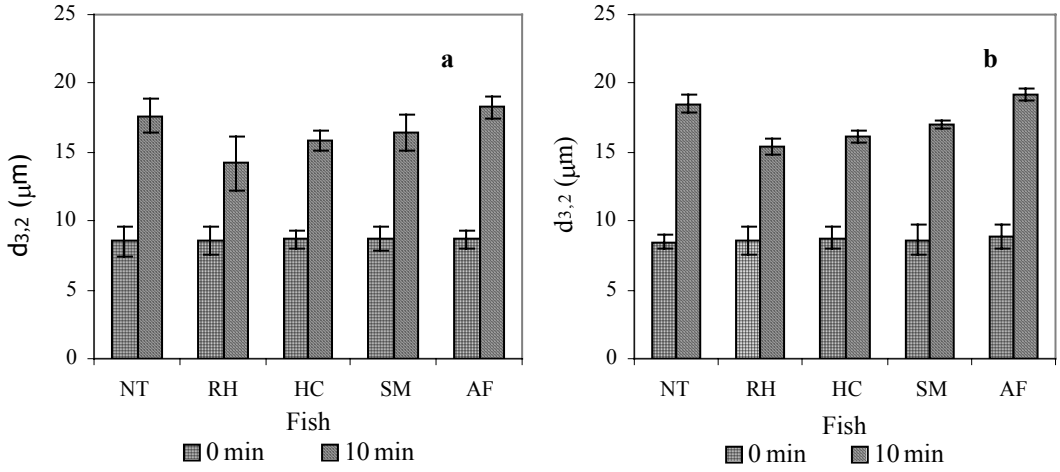

Figure 3.4 Effect of incubation at 50 °C on oil droplet diameter of emulsion stabilized by protein extracted from various freshwater fish species; a, myofibrillar protein; b, mince protein

extensive creaming of the emulsion. Furthermore, droplet flocculation of whey protein stabilized emulsion increased when temperature increased from 50 to 95 °C (Kulmyrzaev , Bryant, and McClements, 2000).

3.3.5 SDS-PAGE of myofibrillar and mince proteins

No severe degradation was observed in both myofibrillar and mince proteins of all species at 50 °C (Figure 3.5). These results indicated that higher surface hydrophobicity (Table 1) observed at 50 °C was not caused by proteolysis. In addition, a decrease of EAI (Figure 3.3) and ES at 50 °C (Table 3.3) was not due to proteolysis of muscle proteins. Incubating muscle proteins of these species at 50 °C for 1 h did not induce proteolysis of myosin heavy chain (MHC) and actin (Figure 3.5). Therefore, incubating at 50 °C during smoking process had no detrimental effect on intregrity of muscle protein of these species.

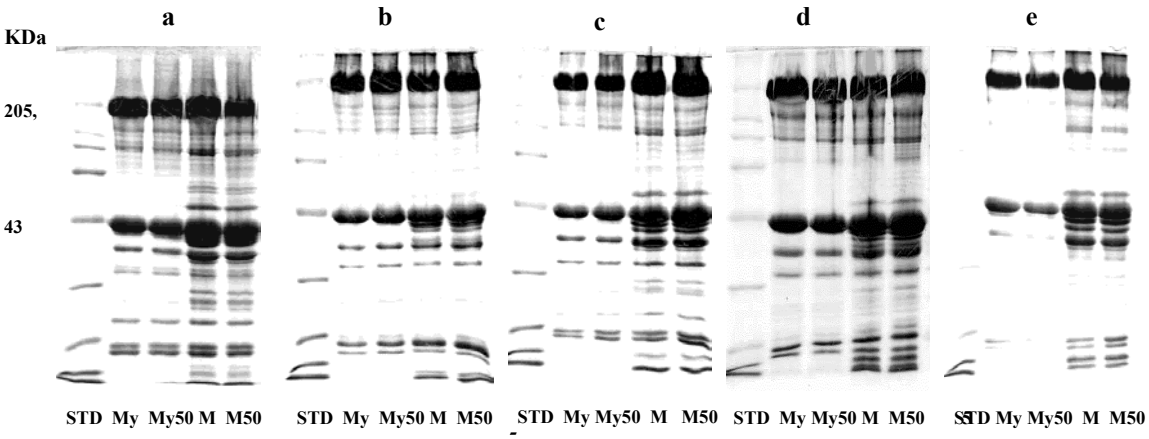

Figure 3.5 Changes in SDS-PAGE pattern of muscle proteins of various fish species incubated at 50 °C for 1 h; a, SM: b, NT: c, RH: d, HC: e, AF; My, myofibrillar protein incubated at 4 °C: My5, myofibrillar protein incubated at 50 °C: M, mince protein incubated at 4°C: M50, mince protein incubated 50 °C

3.4 Conclusion

The studied freshwater fish species had similar ability to stabilize O/W emulsion at 28 °C but only RH proteins exhibited the highest emulsion stability at 50 °C. EAI and ES decreased and oil droplet diameter increased when incubated at 50 °C. These phenomena were not caused by proteolysis as intensity of MHC was unchanged.

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CHAPTHER IV

EFFECT OF POTASSIUM IODIDE (KI) ON EMULSIFYING PROPERTY OF ROHU (*Labeo rohita*) MYOFIBRILLAR PROTEIN AND THE COMBINED EFFECT OF KI AND TUNA OIL ON LIPID OXIDATION OF OIL IN WATER (O/W) EMULSION

Abstract

The objectives of the this study were to investigate the effect of KI concentration (0 -100 mM) on emulsifying activity index (EAI), emulsion stability (ES), and oil droplet diameter ($d_{3,2}$) of oil in water (O/W) emulsion. In addition, to elucidate the effect of KI (50 and 100 mM) combined with tuna oil (5 and 10%) on lipid oxidation of emulsion stabilized by rohu *(Labeo rohita*) myofibrillar protein at 1 mg/ml. EAI and S_0 of myofibrillar protein were relatively constant at 0 –50 mM KI and increased at > 50 mM KI. ES and $d_{3,2}$ remained constant at all KI concentrations studied. Oxidative substances monitored by lipid hydroperoxide (HPV) and thiobarbituric reactive substances (TBARS) increased when emulsions were prepared by 5 and 10% tuna oil ($p<0.05$). Fortification of 100 mM KI to tuna oil emulsion increased HPV and TBARS values ($p<0.05$).

Key words: emulsification activity index (EAI), emulsion stability (ES), oil droplet diameter ($d_{3,2}$), lipid oxidation, tuna oil

4.1 Introduction

Iodine is an essential trace element in human nutrition. The element is an essential part of the thyroid hormones triiodothyronine (T3) and thyroxine (T4), which are necessary for human growth and development. Adolescents and adults need iodine approximately 150 μg per day (National Academy Sciences, 1980). The best known effect of iodine deficiency is endemic goiter. Iodine Deficiency Disorder continues to affect a large number of world population. WHO estimated that 38% and 13% of the world populations live in the area at risk of iodine deficiency and are affected by goiter, respectively (WHO, 2000). Iodine – deficient diet causes a wide spectrum of illnesses. To ensure sufficient iodine uptake, food products can be enriched with iodine.

Iodide is one of the destabilized salts in Hofmeister series, thus could affect protein solubility. Solubility of proteins directly governs other functional properties, particularly emulsifying property. Thus, the effect of iodine fortification on emulsifying property of freshwater fish proteins should be investigated. In addition, some studies indicated that an increase of iodine content resulted in an increased lipid oxidation in animal (Verditti, Deleo, and Dimeo , 1997; Allen, 1996). However, there is little knowledge about the effect of iodine on lipid oxidation in the food system, especially food products containing large amount of unsaturated fatty acids. Therefore, the effect of iodine fortification on lipid oxidation in food model emulsion should be studied.

Tuna oil is a rich source of unsaturated fatty acids, especially omega 3 fatty acids. Research over the past decade revealed the health benefits of omega 3 fatty acids. Omega 3 fatty acids, particularly eicosapentaenoic acid (EPA) and

docosahexaenoic acid (DHA), play a role in the reduction of thombotic tendencies and hypertriacylglycerolemia (Harris, Rothrock, Fanning, 1990; Durrington, et al., 2001). EPA is believed to protect cardiovascular disease, immune disorder, inflammation, allergies, and diabetes (Sardesai, 1992; Terano, Salmon, Higgs, and Moncada, 1986). DHA is essential for proper nervous system and vision development (Salem and Pawlosky, 1992; Jorgensen, Hernell, Hughes, and Michaelsen, 2001). However, the application of oil rich in omega 3 fatty acids to food system was limited because fish oil is susceptible to lipid oxidation. Thus, the effect of tuna oil concentrations on lipid oxidation in O/W emulsion should be investigated.

The objectives of this study were to investigate the effect of potassium iodide (KI) on protein solubility and surface hydrophobicity of myofibrillar protein from rohu. In addition, the effect of KI concentration on lipid flocculation and emulsifying property of rohu myofibrillar protein stabilized emulsion was investigated. The effect of KI fortification on lipid oxidation of O/W emulsion containing tuna oil was also evaluated.

4.2 Materials and methods

4.2.1 Myofibrillar protein preparation

Rohu (*Labeo rohita*) myofibrillar protein was used as an emulsifier. Rohu was obtained from a fish farm of Nakhon Ratchasima. Live fish were transported to the Suranaree University of Technology laboratory and immediately killed upon arrival. The average fish weight was 170.16 ± 2.5 g with the length of 24.5 ± 1.60 cm. Fish was headed, degutted and washed in cold water. Skin and bones were manually removed. Fish fillets were comminuted using a meat grinder (Biro 8-22, Biro

Manufacturing, Marblehead, Ohio, USA). Mince was suspended in 10 volumes of 0.1 M NaCl in 10 mM phosphate buffer (pH 7) at 4 °C and the homogenate was centrifuged at 11,000×g for 30 min. The supernatant was discarded and the precipitates were washed with the same buffer one more time. The precipitates were resuspended in 6 volumes of 0.6 M NaCl in 10 mM phosphate buffer (pH 7) at 4 °C and homogenized using a high-speed blender (Nissei AM-8, NSS Nihonseshi Kaisha Co., Ltd., Kyoto, Japan) for about 30 s. Connective tissue and other non-solubilized proteins were removed by centrifugation at 11,000×g for 30 min. The supernatant was used as myofibrillar protein.

4.2.2. Protein solubility

Protein solubility was determined according to Lin and Park (1989) with slight modifications. KI was added at 0 -100 mM into rohu myofibrillar protein suspended in 0.6 M NaCl in 10 mM phosphate buffer, pH 7 (2 mg/ml). The samples were shaken at 4 °C for 8 h, subsequently were centrifuged at 14,000×g for 30 min. The control was prepared without KI addition. Solubility was expressed as percentage of protein concentration in the supernatant with respect to the original protein content of control after centrifugation. Protein content was determined using Lowry method (1951).

4.2.3. Surface hydrophobicity

Surface hydrophobicity (So) of rohu myofibrillar protein was measured using 8-anilino-1-naphthalene-sulfonic acid (ANS) as described by Hayakawa and Nakai (1985). Rohu myofibillar protein was dissolved in 0.6 M NaCl in 10 mM phosphate

buffer (pH 7) and KI was added at various concentrations ranging from 0 to 100 mM. Subsequently, the samples were incubated at 4 °C and shaken for 8 h. The protein-ANS complex was measured at excitation and emission wavelengths at 375 and 485 nm, respectively. The fluorescence emission intensity of the protein-ANS complex was plotted against protein concentration (0 – 0.1%). So of each sample was calculated from the slope of the fluorescence intensity vs percentage of protein concentration (w/v).

4.2.4 Emulsifying properties

Oil in water (O/W) emulsion was prepared by a slight modification of Fomuso, Corredig, and Akoh (2002). Corn oil (10%, v/v) and rohu myofibrillar protein (1mg/ml dissolved in 0.6 M NaCl in 10 mM phosphate buffer, pH 7) were mixed using a homogenizer (Ystral Scientific, T25, Eastleigh, Hampshire, England) at 60,000 rpm for 15 s. Potassium iodide (KI) was added to emulsion at concentration ranging from 0 to 100 mM. Emulsion activity index (EAI) and emulsion stability (ES) were evaluated by the method of Pearce and Kinsella (1978). Emulsions were diluted in 41 volumes of 0.1% SDS and stirred for 20 s using a vortex mixer before taking absorbance measurement at 500 nm. EAI of myofibrillar and mince proteins was calculated from:

$$\text{EAI } (m^2/\text{g protein}) = \frac{2 \times 2.303 \times A}{L \times C \times \Phi} \times (k)$$

When A = Absorbance at 500 nm L= path length of the cuvette

C = protein concentration in aqueous phase (mg/ml) Φ = oil ratio

K = dilution factor of protein in SDS

Emulsion stability was calculated from:

$$ES\ (\%) = \frac{\text{Absorbance at 1 h} \times 100}{\text{Absorbance at initial time}}$$

4.2.5 Particle size measurements

Emulsions were diluted about 500 folds using 0.6 M NaCl in 10 mM phosphate buffer solution (pH 7) to avoid multiple scattering effect prior to analysis. The particle size of the emulsion was measured using a laser light scattering instrument (Mastersizer S Malvern Instruments, Malvern, Worcestershire, UK). The instrument measures the angular dependence of the intensity of laser light (λ=632.8 nm) scatted by a diluted emulsion. Particle size measurements were reported as the volume average mean diameter ($d_{3,2}$).

4.2.6 Effect of fortified KI and tuna oil on lipid oxidation

Lipid oxidation of emulsion was monitored by measuring lipid hydroperoxide (HPV) and thiobarbituric acid reactive substances (TBARS) values. KI was added to emulsion to contain 50 and 100 mM. Emulsion was prepared by substituting 50 and 100% of soybean oil with the commercial tuna oil, which was equivalent to 5 and 10% of total volume, respectively. The commercial tuna oil contained 31.1 g omega 3 fatty acids/ 100g oil. The control was prepared using 10% corn oil and without KI addition. Emulsions were prepared as described above. All samples were incubated at 40 °C for 100 h. The samples were taken for measurement at 0, 20, 40, 60, 80, and 100 h.

4.2.6.1 HPV measurement

HPV was measured according to the method of Shantha and Decker (1994). Emulsion (0.3 ml) was mixed with 1.5 ml chloroform/methanol (2/1, v/v). After centrifugation (2000×g for 5 min), an aliquot (200 μl) of the lower chloroform layer was mixed with an additional 2.8 ml of chloroform/methanol (2/1, v/v) and then reacted with 50 μl of 3.94 M ammonium thiocyanate and 0.144 M ferrous iron solution. Absorbance was measured at 500 nm after 5 min incubation at room temperature (28°C), and hydroperoxide was quantified using the standard curve prepared by ferrous iron (III) chloride. Hydroperoxides oxidize Fe^{2+} to Fe^{3+} which subsequently forms complex with ammonium thiocyanate, resulting in pink color.

4.2.6.2 TBARS measurement

TBARS was determined using a modified method of Buege and Aust (1978). Emulsion (2 ml) was reacted with 2 ml of 0.02 M 2-thiobarbituric acids and incubated for 30 min in a boiling water bath. Sample was rapidly cool and centrifuged at 2000×g for 5 min. Absorbance of the supernatant was measured at 532 nm. 1,1,3,3-Tetraethyloxypropane (TEP) was used as a standard. TBARS were expressed in μmol of malonaldehyde per ml of emulsion.

4.2.7 Statistical analysis

Experiments were carried out in two replications. Mean values ± standard deviations were presented. The effects of KI concentration on solubility, surface hydrophobicity of rohu myofibrillar protein and emulsifying properties, and changes of oil droplet diameter were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 1993; SAS Institute Inc., Carry, NC) as randomized

complete block design (RCBD). The effect of KI and tuna oil on lipid oxidation was analyzed as factorial design in RCBD. Significant differences of means were determined using the Duncan multiple rang test (DMRT). Level of significance was set at $p \leq 0.05$.

4.3 Results and discussion

4.3.1 Effect of KI concentration on solubility and surface hydrophobicity of rohu myofibrillar protein

Solubility of rohu myofibrillar protein remained constant up to 100 mM KI (Figure 4.1). These indicated that iodide ion (10 - 100 mM) had no effect on protein-water interaction. Addition of KI to myofibrillar protein solution dissolved in 0.6 M NaCl did not affect its protein solubility because high solubility of myofibrillar protein already attained at high ionic strength buffer. Zayas (1997) reported that myosin, the major myofibrillar protein, was soluble in salt solution at concentration

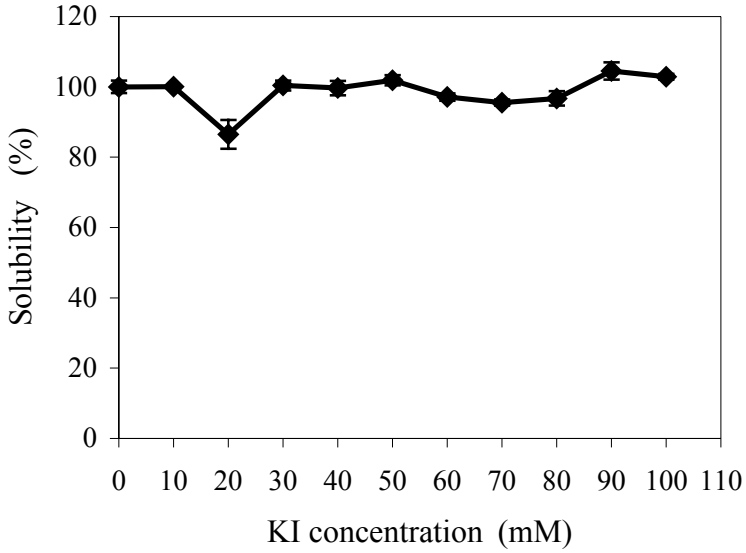

Figure 4.1 Solubility of rohu myofibrillar protein at various KI concentrations

greater than 0.3 M. Lin and Park (1998) found that the solubility of salmon myosin was dramatically increased when KCl concentration increased from 0 to 0.5 M and became less soluble at higher concentration. These were caused by electrostatic repulsion after chloride ion binding to the positive charged protein groups occurring in low KCl concentration (0 –0.5M). At higher salt concentration, chloride ion and protein competed to bind with water, resulting in salting out of protein. Addition of KI up to 100 mM to RH myofibrillar protein in 0.6 M NaCl did not promote the salting out effect.

Surface hydrophobicity was relatively constant at 0 – 50 mM KI and slightly increased at 60 - 100 mM KI (Figure 4.2). Iodide ion could bind to positive charged amino acids (lysine, arginine, histidine) in protein molecule through ionic interactions, resulting in changes of protein conformation accompanied by an increased exposure of hydrophobic amino acids. These results indicated that RH

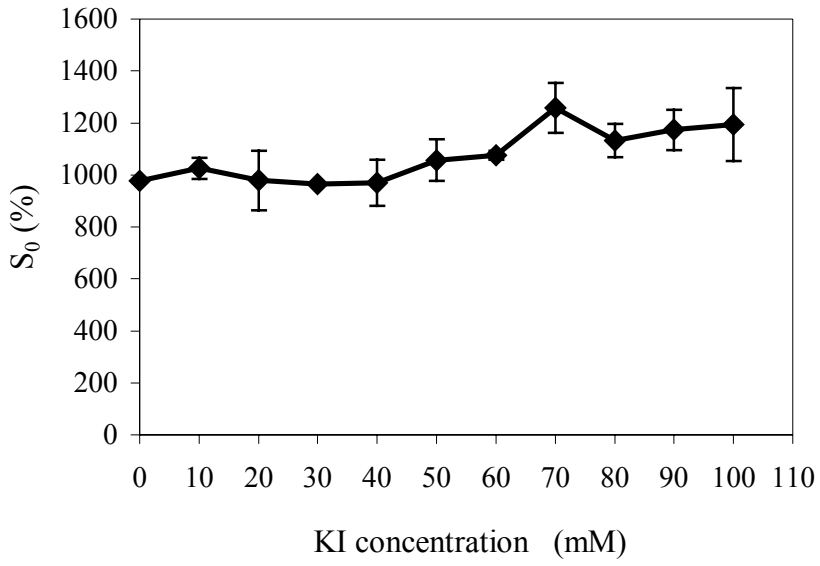

Figure 4.2 Effect of KI concentration on surface hydrophobicity (S_0) of rohu myofibrillar protein

myofibrillar protein unfolded to the greater extent at higher KI concentration (60 – 100 mM). It should be noted that an increased S_0 – ANS did not result in an increased solubility.

4.3.2 Effect of KI concentration on emulsifying properties of O/W emulsion

EAI was constant as KI concentration increased up to 50 mM and slightly increased from 60 to 100 mM (Figure 4.3). Changes of EAI showed similar pattern to those of S_0ANS. Myofibrillar protein exposed more hydrophobic groups > 60 mM KI. The exposure of hydrophobic amino acids enhanced a rapid movement and adsorption of myofibrillar protein at oil-water interface. As a result, EAI of RH myofibrillar protein increased at higher KI concentration. Li-Chan, Nakai, and Wood (1984)

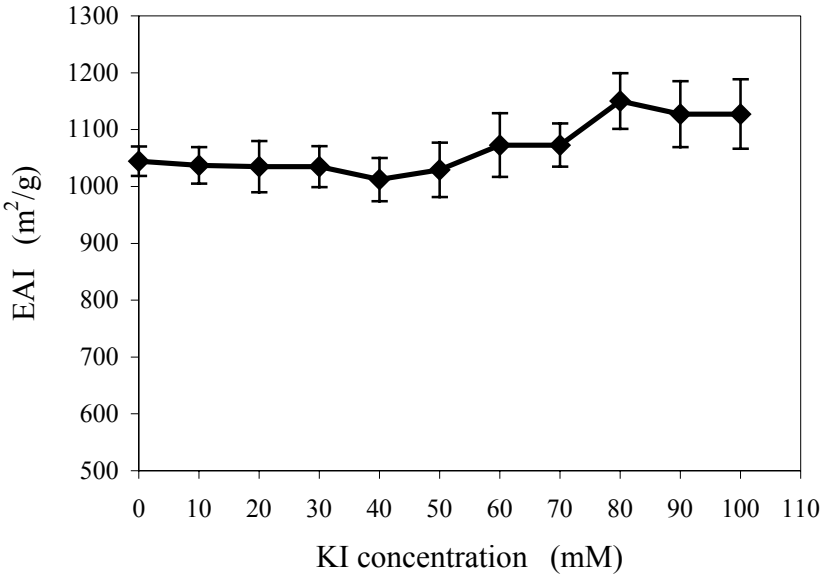

Figure 4.3 Effect of KI concentration on EAI of rohu myofibrillar protein stabilized emulsion

demonstrated that emulsifying ability of salt soluble muscle proteins positively correlated with their surface hydrophobicity. Kristinsson and Hultin (2003) also

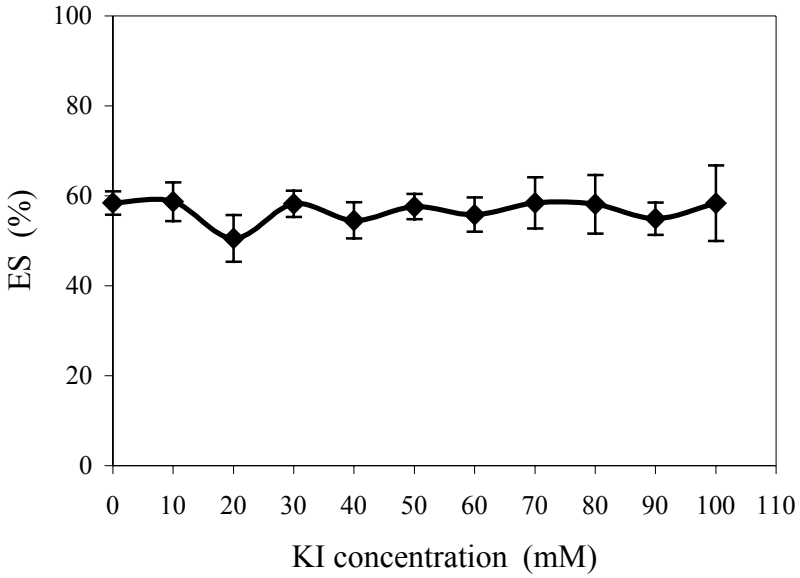

Figure 4.4 Emulsion stability (ES) of rohu myofibrillar protein stabilized emulsion at various KI concentrations

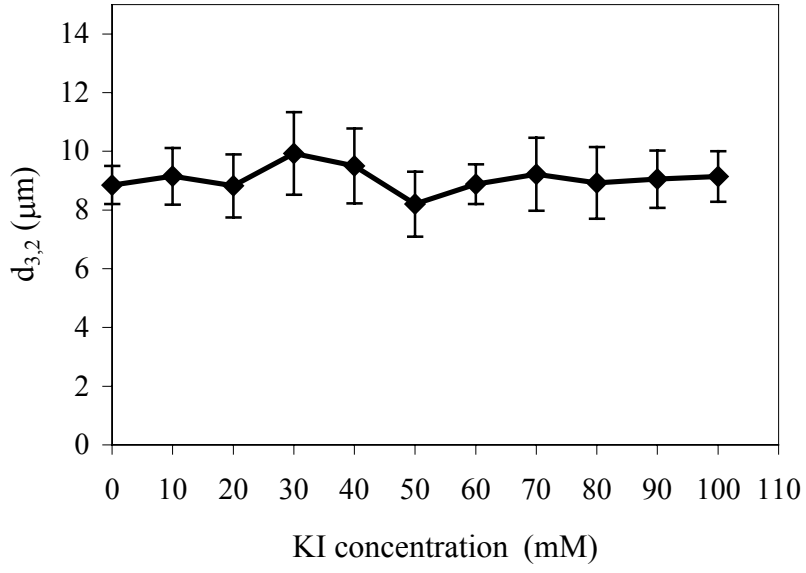

Figure 4.5 Changes of oil droplet diameter of emulsion stabilized by rohu myofibrillar protein at various KI concentrations

suggested that EAI of cod myosin increased with surface hydrophobicity. The influence of KI concentration on ES and oil droplet diameter of emulsion stabilized by 1 mg/ml rohu myofibrillar protein were presented in Figure 4.4 and 4.5,

respectively. KI concentrations (10 to 100 mM) did not affect ES and oil droplet diameter (Figures 4.4, 4.5). These indicated that KI concentration between 10 to 100 mM had no effect on changes of protein conformation around oil droplet. Salts have been reported to have no effect on ES. Demetriades, Coupland, and McClements (1997) suggested that NaCl at concentration of 0 to 100 mM had no effect on particle size diameter of emulsion stabilized by whey protein. In addition, creaming stability of emulsion stabilized by muscle cod protein were not affected by 0 - 100 mM NaCl (Petusson, Decker, and McClements, 2004).

4.3.3 Effect of KI concentration on lipid oxidation of O/W emulsion containing tuna oil

HPV and TBARS values were dramatically increased with incubation time at 40 °C in all samples. HPV and TBARS values also increased with an increase of tuna oil addition ($p<0.05$) (Figures 4.6, 4.7). This was because of an increased polyunsaturated fatty acids in emulsion. Frankel, Gracia, Meyer, and German, (2002) reported that emulsion prepared with fish and algae oil increased lipid oxidation in O/W emulsion. They also found that bulk oils were much more stable to lipid oxidation than the corresponding O/W emulsion. This was due to greater surface area of oil in the emulsion system. Thus, lipid oxidation of O/W emulsion was a concern, particularly emulsion containing high amounts of omega 3 fatty acids.

HPV and TBARS values of sample added 50 mM KI were comparable with those of control (no added KI) ($p>0.05$), and lower than those added 100 mM KI ($p<0.05$). Addition of KI at high concentration appeared to accelerate lipid oxidation. Catalytic effect of iodide on lipid oxidation was unclear. Hatefi and Hanstein (1970)

reported that chaotropic agents (SCN^-, guanidine- HCl, ClO_4^-, I^-, Br^-, NO_3^-, urea) could destabilize the native structure of submitrocondrial particles and microsomes and render these systems susceptible to lipid oxidation by molecular oxygen. They

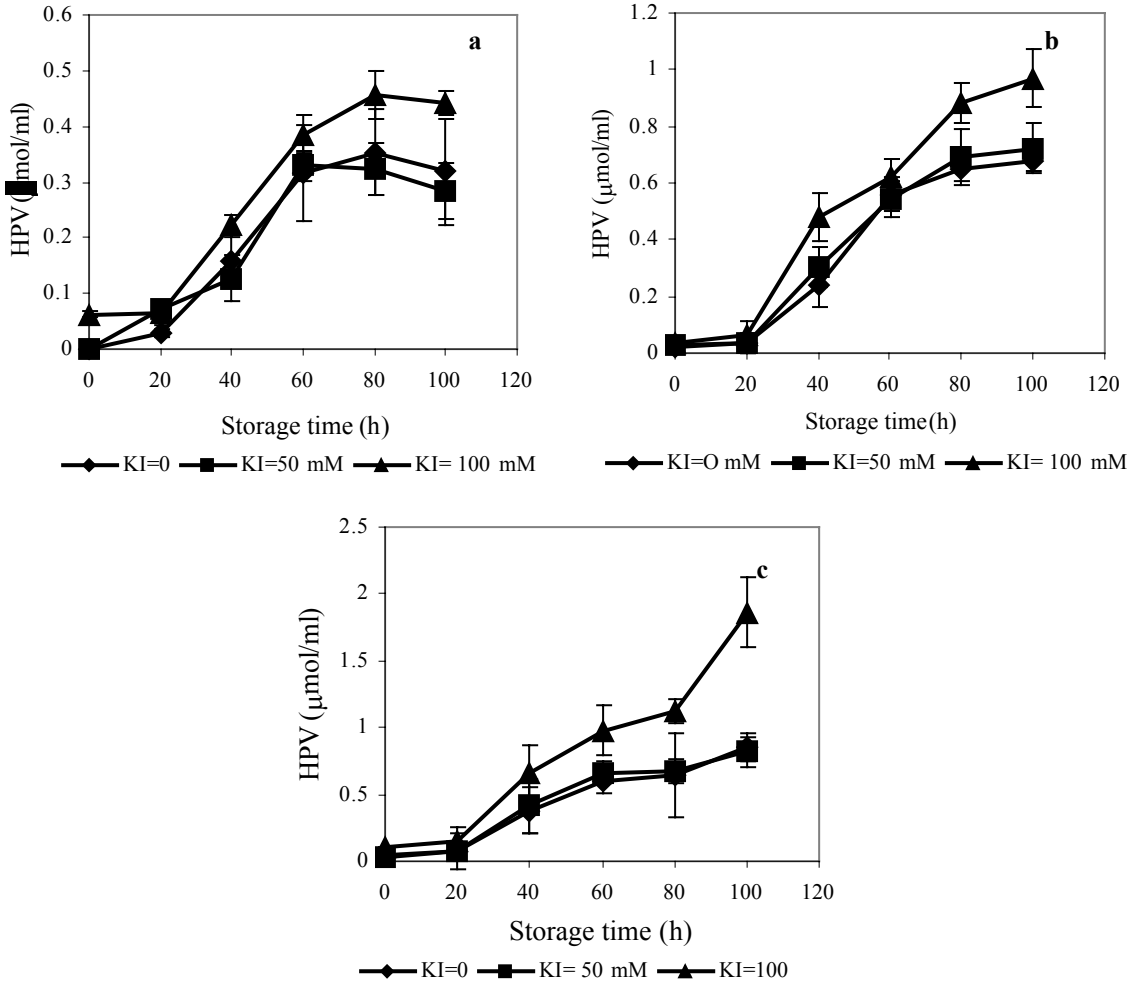

Figure 4.6 Effect of KI concentration on HPV formation in emulsions containing 0% (a), 5% (b), and 10% (c) tuna oil incubated at 40 °C

found that chaotropic agents induced a rapid initial rate of autooxidation. Our results suggested that lipid oxidation catalyzed by KI was concentration - dependent. The oxidative process of iodide might contribute to formation of free radicals. Typically, iodide salt is added to foods at 0.01% (6×10^{-7} mM), thus acceleration of lipid oxidation by iodide rarely occurred in food system.

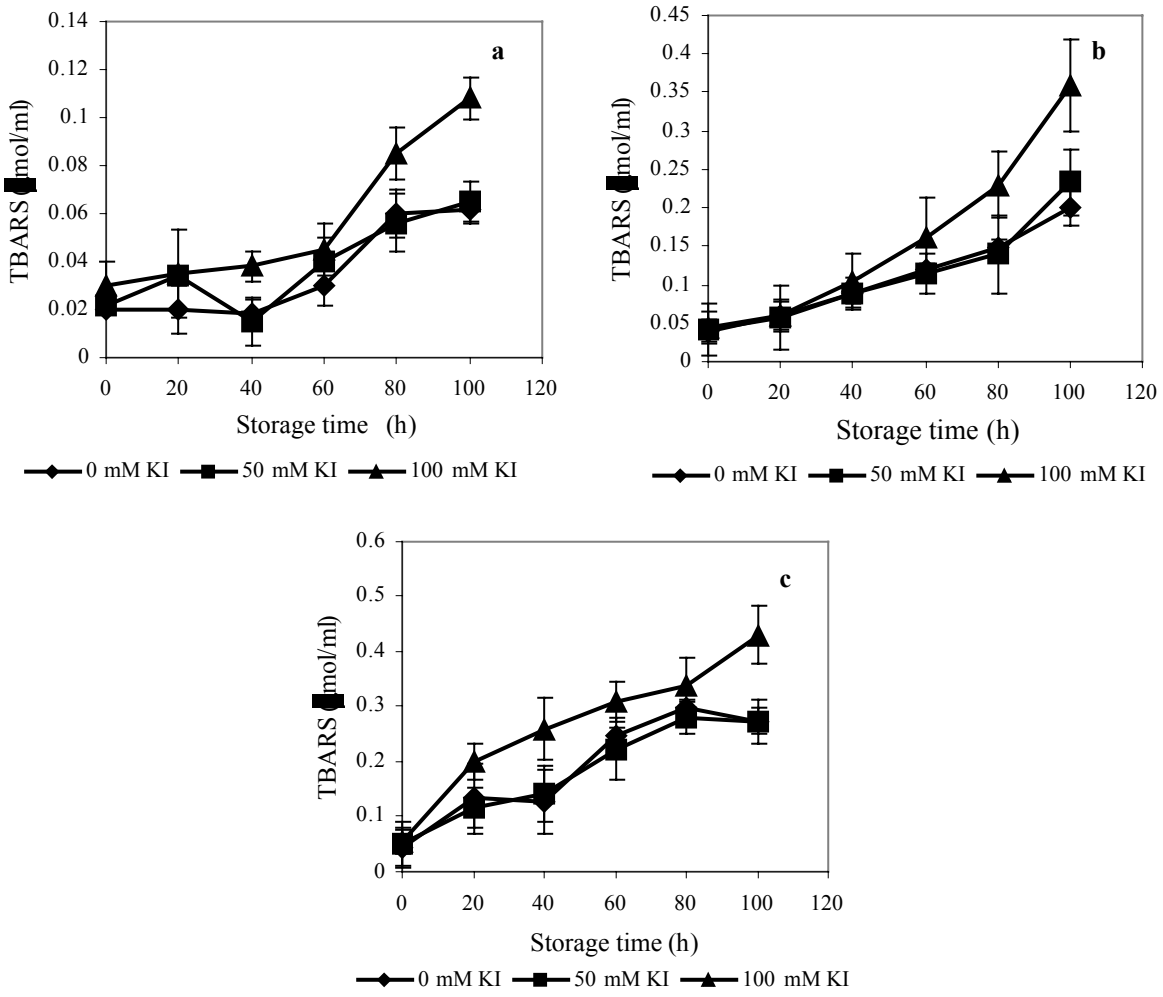

Figure 4.7 Effect of KI concentration on TBARS of emulsions containing 0% (a), 5% (b), and 10% (c) tuna oil incubated at 40 °C

4.4 Conclusions

Addition of KI up to 100 mM had no effect on solubility of rohu myofibrillar protein. ES and oil droplet diameter of emulsion were also not affected by KI. Surface hydrophobicity of myofibrillar protein and EAI of emulsion remained constant up to 50 mM and increased at higher KI concentration (>50 mM). Addition of >50 mM KI promoted the exposure of hydrophobic region and then increased EAI of emulsion

stabilized by RH myofibrillar protein. High KI concentration (100 mM) catalyzed lipid oxidation of O/W stabilized by rohu myofibrillar protein. Therefore, KI addition to O/W emulsion containing 5-10% tuna oil should not exceed 50 mM.

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CHAPTER V

EFFECTS OF POTASSIUM IODIDE AND TUNA OIL FORTIFICATION ON PHYSICAL AND CHEMICAL PROPERTIES OF FRESHWATER FISH SAUSAGES

Abstract

Emulsion sausages from African walking catfish (AF: *Clarias gariepinus)* and rohu *(*RH: *Labeo rohita*) fortified with three levels of tuna oil (2, 6, and 10%) and 150 μg KI/ 100g sample were prepared. The controls were prepared using 20% soybean oil without KI. Samples were vacuum-packed and stored at 4 °C. AF sausages showed higher gel strength and lower lightness than RH sausages ($p<0.05$). Addition of tuna oil and KI concentration had no effect on gel strength and lightness ($p<0.05$). Iodine content decreased approximately 14% after cooking and remained constant throughout storage. Sausages fortified with tuna oil had higher level of omega 3 fatty acids (eicosapentaenoic acid; EPA and docosahexaenoic acid; DHA), but lower levels of omega 6 fatty acids ($p<0.05$) than the control (20% soybean oil without KI). The ratio of omega 6 to omega 3 fatty acids (n6/n3 ratio) of both sausages decreased with an increase of tuna oil addition and was stable throughout storage period. Linoleic acid (LA), linolenic acid (LNA), EPA, and DHA content of products decreased after 3-4 weeks of storage. Hydroperoxide (HPV) and thiobarbituric reactive substances (TBARS) values were increased as addition level of tuna oil increased. Addition of

150 μg KI/ 100 g had no effect on lipid oxidation of fish sausages ($p<0.05$). HPV and TBARS values of AF sausages were lower than those of RH sausages.

Key words: African walking catfish, rohu, iodine stability, lipid oxidation

5.1 Introduction

Production of world freshwater fish aquaculture has increased rapidly in recent years. Freshwater fish account for about 60% of the total fishery production world wide, and have reached more than 20 million tones in 2002 (FAO, 2005). Freshwater fish is a good source of high quality protein, particularly of essential amino acids like lysine and methionine (Sathivel, Bechtel, Babbitt, Prinyawiwatkul, Negulescu, and Reppond, 2004). However, some species of freshwater fish contain tiny bones in the flesh, which hampers the fillet consumption. In order to increase utilization of freshwater fish, their functionalities, namely gelation and emulsifying activity, need to be explored.

The gelling property of fish protein is one of the most important functional properties contributing to textural properties. Gel are formed when partially unfolded proteins interact at specific points to form a three dimensional cross-linked network (Zayas, 1997). Partial unfolded protein with changes in secondary structure is required for gelation. Interactions between protein-protein, protein-water, and protein-lipid are responsible for the formation of a stable gel matrix and structure of processed meat products. Myofibrillar proteins, particularly myosin and actin, are considered as the most important component in binding meat structure (Zayas, 1997).

Freshwater fish contain less iodine and omega 3 fatty acids than marine fish. Iodine concentration of freshwater fish fillet was 5 to 10 times lower than that of marine fish (Eckhoff and Maage, 1997). Haldimann, Alt, Blanc, and Blondeav (2005) reported that iodine content in freshwater fish fillet was about 37.5 μg/100g meat while that of marine fish was about 211.2 μg/100g meat. Iodine is an essential trace element required for synthesis of thyroid hormone, triodothyronine (T3) and

thyroxine (T4). Thyroid hormones regulate a variety of physiological processes, including growth, metabolism, and reproductive function (Pethes, 1980). Adolescents and adults need iodine in the amount of 150 μg per day (National Academy of Sciences, 1980). Marine fish are also a rich source of omega 3 fatty acids. Marine fish contained omega 3 fatty acids of 14 – 30% of total fatty acids whereas only 1 – 11% of total fatty acids were found in freshwater fish (Steffens, 1997; Rahman, Huah, Hassan, and Daud, 1995). Research works over the past decade have revealed the biological importance of omega 3 fatty acids, especially eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The omega 3 fatty acids are important to brain and retina (Simpoulos, 1997). The nutritionists believe that the ratio of omega-6 to omega-3 fatty acids (n6/n3) should be 5 (Moreira, Visentainer, Nilson, Souza, and Matsushita, 2001). Higher values of n6/n3 ratio (>5) are harmful to health and promote cardiovascular diseases. Nutritional values of processed products from freshwater fish could be improved with iodine and tuna oil fortification

One of the main problems facing the application of marine fish oil to food is their high susceptibility to oxidation. The oxidative deterioration involves the formation of hydroperoxides from polyunsaturated fatty acids. The progress of autooxidation gives rise to a complex mixture of secondary oxidation products. Although, lipid hydroperoxide are tasteless and odorless, the secondary oxidation products are responsible for the changes in aroma and flavor (Frankel, 1991). This oxidative deterioration is also related to the adverse changes in texture, appearance, and nutritional value, (Min and Boff, 2002). In addition, some research works indicated the effect of iodine on lipid oxidation in biological systems. Verditti, Deleo, and Dimeo (1997) indicated that lipid peroxidation could be generated in patient with

hyperthyroidism (excess iodine for body need). Similarly, iodine increased lipid peroxidation in goiter rodent (Allen, 1996). Thus, addition of iodine and tuna oil to freshwater fish sausage could have an effect on lipid oxidation of the product.

The objective of this study was to investigate the effect of KI and tuna oil fortification on physical properties and oxidative stability of emulsion sausages prepared from African walking catfish (AF) and rohu (RH).

5.2 Materials and methods

5.2.1 Fish mince preparation

African walking catfish (AF: *Clarias gariepinus*) and rohu *(*RH: *Labeo rohita*) were obtained from the fish farm at Nakhon Ratchasima. Live fish were transported to the Suranaree University of Technology laboratory and immediately killed upon arrival. The average AF and RH weight were 1,650 ± 35.40 g and 170.16 ± 2.5 g with the length of 52.24 ± 6.12 and 24.5 ± 1.60 cm, respectively. Fish were headed, degutted and washed with cold water. Skin and bones were manually removed. Fish fillets were ground using a mincer (Biro 8-22, Biro Manufacturing, Marblehead, Ohio, USA) with a screen size of 1.5 mm perforation.

5.2.2 Proximate composition, iodine content and fatty acids of fish tissues

Fresh samples were analyzed in duplicate for proximate composition. Moisture, crude protein, and ash content were determined according to AOAC (1990). Total fat content was determined as described by Folch, Less, and Stanley (1957). Fifteen grams of mince were homogenized with 90 ml of chloroform – methanol (2:1 v/v) solution for 2 min. After mixing, 30 ml of chloroform and deionized water (DI)

was added, and the mixture was homogenized. Five milliliters of 0.58% NaCl solution were added to the homogenate to separate chloroform phase (containing lipid) from the methanol/water phase. Chloroform fraction was collected and evaporated using a rotary evaporator at 40 °C. The lipid content was determined gravimetrically.

Iodine content was measured by spectrophotometric method according to Moxon and Dixon (1980). Fish mince was charred with 30% potassium carbonate solution at 550 °C for 3 h to eliminate all organic matters. Subsequently, the cooled ash was transferred to centrifuge tube with 50 ml of DI water. The samples were mixed with DI water, 0.023% potassium thiocyanate solution, and 0.077% ammonium iron (III) sulfate. At exactly 90 s intervals, 2.07% sodium nitrite solution was added. The absorbance of mixture was measured at 450 nm after 20 min incubation. Iodine content was calculated using KI as a standard

Fatty acid composition of fish tissue was determined using gas chromatography (GC) according to AOAC method (1997). Total lipids were extracted according to the modified Folch's method as previously described (Folch et al., 1957). The fatty acid methyl esters (FAME) were prepared by the addition of 1.5 ml of 0.5 M sodium hydroxide in methanol to 25 mg of lipid. The mixture was heated in boiling water bath (100 °C) for 2 min. After cooling, 2 ml of 14% boron trifluoride in methanol was added in the mixture and reheated in a boiling water bath for 30 min. Subsequently, 1 ml of isooctane was added. Five milliliters of saturated NaCl was added to the mixture to separate isooctane phase (containing FAME) from methanol/water phase. Isooctane fraction was collected and 1 ml of isooctane was added in the mixture for re-extraction. Two milliliters of isooctane (containing

FAME) was dried under nitrogen gas at room temperature. Subsequently, 1 ml of isooctane was added to solubilized the dried FAME. The FAME was analyzed using gas chromatography (6890 GC, Agilent Technology Co, Ltd., Palo Alto, CA, USA), equipped with a flame ionization detector. Fused silica capillary column (Supelcowax 10 column, SUPELCO Co., Ltd., Bellefonete, PA, USA) was used. Temperature was programmed from 140 °C to 240 °C at a rate of 4.0 °C /min. Temperature of both injector and detector was set at 260 °C. Carrier gas was helium and a flow rate of 0.9 ml/min was maintained constant throughout the experiment. Identification of FAME was based on comparison of retention time of tested samples to methyl ester standards from Sigma (St. Louise, MO, USA). Absolute response factors were calculated for each identified fatty acid peak. Fatty acid composition was expressed as μg/100 g sample and was determined in duplicate.

5.2.3 Formulation and preparation of freshwater fish sausage

Emulsion sausages were prepared to contain 60% fish mince and 20 % soybean oil. Commercial tuna oil was added at 10, 30, and 50% of added soybean oil which was equivalent to 2, 6 and 10% of the total weight, respectively. Potassium iodide (KI) was added at 0 and 150 μg/ 100 g sample. The controls were prepared using 20% soybean oil without KI. Mince was chopped using a Stephan vacuum cutter (UM5, Stephan Machinery Co., Columbus, Ohio, USA). Sodium chloride was added at 2% of total weight, subsequently oil was added. Moisture content of all samples was adjusted to 86%. Other ingredients (0.2% tripolyphosphate, 1.6% spice, 2.4% starch, 1% soy protein isolate, and 1% sugar) were added. The raw paste was stuffed into a 28 mm-diameter cellophane casing and pre-incubated at 55 °C for 40

min prior to boiling at 80 °C for 15 min. Subsequently, samples were cooled for about 30 min in ice water, vacuum packaged and stored at 4°C. Two replications were performed on separated days. Samples were randomly selected at 0, 1, 2, 3, and 4 weeks of storage for chemical and physical analyzes.

5.2.4 Chemical analyses

Changes of iodine, unsaturated fatty acids content, ratio of n6 to n3, hydroperoxide (HPV), and thiobarbituric reactive substances (TBARS) values during storage were monitored.

5.2.4.1 Lipid oxidation

Changes of linoleic acid (LA), linolenic acid (LNA), EPA, and DHA were measured using gas chromatography (GC) according to the method of AOAC (1997) as described above. The content of all fatty acids was expressed as μg/g sample.

HPV was monitored using spectrophotometic method as described by Shantha and Decker (1994). One gram of sample was homogenized with 10 ml of chloroform/methanol (2/1, v/v). After centrifugation (2000g for 5 min), an aliquot (2 ml) of the lower chloroform layer was mixed with an additional 1.3 ml of chloroform/methanol (2/1, v/v) and then reacted with 50 μl of 3.94 M ammonium thiocyanate and 0.144 M ferrous iron (II) solution. The reaction mixture was incubated at ambient (28 °C) for 5 min and absorbance at 500 nm was measured. Hydroperoxide was quantified using a standard curve prepared by various concentrations of ferrous iron (III) chloride. Hydroperoxides oxidize Fe^{2+} to Fe^{3+}

which subsequently formed complex with ammonium thiocyanate, resulting in pink color.

TBARS measurement was performed as described by Ahn, Lutz, Cherian, Wolfe, and Sim (1995). Ten grams of sample were mixed with 50 ml of 3.86% perchloric acid, butylated hydroxyanisole (BHA) to contain 125 μg/mg fat using a homogenizer (Nissei AM-8, NSS Nihonseiki Kaisha co., Ltd., Kyoto, Japan) at high speed for 30 s. The homogenate was then filtered through a Whatman No. 1 filter paper, and a 2 ml aliquot of the filtrate was mixed with 2 ml 0.02 M thiobarbituric acid (TBA). Samples were heated in boiling water for 30 min. The absorbance was determined at 531 nm. DI water was used as a blank. 1,1,3,3-Tetraethyloxypropane (TEP) was used as a standard. TBARS was expressed as mg malonaldehyde per 100 g sample.

5.2.4.2 n6/ n3 ratio

The ratio of n6 to n3 was calculated. Omega 6 fatty acids used in the calculation were LA, eicosatrienoic, and archidonic acid, while LNA, EPA, and DHA were used for calculation of omega 3 fatty acids.

5.2.5 Texture analysis

Texture Analyzer (Stable Micro System, Surrey, England) was used to evaluate textural properties of sausages. Sausages were cut into pieces of 2 cm length. Breaking force (g) and deformation (mm) were determined using a 5 mm spherical plunger probe at a test speed of 1 mm/sec. The results were expressed as the average of ten samples.

5.2.6 Color measurement

Color measurement was conducted using CIE (1978) *L* a* b** color system. Minolta Chroma Meter CR-300 (Minolta Camera Co Ltd, Osaka, Japan) was used to register the *L** (lightness), *a** (redness), and *b** (yellowness) values. The results were expressed as the average of five samples.

5.2.8 Sodium Dodecyl Sulflate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used to monitor degradation of muscle proteins. All samples were dissolved in 10 volumes of hot 5% SDS and homogenized until complete solubilization. The solution was centrifuged at 13000×g for 30 min. The supernatants were collected and mixed with a treatment buffer (0.125 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol) and heated in boiling water for 5 min. Molecular weight standards ranging from 4500 to 200000 Da were used (Bio-Rad Laboratories, Hercules, CA). SDS-PAGE was performed on 4% stacking gel and 10% running gel (Laemmli, 1970).

5.2.9 Statistical analysis

Experiments were conducted in two replications. Mean values and standard deviations were presented. The effects of iodine and tuna oil fortification on gel strength, color, iodine stability and lipid oxidation were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 1993; SAS Institute Inc., Carry, NC) as a factorial design in randomized complete block design (RCBD).

Significant differences of means were determined using the Duncan multiple rang test (DMRT). Level of significance was set at $p \leq 0.05$.

5.3. Results and discussion

5.3.1 Proximate composition, iodine content and fatty acid composition of fish tissues

RH mince contained lower lipid content than AF mince ($p<0.05$, Table 5.1). Protein, ash, and iodine content of both fish were similar ($p>0.05$). Iodine content of AF and RH mince was higher than those of freshwater fish species previously reported. Eckhoff and Maage (1997) found that iodine content of catfish flesh in the East and West Greenland was 7.8 μg/100 g meat. Some variation in iodine content was influenced by age, diet, and habitat of fish. Hunn and Fromm (1966) demonstrated that fish could adsorb iodine from both water and diet. Hunt and Eales (1979) found that 84% of the plasma iodine in rainbow trout came from water, 16% from the diet, and less than 1% from thyroid hormone degradation. However, iodine concentration of freshwater fish fillet was lower than that of marine fish (Eckhoff and Maage, 1997). Hamre, Lie, Mage, and Julshamn (1995) reported that iodine content of marine fish from the Barents Ocean in Norway varied from 50 to 560 μg/100g meat.

Table 5.1 Proximate composition and iodine content of AF and RH muscle

Composition	AF	RH
Moisture content (%)	80.68 ± 0.31^{b}	84.76 ± 0.48^{a}
Protein content (%)	14.63 ± 0.32^{a}	13.31 ± 0.30^{a}
Lipid content (%)	9.10 ± 0.10^{a}	1.76 ± 0.02^{b}
Ash content (%)	1.12 ± 0.05^{a}	1.14 ± 0.10^{a}
Iodine content (μg/100 g)	12.67 ± 0.30^{a}	12.83 ± 0.10^{a}

** $^{a, b}$ in the same row indicated statistical difference at $p<0.05$

AF muscle contained higher saturated fatty acids (palmitic and stearic acid) and LA content than RH muscle ($p<0.05$, Table 5.2). Omega 3 fatty acids content of AF was lower than that of RH ($p<0.05$, Table 5.2). Omega 3 fatty acids content of AF and RH obtained in this study was lower than those of salmon (1000 – 1400 mg/100g) and trout (500 – 1600 mg/100 g) (Shihu, 2003). The n6/ n3 ratio of AF was higher than the recommended value (=5) whereas ratio of RH was in the recommendation range (Table 5.2). The n6/n3 ratio >5 are harmful to health and promote cardiovascular disease (Moreira et al., 2001). Moreira et al. (2001) found that n6/n3

Table 5.2 Fatty acid composition of extracted lipid from freshwater fish muscle and tuna oil

Fatty acid	Content (mg/100g sample)		
	AF	RH	Tuna oil
Myristic acid (C14:0)	12.92	22.31	2645.62
Pentadecanoic acid (C15:0)	3.13	7.59	863.79
Palmitic acid (C16:0)	652.18	148.02	14251.20
Palmitoleic acid (C16:1)	100.67	41.04	5045.58
Stearic acid (C18:0)	196.35	54.64	4255.87
Oleic acid (C18:1n9c)	980.07	200.20	8532.93
Linoleic acid (C18:2n6c)	821.41	60.20	1290.22
Arachidic acid (C20:0)	4.02	1.88	329.75
Eicosenoic acid (C20:1)	10.26	2.93	437.28
Octatrienoic acid (C18:3n6)	94.65	2.62	449.50
Linolenic acid (C18:3n3)	38.47	57.83	391.98
Eicosatrienonic acid(C20:3n6)	38.83	5.88	108.17
Arachidonic acid (C20:4n6)	59.77	86.35	82.46
Eicosapentaenoic acid (C20:5n3)	1.19	9.59	4023.14
Docosahexaenoic acid (C22:6n3)	99.07	256.22	26680.54
n-6 fatty acids	1014.66	155.05	1930.36
n-3 fatty acids	138.73	323.64	31095.65
n-6/n-3 ratio	7.31	0.48	0.06

ratio of wild freshwater fish was 1.14 – 1.79, which were higher than that of marine fish (0.05 - 0.08) (Andrade, Visentainer, Matsushita, and Souza, 1996). Commercial

tuna oil contained low amount of omega 6 fatty acids but high level of omega 3 fatty acids content (Table 5.2). The n6/n3 ratio of tuna oil was much lower than the recommended ratio. Thus, commercial tuna oil is a good source of omega 3 fatty acids and can be fortified into food products. Based on iodine and omega 3 fatty acids content, fortification of KI and tuna oil rich in omega 3 fatty acids into products made from these species would improve nutritional quality of the finished products.

5.3.2 Chemical properties of sausages

5.3.2.1 Iodine stability

Iodine content decreased about 14 to 16% in the finished product after cooking (week 0)(Table 5.3). Iodine content remained constant throughout the experiment ($p>0.05$, Table 5.3). It should be noted that iodine content of sausages also decreased about 15 – 20 % in samples without fortification (data not shown). The loss of iodine after sausage manufacturing process might be caused by breakdown of iodine by high temperature. Diosady, Alberti, and Mannar (2002) indicated that the critical problem of iodine stability is the potential for reducing or oxidizing the iodide to elemental iodine, I_2. Elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion at room temperature, or >40 °C. High stability of iodine up to 4 weeks was attributed from low temperature storage (4 °C). Chauhan, Bhatt, Bhatt, and Majeethia (1992) reported that iodine loss of 9-10% was found within the first month in common iodized salt.

Table 5.3 Iodine stability of AF and RH sausages fortified with 150 μg KI / 100 g

Fish	Tuna oil addition (%)	Iodine content (μg/100 g sample)				
		wk 0	wk 1	wk 2	wk 3	wk 4
AF	0	130.05 ± 0.19	130.56 ± 0.26	130.46 ± 0.18	130.25 ± 0.28	129.78 ± 0.27
	2	131.45 ± 0.31	130.42 ± 0.11	130.25 ± 0.05	130.45 ± 0.31	129.15 ± 0.34
	6	131.85 ± 0.22	131.25 ± 0.52	130.28 ± 0.50	130.87 ± 0.14	129.54 ± 0.15
	10	130.65 ± 0.45	131.35 ± 0.61	130.87 ± 0.16	130.86 ± 0.42	130.15 ± 0.24
RH	0	132.25 ± 0.47	131.86 ± 0.17	130.24 ± 0.25	130.45 ± 0.43	129.24 ± 0.58
	2	129.65 ± 0.56	130.25 ± 0.75	130.58 ± 0.19	130.28 ± 0.37	129.45 ± 0.64
	6	131.67 ± 0.23	130.25 ± 0.32	130.45 ± 0.23	131.24 ± 0.16	130.45 ± 0.24
	10	130.24 ± 0.15	131.25 ± 0.46	130.25 ± 0.57	130.54 ± 0.24	129.26 ± 0.26

5.3.2.2 Lipid oxidation

5.3.2.2.1 Changes of individual unsaturated fatty acids

LA, LNA, EPA and DHA stability in AF and RH sausages were investigated (Figures 5.1, 5.2). In contrast, LA and LNA content were high in all samples because 20% of soybean oil was used as one of the major ingredients. EPA and DHA were also high in samples added tuna oil. Addition of tuna oil increased EPA and DHA content and decreased LA (omega 6 fatty acid family) content of sausages made from two species (Figures 5.1, 5.2). KI fortification had no effect on stability of individual fatty acids because low amount of KI was added (≈ 0.009 mM). From the previous study, addition of ≤ 50 mM KI had no effect on lipid oxidation of O/W emulsion prepared from tuna oil. LA and LNA content dramatically decreased after 3 weeks of storage in the controls (10% soybean oil) and sample with 2% of tuna oil addition (Figure 5.1 and 5.2, a, b, c, d). EPA and DHA content of controls and sausage fortified by 2% tuna oil remained constant throughout the studied period. However, they decreased after 3 weeks of storage in samples added 6 and 10% tuna oil ($p<0.05$). These results suggested that LA and LNA underwent lipid oxidation in

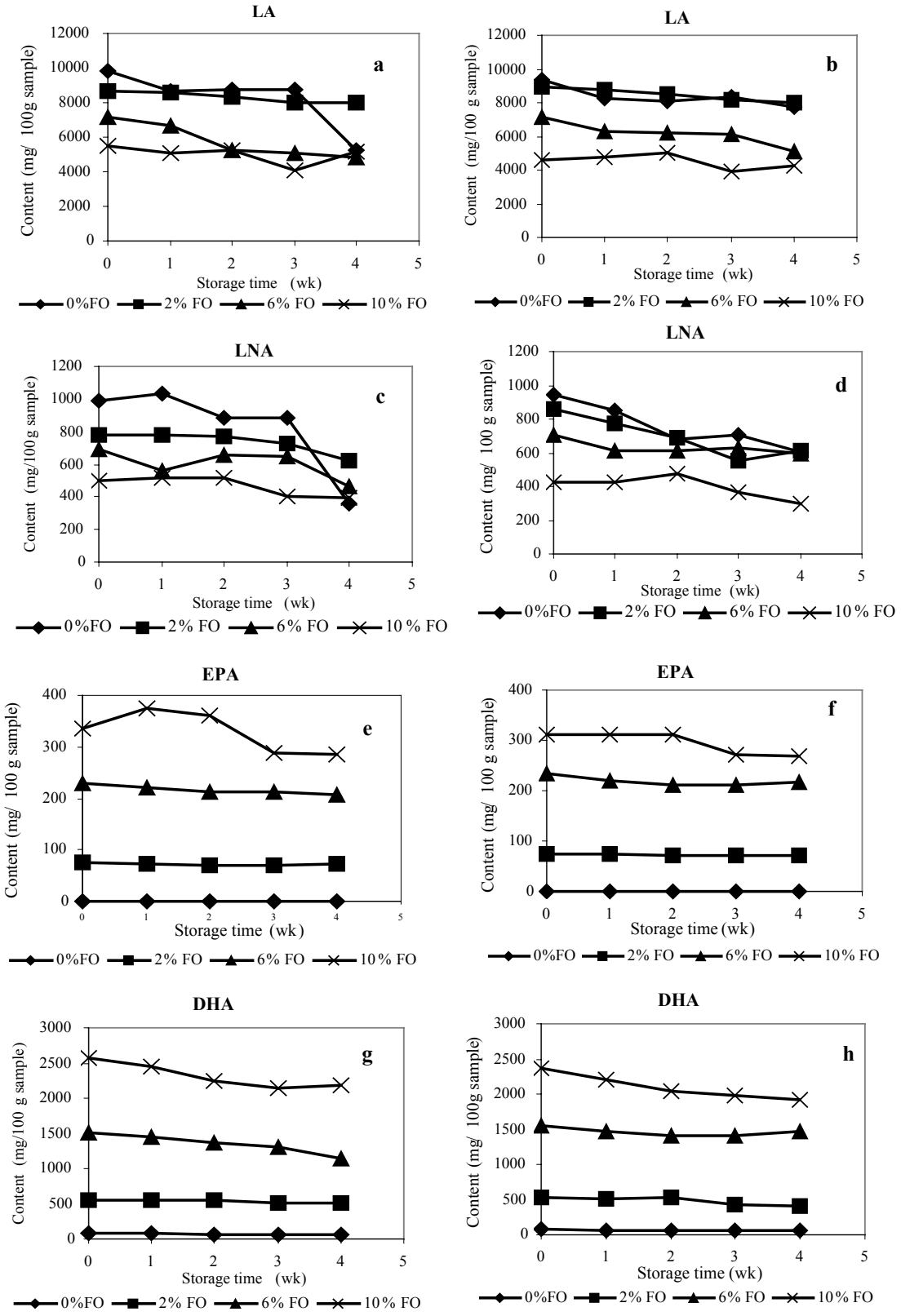

Figure 5.1 Changes of individual unsaturated fatty acids of AF sausage; LA= linoleic acid, LNA=linolenic acid, EPA=eicosapentaenoic acid. DHA=docosahexaenoic acid; a, c, e, g = no added KI; b, d, f, h = 150 µg KI/100 g sample; FO= Tuna oil

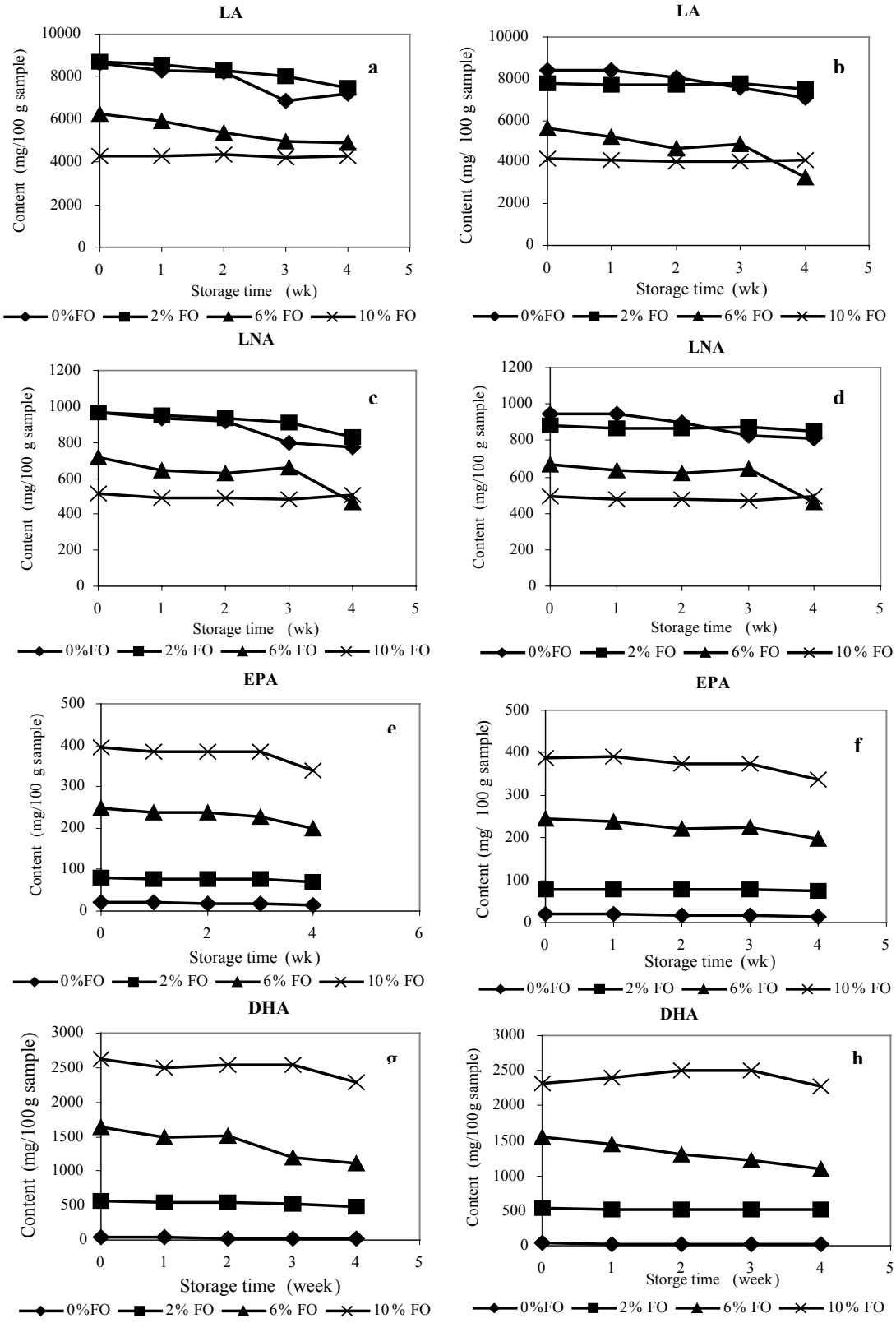

Figure 5.2 Changes of individual unsaturated fatty acids of RH sausage; LA= linoleic acid; LNA=linolenic acid; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid; a, c, e, g no added KI; b, d, f, h = 150 µg KI/100 g sample; FO= Tuna oil

samples containing low amount of tuna oil (control and 2% tuna oil)l, while EPA and DHA were substrates for lipid oxidation in samples containing 6 and 10% tuna oil.

5.3.2.2.2 HPV and TBARS values

HPV and TBARS values increased when addition level of tuna oil increased in both sausages ($p<0.05$)(Figures 5.3, 5.4). This was due to higher omega 3 polyunsaturated acids content, especially EPA and DHA (Figures 5.3, 5.4). HPV and TBARS gradually increased with storage times. Lin, Lin, and Kua (2002) reported that frozen breast and thigh from broilers fed 2 and 4% supplemental fish oil showed

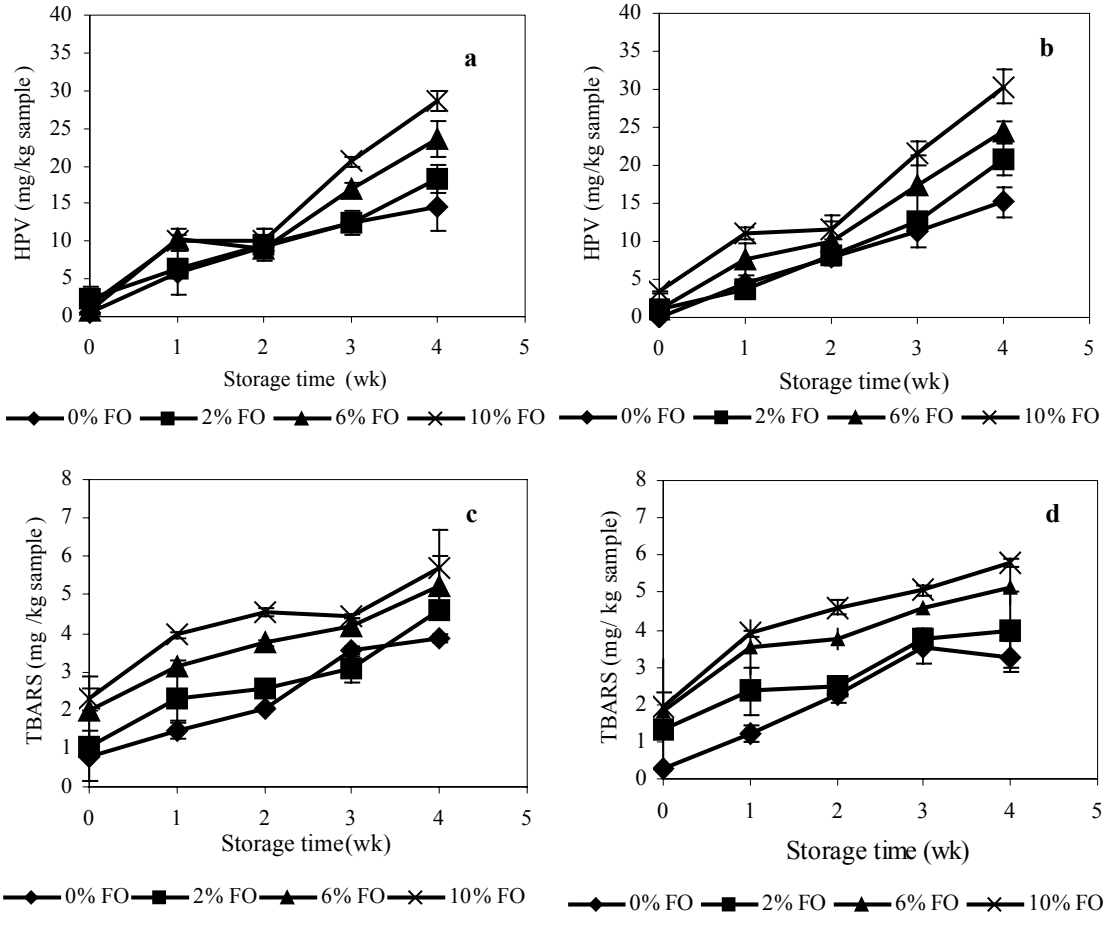

Figure 5.3 Changes of HPV (a, b) and TBARS values (c, d) of AF sausage stored at 4 °C; a, c, no added KI; b, d, 150 µg KI/ 100 g

higher TBARS than the controls (0% supplementation) during storage for 30 days. They also demonstrated that chicken frankfurters made from 2 and 4% supplemental fish oil had higher TBARS than the control. KI had no effect on HPV and TBARS values of all sample ($p>0.05$). It was suggested that fortification of KI (150 μg/100 g sample) had no effect on lipid oxidation. KI did not accelerate lipid oxidation of tuna oil. This was probably due to low amount of KI added (150 μg/ 100 g sample).

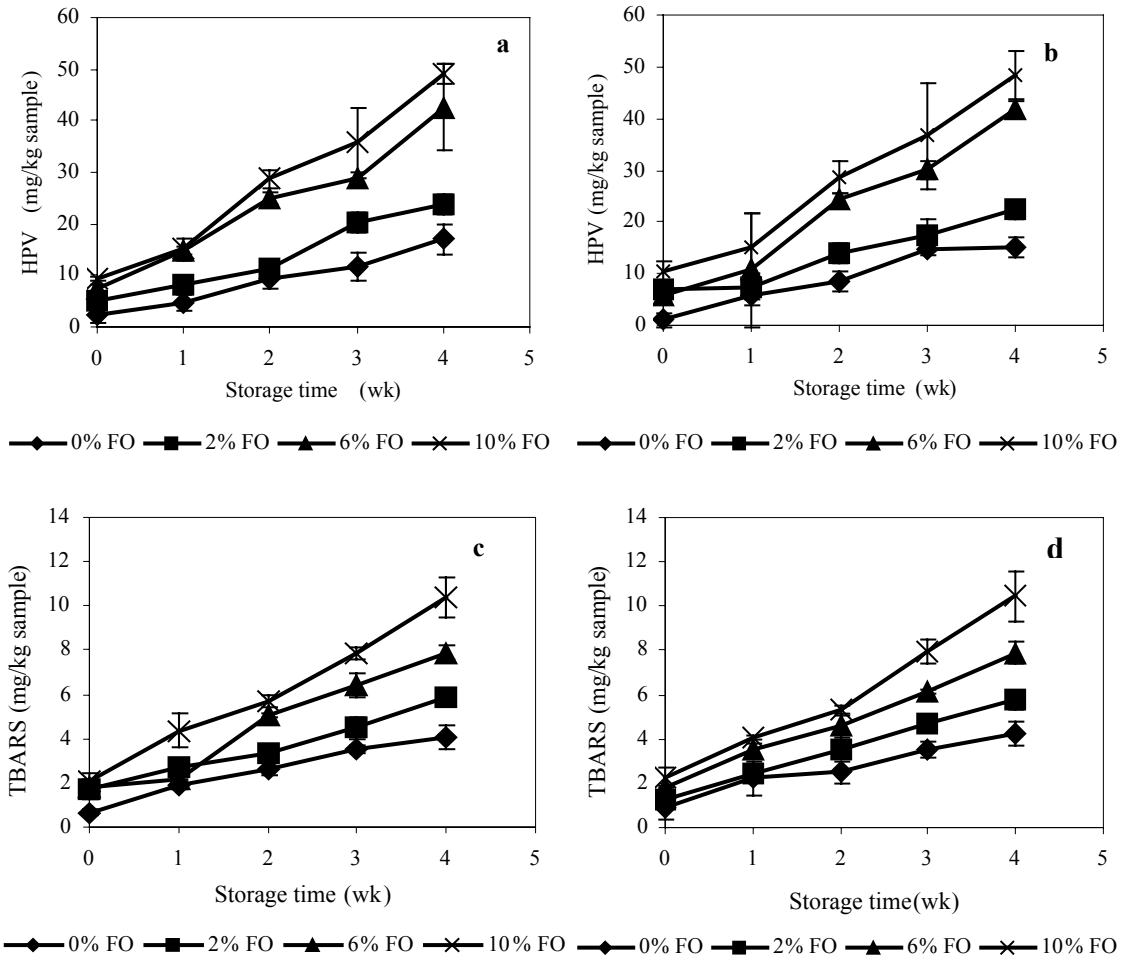

Figure 5.4 Changes of HPV (a, b) and TBARS values (c, d) of RH sausage stored at 4 °C; a, c, no added KI; b, d: 150 μg KI/ 100 g

However, HPV and TBARS values of RH sausages were higher than those of AF sausages. This was because RH flesh contained higher unsaturated fatty acids content, especially omega 3 polyunsaturated fatty acids (Table 5.2).

5.3.2.3 Changes of n6/n3 ratio

The n6/n3 ratio was dramatically decreased with added levels of tuna oil in both sausages (Figure 5.5). The n6/n3 ratio was constant during storage time (Figure

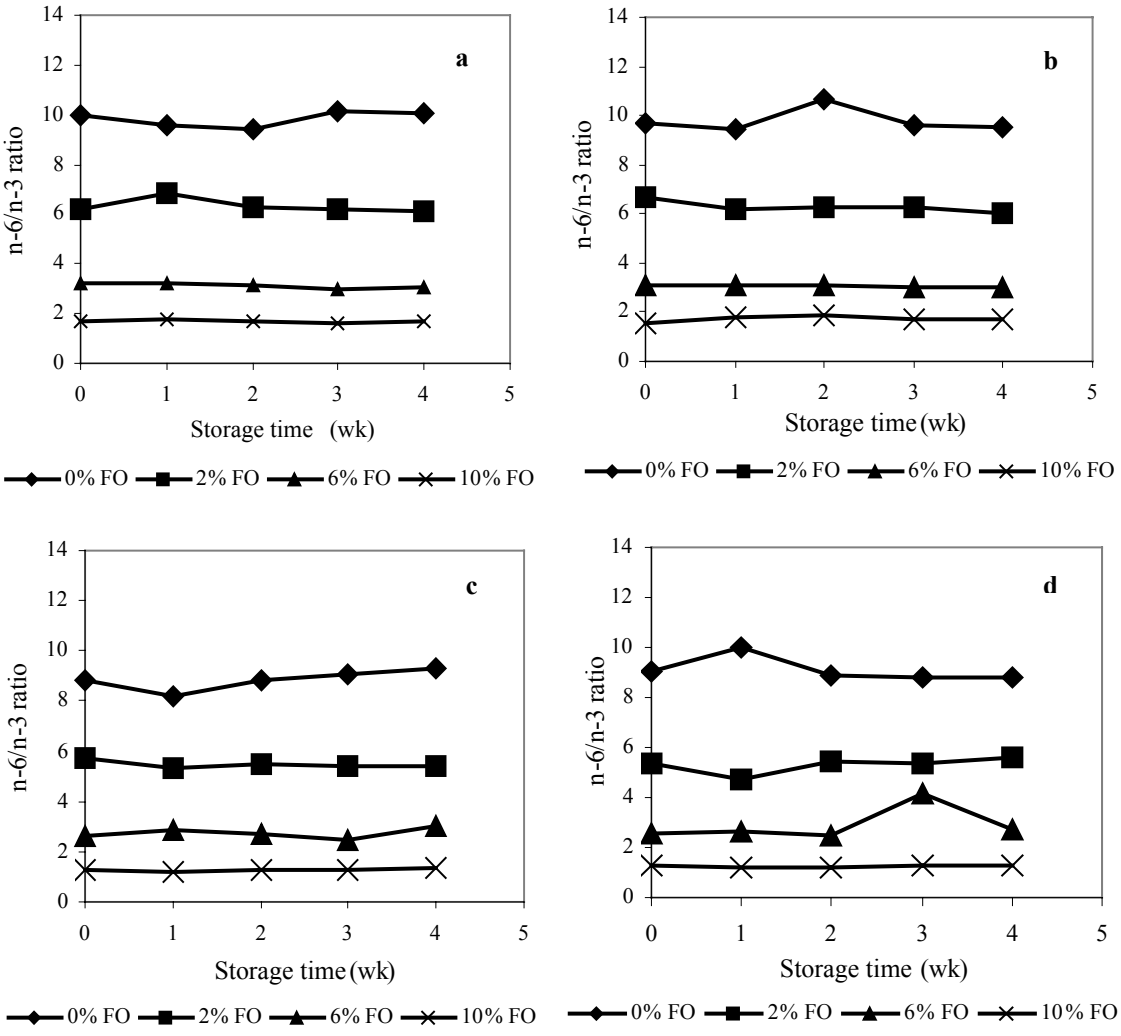

Figure 5.5 Changes of n6/ n3 ratio of AF (a, b) and RH sausage (c, d);
a, c = no added KI; b, d = 150 μg KI/100 g sample

5.5). The addition of 2% tuna oil showed higher n6/n3 ratio than the value recommended by U.K Department of Health (maximum at 5), whereas fortification of tuna oil at 6 and 10% reduced n6/n3 ratio to 3.20 and 1.72 for AF sausages, and 2.5 and 1.25 for RH sausages, respectively. Kouba, Enser, Whittington, Nute and Wood (2003) reported that feeding a diet containing 6% whole crushed linseed (rich source of omega 3 fatty acids) reduced the n6/n3 ratio in longissimus muscle from 7.6 to 3.9. KI fortification had no effect on changes of n6/n3 ratio in all samples (Figure 5.5). Thus, KI at 150 μg / 100 g sample did not accelerate lipid oxidation of n-3 and n-6 fatty acids.

5.3.3 Color and textural properties

Addition level of KI (150 μg/ 100 g sample) and tuna oil (2, 6, 8, and 10%) had no effect on changes of lightness (L^*) and yellowness (b^*) throughout storage period ($p<0.05$) (Figure 5.6). L^* and b^* values remained constant in all samples (Figure 5.6). Ponnampalam Trout, Sinclair, Egan, and Leury (2001) reported that substantial increase of omega 3 and omega 6 fatty acids had no effect on color values (L^*, a^*, b^*) of fresh and vacuum packaged lamb over a 6-day display period. The effect of KI and tuna oil fortification on color changes (L^* *and* b^*) of AF sausage over the entire storage time was similar to that of RH sausage (Figure 5.7). However, L^* values of RH sausages were higher than those of AF sausages. This was probably because RH muscle contained lower myoglobin content than AF. It should be noted that lipid oxidation (Figure 5.3 and 5.4) did not affect color (L^* and b^* values) of fish sausage during storage.

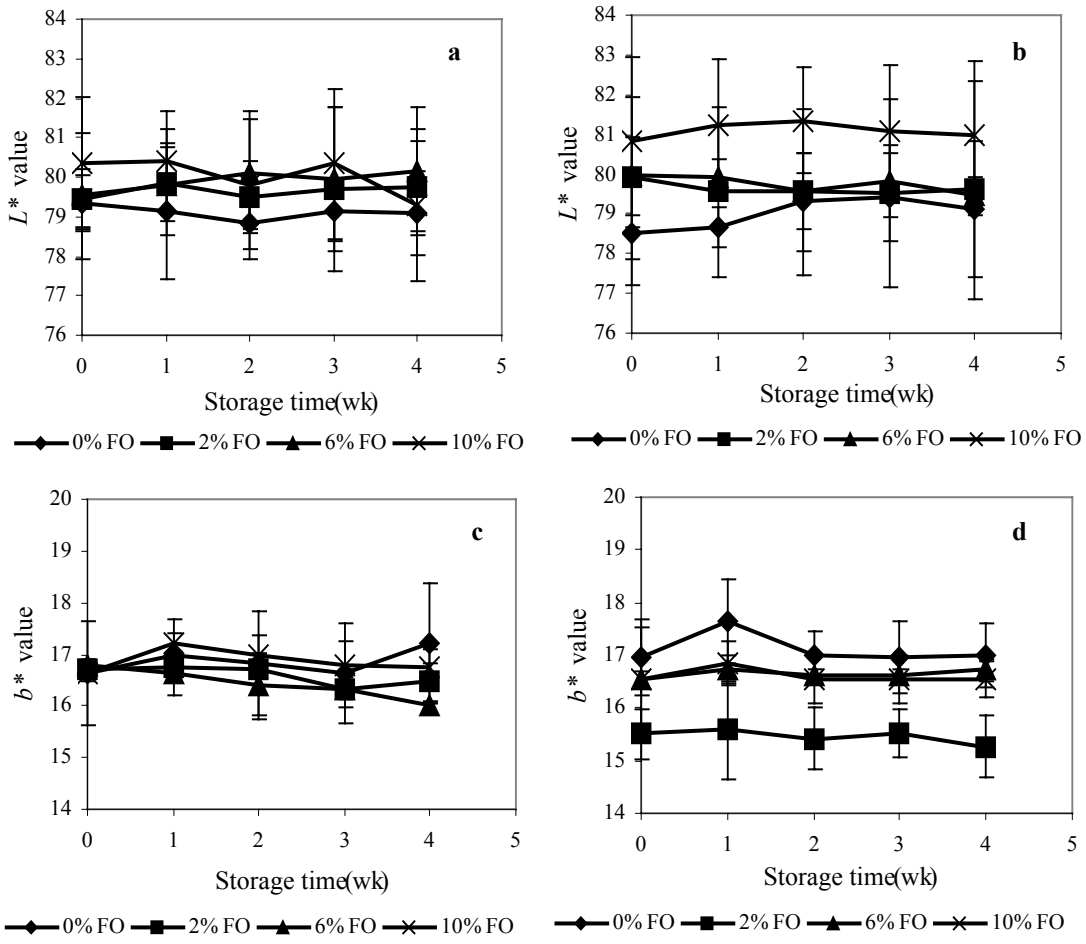

Figure 5.6 Changes in *L** (a, b) and *b** (c, d) values of AF sausage;
a, c, no added KI; b, d , 150 μg KI/100 g sample

Fortification of KI and tuna oil had no effect on breaking force (Figure 5.8a, b) and deformation of AF sausages (Figure 5.8c, d). Breaking force and deformation remained constant during storage. Perez-Mateos, Boyd, and Lanier (in press) suggested that fortification of fish oil to attain 1.5 and 2.5% omega 3 fatty acids had no effect on textural property of surimi seafoods during 2 months storage. Similarly, KI and tuna oil did not affect breaking force and deformation of RH sausage ($p>0.05$, Figure 5.9). Breaking force values of RH sausage were lower than those of AF suggested that fortification of fish oil to attain 1.5 and 2.5% omega 3 fatty acids had no effect on textural property of surimi seafoods during 2 months storage. Similarly,

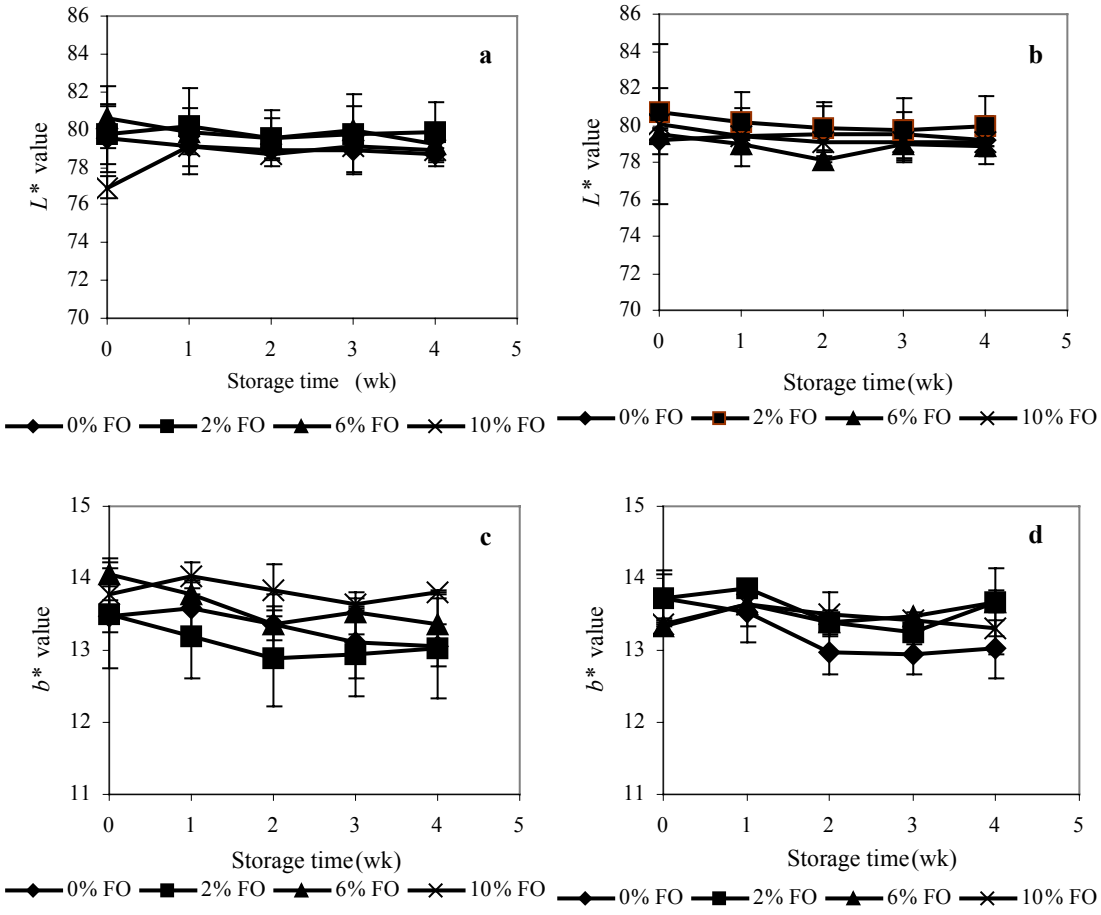

Figure 5.7 Changes in L^* (a, b) and b^* (c, d) values of RH sausage; a, c, no added KI; b, d, 150 µg KI/100 g sample, FO=tuna oil

KI and tuna oil did not affect breaking force and deformation of RH sausage ($p>0.05$, Figure 5.9). Breaking force values of RH sausage were lower than those of AF sausage, indicating that gel-forming ability of RH muscle was inferior to that of AF muscle. Chan, Gill, and Paulson (1992) indicated that the differences in gel-forming ability of fish proteins were attributed to different ability of myosin heavy chain cross-linking among varied species. Lipid oxidation did not affect textural properties of sausages in this study. This was because gel network already established before

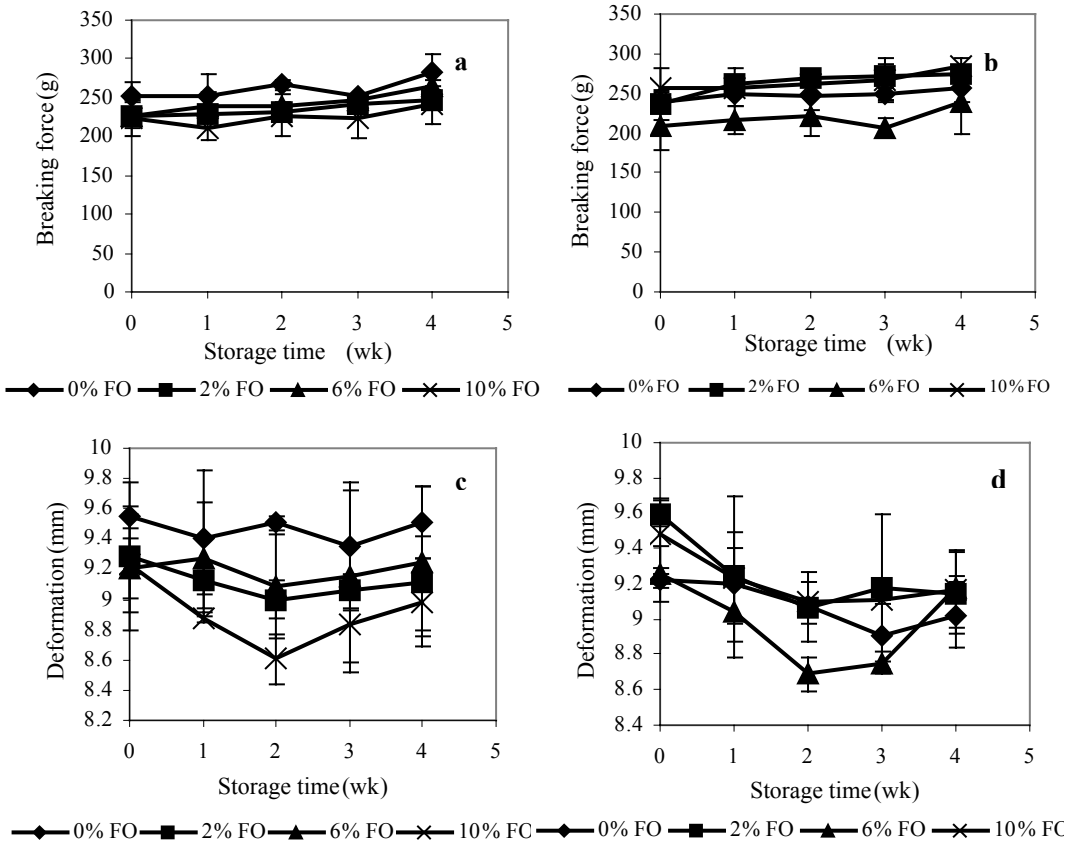

Figure 5.8 Breaking force (a, b) and deformation (c, d) of AF sausage:
a, c = 0 μg KI, b, d = 150 μg KI/100 g sample, FO=Tuna oil

formation of hydroperoxides. Recent studies revealed the effect of lipid oxidation on gel forming ability of proteins. Careche, Cofrades, Carballo, and Colmenero (1998) reported that gel strength of hake, pork, and chicken muscle proteins treated with formaldehyde (lipid oxidation product) remained constant during 10 weeks of frozen storage. In contrast, Liu and Xiong (2000) and Liu, Xiong, and Butterfied (2000) suggested that a substance generated from lipid oxidation, malonaldehyde, decreased gel forming ability of chicken myofibrillar proteins. These experiments were carried out by direct addition of malonaldehyde to protein. Lipid oxidation decreased stability of myosin conformation, increased protein carbonyls, and promoted myosin heavy chains cross-linking. As a result, gel forming ability of myosin heavy chain decreased.

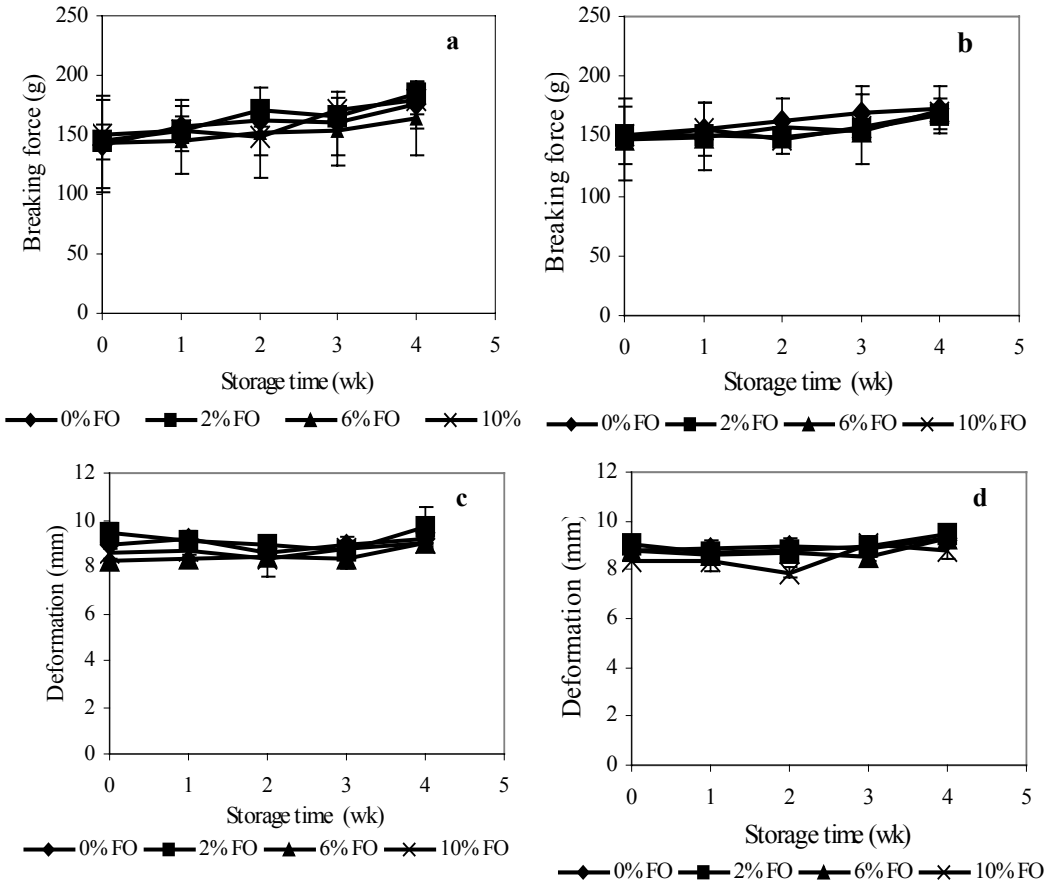

Figure 5.9 Breaking force (a, b) and deformation (c, d) of RH sausage; a, c, no added KI; b, d, 150 µg KI/100 g sample

Protein patterns of raw mince, samples incubated at 55 °C for 40 min, and finished sausage were comparable among paste, incubation at and 80 °C for 15 min for AF (data not shown). Incubation at 55 °C for 40 min followed by 80 °C for 15 min did not promote proteolysis in AF. However, degradation of myosin heavy chain (MHC) to smaller polypeptide chain was observed in RH sausage (Figure 5.10). This could be resulted from endogenous proteinases. Proteolysis in RH also contributed to poorer textural properties as compared to AF.

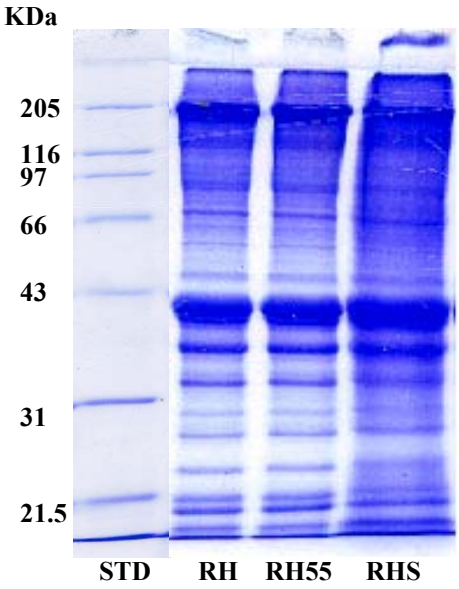

Figure 5.10 Changes in SDS-PAGE patterns of RH proteins; RH, raw RH meat; RH55 , RH meat incubated 55 °C for 40 min and 80 °C 15 min; RHS, RH sausage

5.4. Conclusion

AF and RH flesh contained low level of iodine and omega 3 fatty acids. Fortification of KI (150 μg/ 100g sample) and tuna oil (2, 6, and 10%) had no effect on color (L^* and b^*) and gel strength (breaking force and deformation) of sausages prepared from both species during storage at 4 °C. AF sausage exhibited higher gel strength than RH sausage because proteolysis took place during sausage preparation of RH. KI had no effect on lipid oxidation of sausages prepared from both species. Therefore, addition of 150 μg/ 100g samples did not effect on lipid oxidation. Lipid oxidation increased with addition of tuna oil. Addition of 6 and 10% tuna oil showed lower n6/n3 ratio than 5, resulting in a reduced risk of cardiovascular disease. However, the fortified tuna oil sausages were more prone to lipid oxidation.

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CHAPTER VI

CONCLUSIONS

Rohu (RH) muscle proteins exhibited the highest ES at 50 °C. Emulsifying activity index (EAI) and emulsion stability (ES) values at 50 °C were lower than those at room temperature (28 °C). EAI values of various fish species were similar when incubated at either 28 or 50 °C. Myofibrillar protein was important protein component exhibiting emulsifying property. KI concentration up to 100 mM had no effect on ES, $d_{3,2}$, and myofibrillar protein solubility. EAI and surface hyrophobicity (S_0) slightly increased as KI increased from 60 to 100 mM. KI concentration at 100 mM appear to catalyze lipid oxidation of oil in water (O/W) emulsion. Addition of tuna oil (5 and 10%) also increased lipid oxidation of O/W emulsion.

In emulsion sausages, lipid oxidation as measured by hydroperoxide (HPV) and thiobarbituric reactive substances (TBARS) values increased and omega 6 to omega 3 fatty acid ratio (n6/n3) decreased with an increased tuna oil concentrations. Iodine content was readily reduced about 14% after cooking at 55 °C for 40 min and 80 °C for 15 min and remained constant during storage at 4 °C for 4 weeks. Fortification of KI (150 μg/ 100g sample) had no effect on lipid oxidation of sausages. AF sausage showed higher gel strength and lower lightness than of RH sausage. Addition of tuna oil (2, 6, and 10%) and KI levels (150 μg/100g sample) had no effect on changes of color and gel strength of fish sausage.

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